Title: A Prospective Study of Tocilizumab for the Prevention of Graft Failure and Graft-versus-Host Disease in Haplo-Cord Transplantation

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Statement of Compliance

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCM.

List of Abbreviations

AE	Adverse Event
ALL	Acute Lymphoblastic Leukemia
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
APC	Antigen-Presenting Cell
AST	Aspartate Aminotransferase
B-HCG	Beta-Human Chorionic Gonadotropin
CAR-T	Chimeric Antigen Receptor T-Cell
CBT	Cord Blood Transplantation
CFR	Code of Federal Regulations
CI	Continuous Infusion
CIBMTR	Center for International Blood and Marrow Transplant Research
CMV	Cytomegalovirus
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRS	Cytokine Release Syndrome
CT	Computed Tomography
CTSC	Clinical Translational Science Center
Cy	Cyclophosphamide
CyTOF	Mass Cytometry by Time of Flight
DAMPS	Danger-Associated Molecular Patterns
DFS	Disease-Free Survival
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
EBV	Epstein-Barr Virus
eGFR	Estimated Glomerular Filtration Rate
FDA	Food and Drug Administration
Flu	Fludarabine
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor
GFRS	GVHD-Free/Relapse-Free Survival
GI	Gastrointestinal
GVHD	Graft-versus-Host Disease
GVL	Graft-versus-Leukemia
GVT	Graft-versus-Tumor

Gy	Gray
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HRBFA	Human Research Billing Analysis Form
HSCT	Hematopoietic Stem Cell Transplantation
HUD	Humanitarian Use Device
ICF	Informed Consent Form
IDE	Investigational Device Exemption
IFN-y	Interferon gamma
IL	Interleukin
IND	Investigational New Drug
IPA	Inherited Paternal Antigen
IQR	Interquartile Range
IRB	Institutional Review Board
IV	Intravenously
JAK	Janus Kinase
KPS	Karnofsky Performance Status
LVEF	Left Ventricular Ejection Fraction
MDRD	Modification of Diet in Renal Disease Study
Mel	Melphalan
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetil
MNC	Mononuclear Cell
MRD	Measurable Residual Disease
MSKCC	Memorial Sloan Kettering Cancer Center
MUD	Matched Unrelated Donor
NIMA	Non-Inherited Maternal Antigen
NMDP	National Marrow Donor Program
OS	Overall Survival
PAMPS	Pathogen-Associated Molecular Patterns
PB	Peripheral Blood
PBSC	Peripheral Blood Stem Cell
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PFS	Progression-Free Survival

PHI	Protected Health Information
PI	Principal Investigator
PO	Per os
PTCy	Post-Transplant Cyclophosphamide
PTLD	Post-Transplant Lymphoproliferative Disease
rATG	Rabbit Antithymocyte Globulin
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SGPT	Serum Glutamate-Pyruvate Transaminase
SNP	Single Nucleotide Polymorphisms
SR	Steroid-Refractory
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBI	Total Body Irradiation
TBV	Total Blood Volume
TNC	Total Nucleated Cell
TNFa	Tumor Necrosis Factor alpha
Tregs	Regulatory T-cells
TRM	Transplant-Related Mortality
UCB	Umbilical Cord Blood
UIRTSO	Unanticipated Problem Involving Risks to Subjects or Others
WCM	Weill Cornell Medicine
WRG-CT	Weill Research Gateway – Clinical Trials

1.0 Protocol Summary

Full Title:	**A Prospective Study of Tocilizumab for the Prevention of Graft Failure and Graft-versus-Host Disease in Haplo-Cord Transplantation**
Short Title:	Tocilizumab in Haplo-Cord Transplant
Clinical Phase:	Phase II
Principal Investigator:	Alexandra Gomez Arteaga, MD
Study Description:	Haplo-Cord transplantation – the co-infusion of a single umbilical cord blood graft with CD34-selected cells from a haplo-identical adult donor, provides an alternate source of stem cells for patients with high-risk hematologic malignancy in need of an allogeneic transplant but lacking a suitable HLA-matched donor. Traditionally, anti-thymocyte globulin (ATG) is administered prior to haplo-cord transplantation to reduce the incidence of graft failure, graft-versus-host disease (GVHD) and graft versus graft reactions. However, excessive ATG-mediation of cord lymphocytes may compromise immune reconstitution leading to increased infections, viral reactivations and relapse – due to impaired graft versus tumor (GVT) effects. Tocilizumab is an IL-6 receptor antibody with demonstrated efficacy in the prevention of GVHD. In this prospective investigator-initiated study, we will assess the safety of reducing and ultimately eliminating ATG from our haplo-cord preparative regimen while using tocilizumab as an alternative immunomodulatory agent. We hypothesize that this will improve immune reconstitution while preserving the low rates of graft failure and GVHD that we currently enjoy.
Study Population:	Adult patients with hematologic malignancy in need of an alternate donor transplant
Sample Size:	The smallest sample size for the trial is 10 subjects and the largest is 70 evaluable subjects. Four successive subject cohorts will accrue up to 10 subjects. The final group will be expanded to a total subject size of 40 (See statistical plan section 11.2)
Enrollment:	Subjects who undergo study treatment but are not evaluable for the primary endpoint due to death prior to day +21 will be replaced. Assuming that no more than 5% of subjects are non-evaluable, the largest accrual would be 74 subjects (=70 x 1.05) (See statistical plan section 11.2)

Enrollment Period:	Two years (projected)
Study Design:	In this prospective study, all subjects will receive haplo-cord transplantation, conditioned with fludarabine, melphalan and total body irradiation (TBI), followed by a single dose of tocilizumab 8 mg/kg on day -1 of their regimen. Subjects will be recruited on to four successive cohorts – with subjects initially receiving our current standard 3 doses of ATG 1.5 mg/kg (total dose 4.5 mg/kg). In the absence of safety signals, we will drop one dose of ATG in successive cohorts until the drug ultimately has been eliminated entirely. We use a single arm, Bayesian study design. Each dose group will accrue up to 10 subjects. If there are 4 or fewer successes, that dose group will be deemed unacceptable and the next higher ATG dose for which there were 5 or more successes will be expanded. In other words, there will be 10 subjects initially enrolled in the first group (total ATG dose of 4.5 mg/kg). At the point of 5 or more successes are observed, subjects will be enrolled at dose group II (even if less than 10 subjects were accrued to group I). This will continue until there is a point where there are 4 or fewer successes out of 10 subjects. At this point, the next lower group (higher dose) will be expanded to a total of 40 subjects with interim analyses done after each 10 subjects for futility. An expansion cohort sample size of 40 has 80.9% power of declaring the regimen active if the true success probability is 80%. Planned correlative studies are outlined in section 2.12
Study Duration:	Projected end date for enrollment January 2022. Final data analysis to be completed one year after the last subject accrual – projected January 2023**.**
Participant Duration:	Following discharge from the in-patient transplant service, regular study visits will take place until 12 months post-transplant. After this time, subjects will continue to be followed for long-term outcomes at a frequency determined b the primary transplant physician for up to 5 years.
Study Agent/Device Name	Tocilizumab
Intervention Description:	Tocilizumab 8 mg/kg IV administered as a single dose on Day -1 of transplant conditioning regimen

Primary Objective: To assess the safety and efficacy of tocilizumab in preventing early failure of the haplo-identical graft in haplo-graft transplantation

Primary Endpoint: The primary endpoint is the percentage of successful haplo-derived neutrophil engraftment as defined by:

- An absolute neutrophil count (ANC) of ?500 cells/microL for three consecutive days with the first on or prior to Day +21 post-transplant
- Absence of a second nadir, i.e. – a drop in the ANC to <0.3 cells/microL for 5 consecutive days occurring after initial engraftment within the first 3 weeks post-transplant

Secondary Objectives: To evaluate transplant outcomes in subjects undergoing haplo-cord transplantation using tocilizumab as an alternative to ATG – including overall survival, progression-free survival, relapse, transplant-related mortality, GVHD, toxicities, platelet engraftment, infections, post-transplant lymphoproliferative disorders and viral reactivations (See section 9 for definitions and section 11.3.2 for analysis)

Exploratory Objectives: To characterize the effects of tocilizumab used as an alternative to ATG in haplo-cord transplantation on immune reconstitution, serum cytokine profiles, novel predictive biomarkers of GVHD and on measurable residual disease (MRD)

1.1 Schema

INCLUSION/EXCLUSION: See Section 4.

ENROLLMENT: All potential participants will have complete human leukocyte antigen (HLA) typing and determination of HLA antibodies.

TREATMENT PLAN:

Conditioning Regimen: Age <60yrs

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1 or day 0
Fludarabine	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2			Haplo Infusion	UCB Infusion
Melphalan						140 mg/m^2			
Tocilizumab							8 mg/kg		
TBI				2Gray	2Gray				
ATG Group I			1.5 mg/kg		1.5 mg/kg		1.5 mg/kg		
ATG Group II			1.5 mg/kg		1.5 mg/kg		_		
ATG Group III			1.5 mg/kg		_		_		
ATG Group IV			_		_		_		

Conditioning Regimen: Age >60yrs

	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1 or day 0
Fludarabine	30 mg/m^2	30 mg/m^2	30 mg/m^2			Haplo Infusion	UCB Infusion
Melphalan				140 mg/m^2			
Tocilizumab					8 mg/kg		
TBI		2Gray	2Gray				
ATG Group I	1.5 mg/kg		1.5 mg/kg		1.5 mg/kg		
ATG Group II	1.5 mg/kg		1.5 mg/kg		_		
ATG Group III	1.5 mg/kg		_		_		
ATG Group IV	_		_		_		

Fludarabine:
Age <60yrs: 30 mg/m^2/day IV x 5 days (Total dose 150 mg/m^2).
Age >60yrs: 30 mg/m^2/day IV x 3 days (Total dose 90 mg/m^2).
Fludarabine will be dosed according to actual body weight.

Melphalan: 140 mg/m^2/day IV x 1 day for all patients. Melphalan will be dosed according to actual body weight. Cryotherapy with ice chips will be administered to prevent mucositis.

Tocilizumab: 8 mg/kg/day IV x 1 day. Tocilizumab will be dosed according to actual body weight. Patients >100kg will receive a capped maximum dose of 800 mg.

TBI: Conditioning will be intensified with 2 doses of TBI (4 Gy) for all patients. This serves to decrease graft rejection and reduce the risk of relapse of malignant disease.

Rabbit ATG (rATG)*: In the absence of safety signals, dosing will be reduced as the study proceeds as follows –

- Cohort I: 1.5 mg/kg/day IV x 3 days (Total dose 4.5 mg/kg) on days -5, -3 and -1.
- Cohort II: 1.5 mg/kg/day IV x 2 days (Total dose 3 mg/kg) on days -5 and -3.
- Cohort III: 1.5mg/kg/day IV x 1 day (Total dose 1.5 mg/kg) on day -5 only.
- Cohort IV: No rATG to be administered.

ATG will be dosed according to actual body weight. The first dose will be infused over at least six hours, and any subsequent doses over at least 4 hours. Pre-medications include acetaminophen 650 mg PO, diphenhydramine 25-50 mg PO/IV, and methylprednisolone 2 mg/kg (1 mg/ kg at the initiation and 1 mg/kg half-way through anti-thymocyte globulin administration).
Circumstances may require minor changes in scheduling of chemotherapy. Variations of up to 24 hours in scheduling will be acceptable.

GVHD Prophylaxis:

Tacrolimus: 0.03 mg/kg/day IV continuous infusion (CI) over 24 hr from 4 PM Day -2 until engraftment or when subject is able to take PO, then tacrolimus approximately 0.09 mg/kg PO in 2 divided doses. Tacrolimus should be given at full dose to maintain levels of 5-15 ng/mL through day 180, tapered by 20% every week thereafter. PO tacrolimus can be used when IV access for CI tacrolimus is unavailable.

Cyclosporine: May be used as an alternative to Tacrolimus. 3 mg/kg/day IV over 24 hr from 9 PM Day -2 until engraftment or when subject is able to take PO, then cyclosporine 3.5 mg/kg PO twice a day. Cyclosporine should be given at full dose to maintain levels of 150-400 ng/mL through day 180 and tapered by 20% every week thereafter. PO cyclosporine can be used when IV access for infusion is unavailable.

Infection, toxicity or other clinical circumstances may prompt earlier discontinuation or adjustment of tacrolimus/cyclosporine doses. In the presence of GVHD, a clinical decision by the attending physician will determine if tacrolimus/cyclosporine can be tapered or should be continued.

Mycophenolate Mofetil (MMF): will be started on day -2 and given at a dose of 1000 mg q8 hours until day 28. MMF can be given PO or IV. Infection, toxicity, very low patient weight (<50kg) may prompt earlier discontinuation or adjustment of doses.

Note: Patient specific circumstances may mandate minor variations in conditioning regimen and/or GVHD prophylaxis, such as reduction of some of the doses of medications, changes in schedule or – rarely - substitution of a particular drug. These changes will be noted and justified, but will not require IRB approval.

Stem Cell infusion:
The infusion of UCB and the haplo-identical units will be separated by at least 2 hours and preferably they will occur on successive days (Day 0 and Day 1). The order of infusion is not specified.

Supportive care:

- **CMV Prophylaxis**
  - Prophylaxis will follow institutional unit policy and will be guided by the recipient and donor CMV serostatus tested during pre-transplant screening.

- **Cytomegalovirus (CMV) monitoring:**
  - CMV viral load monitoring by PCR at least weekly until Day 100 and at least monthly until Day 210, regardless of donor/recipient CMV status.
  - Pre-emptive CMV treatment should be strongly considered for any positive result on by DNA testing (i.e, viral load) within the first 100 days of transplant.

- **PTLD Prophylaxis**
  - **Rituximab:** All patients with prior EBV exposure not previously exposed to rituximab or who have not received rituximab in the six months prior to transplant, will receive one dose of rituximab 375 mg/m^2 prior to or upon admission.

- **Epstein-Barr virus (EBV) monitoring:**
  - EBV viral load monitoring by PCR at least weekly until Day 100 and at least monthly until Day 365 is strongly recommended.
  - Rising EBV titers should warrant investigation for EBV post-transplant lymphoproliferative disorder (PTLD) including CT imaging and PET scanning for positive CT findings. A bone marrow evaluation is recommended if any evidence of PTLD is found on imaging or peripheral blood analysis.
  - Evidence of PTLD or consecutive increases in EBV PCR should lead to treatment with rituximab at a dose and schedule per institutional policy.

- A prophylactic broad-spectrum antifungal with anti-mold activity is strongly recommended.
- Other infection prophylaxis and supportive care will be as per institutional unit policy.
- In some cases, a drug called filgrastim (G-CSF) may be administered to hasten the recovery of blood counts.[1-3] Generic formulations of filgrastim may be utilized as required by insurance or pharmacy.
- Blood transfusion policy should follow institutional policy.

1.2 Study Objectives

1.2.1 Primary Objectives

- To assess the safety and efficacy of the humanized anti-IL6 receptor monoclonal antibody tocilizumab, used as an alternative to ATG, in preventing early failure of the haplo graft in haplo-cord hematopoietic cell transplantation.

1.2.2 Secondary Objectives

- To assess the safety and efficacy of tocilizumab, used as an alternative to ATG, in preventing graft versus host disease after haplo-cord transplantation.

- To evaluate transplant outcomes in subjects undergoing haplo-cord transplantation using tocilizumab as an alternative to ATG - including overall survival (OS), progression-free survival (PFS), relapse incidence, transplant-related mortality (TRM), GVHD, organ toxicities, infections, PTLD and viral reactivations.

- To characterize the effects of tocilizumab used as an alternative to ATG on immune reconstitution, serum cytokine profiles and on detectable measurable residual disease (MRD) after haplo-cord transplantation in the presence and absence of ATG.

1.2.3 Exploratory Objectives

- To characterize the effects of tocilizumab used as an alternative to ATG in haplo-cord transplantation on immune reconstitution, serum cytokine profiles, novel predictive biomarkers of GVHD and on measurable residual disease (MRD).

2. Background

2.1 Histocompatibility in Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the most definitive and best-established curative strategy for high-risk hematologic malignancy. The success of this therapy relies on the immunologic effects of a donor-derived hematopoietic system on residual tumor cells – a so-called 'graft versus tumor' (GVT) effect.

Initial attempts at HSCT in the 1950s and 1960s were undertaken without any understanding of histocompatibility and as a result were invariably unsuccessful due to disease relapse or severe immunologic reactions in the host.[4] Studies initially in mice[5] and later in dogs[6] led to the discovery of the human leukocyte antigen (HLA) system that we now recognize as the greatest barrier to successful HSCT.[7] HLA molecules are proteins expressed on the surface of cells, encoded by a series of over 250 highly polymorphic genes on chromosome 6 that form the major histocompatibility complex (MHC).[7] They are broadly divided into three categories with differences in structure and function - class I, class

II and class III. For transplant purposes, the most important molecules are HLA-A, HLA-B and HLA-C from Class I and HLA-DR, HLA-DQ and HLA-DP from Class II. Class I molecules are expressed on the surface of all nucleated cells and interact with CD8+ T lymphocytes to activate their cytotoxic or cytokine effector function. Class II molecules are expressed on antigen-presenting cells and their expression can be either up- or down-regulated by cytokines. These molecules interact with CD4+ T lymphocytes to activate cytokine-mediated helper or regulatory function.[7]

An individual's HLA type determines their immunologic identity and enables the distinction of self from non-self - thus governing the reciprocal immune reactions that occur when HSCs from one individual are transplanted into another. Alloreactive T cells in transplant recipients may interact with donor HLA molecules to cause graft rejection. The interaction of donor T-lymphocytes with recipient HLA molecules mediates the desired GVT effect but also the major transplant complication graft versus host disease (GVHD).

HLA genes are co-dominantly expressed and inherited in a Mendelian fashion such that there is a 25% probability of any two siblings being HLA-identical. According to the National Marrow Donor Program (NMDP), only roughly 30% of patients will have an HLA-matched sibling eligible to donate. Our national volunteer registry now contains over 19 million potential domestic unrelated donors and through international partnerships provides access to over 30 million. An optimal unrelated donor will match the recipient for each allele at least at HLA A, B, C and DRB1 – an 8/8 match. For every allelic mismatch at these loci, transplant survival probability decreases by 10%.[8] The probability of identifying a suitable 8/8 HLA-matched unrelated donor varies widely according to patient ethnicity. Whites of European descent have the highest likelihood at 75%, while blacks of South or Central American descent have only a 16% chance.[9] In our experience treating a uniquely diverse population, 30% of patients requiring HSCT will be unable to find a fully matched donor and will be in need of an alternate stem cell source.

2.2 Umbilical Cord Blood as a Hematopoietic Stem Cell Source

Umbilical cord blood (UCB) is rich in hematopoietic stem cells and has been used in HSCT since the 1980s. Cord stem cells are highly proliferative and are capable of securely engrafting despite the average graft containing 1-log fewer progenitor cells than an adult donation. They have the added advantage of being rapidly available, avoiding unnecessary delays in time to transplant for patients with high-risk malignancy.

HLA-matching requirements are less stringent for cord blood transplantation (CBT) than they are for adult unrelated donor transplants. Even in the mismatched setting, CBT is associated with low incidences of acute and chronic GVHD.[10] Traditionally matching has been limited to the examination of 6 HLA loci – HLA-A and HLA-B at intermediate resolution and HLA-DRB1 at high resolution, with 4-6/6 matched units being acceptable. HLA-C has generally been ignored. These criteria extend the option of transplant to minority patients with no readily available adult donor. In 2014, a registry study of 1568 patients reported progressively increasing TRM with increasing 8-allele mismatches. While mismatches at 1 or 2 HLA alleles were still better tolerated than they are in adult unrelated donor transplants, the study gives cause to consider more rigorous matching criteria.11 This however would restrict the availability of cord units for minority populations and at present is only adopted when multiple cord units are available for a particular patient.

The major limitation of CBT is delayed engraftment, with a median time to neutrophil recovery of 16 to 24 days reported across most series. This prolonged cytopenia translates into increased hospital stays, greater transfusion requirements, increased rates of opportunistic infection and ultimately - increased early transplant-related mortality (TRM). The total nucleated cell (TNC) dose per kilogram of recipient weight is a predictor of hematopoietic recovery after CBT with minimum doses of 2.5 x 10^7 TNC/kg being standard practice and even higher doses of 3 x 10^7 TNC/kg being more recently recommended.11 Higher TNC doses again may be required to compensate for increasing degrees of HLA mismatching.[11,12] The cord blood unit CD34+ count per kilogram of recipient weight measured at cryopreservation is an emerging factor guiding unit selection and may be a better predictor of post-thaw CD34+ dose and hematopoietic recovery than TNC.[13] A minimum count of 1.5 x 10^5 CD34+ cells/kg is considered preferable in most centers performing single unit CBT.

Using a minimum cell dose of 2.5 x 10^7 TNC/kg, the likelihood of identifying a 6/6 or 5/6 HLA-matched cord unit for a patient over 20 years of age is highest at 66% for whites of European descent but only 24% for the African American population with other ethnic groups falling within this range.[9] A recently published analysis of 126,341 cord blood units in the US inventory showed that adding to this a CD34+ cell dose requirement of 1.5 x 10^5 CD34+ cells/kg, only 4% of stored cord units would qualify for use as single-unit grafts for 70-kg patients.[14] Thus stringent implementation of these dosing requirements precludes the majority of adult minority patients from single unit CBT.

2.3 Haplo-Cord Transplantation

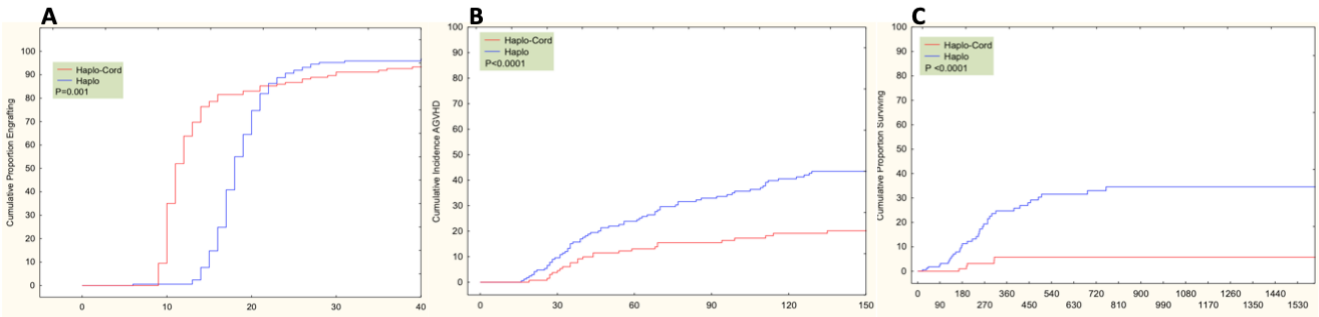
Disappointed with the delayed engraftment after single unit CBT, several groups have tried to enhance recovery by in vitro expansion of cords, the infusion of multiple cords or the combination of CBT with cells from other origins.[15] Our favored approach is a technique originally pioneered in Spain known as haplo-cord transplantation - the co-infusion of a single cord blood graft with CD34-selected cells from a haploidentical related or unrelated donor.[16] In this scenario the T-cell depleted haploidentical graft acts as a myeloid bridge until its eventual replacement by durable cord hematopoiesis.

In 2011, we published our initial experience using this technique after reduced intensity conditioning in 45 patients with hematologic malignancy. The median age of the cohort was 50 years, the median weight 80kg and 58% of the patients had active disease at the time of transplantation.[17] Cords were matched at a minimum of 4/6 HLA loci and the minimum cell dose was lowered to 1 x 10^7 TNC/kg. At this lower dose, suitable cord blood units could be more easily identified for overweight patients and for those of minority descent. Haplo-donations were T-depleted and capped at 3 x 10^6 CD34+ cells/kg. Neutrophil engraftment occurred at 11 days (Interquartile range (IQR), 9 -15) and platelet engraftment at 19 days (IQR, 15-33). In the majority of subjects, early haplo-engraftment was replaced by durable engraftment of umbilical cord blood by 100 days, with regular persistence of minor cell populations of host- and/or haplo-hematopoiesis. Higher haplo-chimerism at Day 100 correlated with greater haplo-CD34 dose (p=0.003). The cumulative incidence of acute GVHD was 25% and chronic GVHD was 5%. Actuarial survival at one year was 55%, progression-free survival was 42%, non-relapse mortality was 28% and relapse was 30%. The reduced UCB cell dose had no impact on time to hematopoietic recovery. We concluded that reduced intensity conditioning and haplo-cord transplant result in fast engraftment of neutrophils and platelets, low incidences of acute and chronic GVHD, low frequency of delayed opportunistic infections and promising long-term outcomes.[17] We have established a minimum UCB cell dose of 1.2 x 10^7 TNC/kg. Using this threshold, we can identify at least 5/8 HLA-matched cord units for over 95% of our transplant candidates.

We have continued to offer haplo-cord transplantation to patients lacking sibling or fully matched unrelated donors and now have considerable experience in this technique. In older adults (median age 63) with acute myeloid leukemia and myelodysplastic syndrome, we report the 3-year probability of overall survival at 45%, progression free survival at 30.2%, non-relapse mortality at 35.1% and relapse incidence at 34.7%. These outcomes are comparative to outcomes after matched unrelated donor transplantation.[18] Our experience in treating high-risk lymphoma and chronic lymphocytic leukemia has been similarly encouraging – in a heterogeneous but for the most part heavily pre-treated patient cohort, we report a 3-year overall survival of 65% and progression-free survival of 62%. The 1-year cumulative incidence of relapse was 19% and of non-relapse mortality was 19%.[19] In both of these studies, the rate of chronic GVHD after haplo-cord transplantation was low – 4.9% and 8% respectively.

Haplo-identical HSCT with post-transplant cyclophosphamide is a competing approach to haplo-cord transplantation in situations where fully HLA-matched donors are unavailable. We recently compared our haplo-cord outcomes with the outcomes of haplo-identical transplants performed at the MD Anderson Cancer Center.[20] All patients studied were under 60 years of age and all received reduced-intensity conditioning with fludarabine and melphalan with or without total body irradiation (TBI). GVHD prophylaxis consisted of cyclophosphamide, tacrolimus and mycophenolate for haplo-identical transplantation and ATG, tacrolimus and mycophenolate for haplo-cord recipients. Bone marrow was used as the preferred graft source in haploidentical transplants whereas for haplo-cord transplants peripheral blood stem cells were used. Despite haplo-cord patients being older and having more advanced disease, transplant outcomes were similar, with no significant difference in 4-year overall survival (Haplo 50% vs. Haplo-cord 49%), 1-year progression free survival (Haplo 45% vs. Haplo-cord 40%), 1-year relapse incidence (Haplo 27% vs. Haplo-cord 27%) or non-relapse mortality (Haplo 19% vs. Haplo-cord 18%). However, haplo-cord transplantation was associated with earlier neutrophil recovery (11 vs. 18 days, p=0.001), earlier platelet recovery (22 vs. 25 days, p=0.03) and lower rates of grade II-IV acute GVHD by day 100 (16% vs. 33%, p<0.0001) and a lower incidence of chronic GVHD at one year (4% vs. 16%, p<0.0001) **(Figure 1).**[20]

Figure 1: Comparison of outcomes between HC and haplo transplant over time in days. (A) Neutrophil



recovery, (B) Acute GVHD Grade 2-4 and (C) Chronic GVHD.[20]

2.4 Cord Blood Transplantation and the Graft versus Tumor effect

In retrospective analyses, similar rates of disease-free survival (DFS) are seen when comparing umbilical cord blood transplants to fully matched unrelated donor transplants in both the adult and

pediatric setting.[21-24] Umbilical cord blood appears to mount a robust graft versus tumor (GVT) effect that may be superior to that seen from other graft sources and may compensate for the complications of delayed engraftment.

Kwon et. al. conducted a comparison of haplo-cord transplantation and haploidentical transplantation with post-transplant cyclophosphamide (PTCY-haplo) in patients with AML following myelo-ablative conditioning. Similar rates of relapse and survival were observed between the groups despite the haplo-cord cohort having a higher proportion of patients with a high disease risk index – suggesting a more powerful GVT effect from the cord graft.[25] In keeping with this, we have demonstrated that UCB chimerism after haplo-cord transplantation directly correlates with relapse incidence. At 2 months post-transplant, UCB chimerism levels under 20% in the CD33 lineage were associated with increased disease recurrence (54% versus 11%, p<0.0001). Less than 100% UCB chimerism in the CD3 lineage also associated with increased recurrence (46% versus 12%, p=0.007)[26]

A retrospective study from the Fred Hutchinson Cancer Research Center published in the New England Journal of Medicine showed that in patients with acute leukemia or myelodysplastic syndrome who entered transplant with minimal residual disease, the risk of relapse was higher after MUD than CBT (Hazard ratio 2.92, p=0.0007).[27] These observations are supported by experiments using a mouse model of B-cell lymphoma, which demonstrate that cord blood T-cells mediate stronger antitumor responses than adult peripheral blood T cells administered at equal numbers.[28]

The mechanisms behind the superior GVT effect of UCB are under continued investigation. Cord blood T-cells have unique immunologic properties – the majority have a naïve phenotype and are antigen inexperienced but once stimulated, they transform more quickly than adult T cells into central memory T-cells. CBT recipients also demonstrate faster B-cell and NK-cell reconstitution than other cell sources.[29]

UCB may contain a substantial number of lymphocytes of maternal origin that are primed against the paternal HLA-haplotype of the fetus – the inherited paternal antigen or IPA. These lymphocytes are protected from immune mediated destruction by the presence of a very high number of fetal T-regulatory cells. In the large majority of cases, the IPA HLA haplotype is not present in these maternal cells and are recognized as foreign. Van Rood et. al. speculated that the IPA-primed maternal cells were responsible for the graft versus leukemia (GVL) activity of UCB and that a similar mechanism explained why haplo-identical transplant from a mother to her child is associated with less relapse than that from a father. In an elegant retrospective study, they demonstrated that patients with acute leukemia who shared on or more HLA-A, -B or DRB1 antigens with their UCB donor's IPAs had a reduced incidence of relapse compared to patients with no shared IPA (Hazard ratio 0.38, p <0.001). Relapse risk was lower when the CB came from a second or subsequently born child than from firstborn children, perhaps correlating with maternal lymphocyte memory from prior priming.[30]

Post-transplant disease recurrence can occur if tumor cells develop a means to evade immune recognition. In the case of haplo-identical transplantation, disease control depends on donor T-cells recognizing a fully mismatched haplotype as foreign. Loss of genomic heterozygosity through acquired uniparental disomy can eliminate mismatched HLA alleles from tumor cells and render them invisible to immune surveillance. This phenomenon of 'HLA loss' accounts for a third of relapses after haploidentical HSCT.[31] A HLA loss collaborative study group is currently examining data from a global cohort of post-transplant relapses across 20 transplant centers – to date no events have been detected in relapse after umbilical CBT.[32] Due to less stringent HLA matching requirements, CBT is

commonly performed across multiple HLA incompatibilities potentiating a GVT effect – however these are less likely to be in cis on the same chromosome. Even with loss of one haplotype, HLA mismatches will likely persist, that allow tumor cell immune recognition and elimination.

2.5 ATG in Cord Blood Transplantation

The established standard of GVHD prophylaxis is the combination of a calcineurin inhibitor – tacrolimus or cyclosporine, with an anti-proliferative agent – methotrexate or mycophenolate mofetil (MMF). The addition of anti-thymocyte globulin (ATG) to this standard regimen has been shown in randomized trials to reduce both acute and chronic GVHD.[33] In addition, ATG suppresses the recipient immune system to reduce the odds of graft rejection. ATG is a polyclonal product developed in rabbits with IgG activity against many immune cells but with a greatest affinity for naïve T-lymphocytes. The beneficial effects of this therapy are tempered by its adverse effects on immune reconstitution. Excess toxicity to incoming donor T-lymphocytes is associated with an increased probability of infection and viral reactivation, higher relapse rates in malignant disease and poorer overall survival.[34,35]

The pharmacokinetics of ATG display substantial interpatient variability and with standard weight-based dosing, a half-life of up to 6 weeks is seen in some patients.[36] Recent work in pediatric cohorts has shown that T-cell reconstitution in the presence of post-transplant residual ATG is more suppressed in cord blood transplantation (CBT) than it is in either bone marrow or peripheral blood HSCT.[34] In the absence of ATG however, cord blood immune reconstitution can be surprisingly fast despite the low T-cell dose infused, with restoration of adaptive immunity within 2 months after transplant.[37,38] A striking early recovery of CD4 counts is observed, which seems to occur via thymic-independent peripheral expansion and may be more robust than the recovery seen after adult donor transplantation. Improved CD4+ immune reconstitution directly correlates with event-free and overall survival.[39]

Several retrospective studies have demonstrated improved outcomes of CBT when ATG is omitted from conditioning regimens. Pascal et. al. examined adult patients with hematologic malignancy undergoing CBT after reduced intensity conditioning with cyclophosphamide, fludarabine and total body irradiation with 200cGy (Cy/Flu/TBI200) with or without ATG. ATG-treated patients had a higher non-relapse mortality (HR 1.68, p=0.0009) and a reduced overall survival (HR 1.69, p=0.03).[40] Wakamatsu et. al. recently compared transplant outcomes in a large series of patients with acute leukemia who underwent HSCT from various graft sources with or without ATG. In CBT, the use of ATG significantly increased NRM and while it reduced the incidence of acute GVHD, it had an overall detrimental effect on GVHD free/Relapse free survival (GRFS) and on overall survival.[41] In each of these studies, the incidence of acute GVHD increased in the absence of ATG but no statistically significant increase in chronic GVHD was observed and neutrophil engraftment was unaffected.[40,41] In a pediatric analysis of myelo-ablative CBTs, Zheng et. al. demonstrated higher incidences of CMV infection in children who received ATG compared with those who did not. A higher 5-year cumulative incidence of relapse was seen in the ATG cohort (30.7% vs. 15.4%, p=0.009), which translated into poorer leukemia-free survival (37.7% vs. 56.6%, p=0.015). In this study, no significant change in acute or chronic GVHD was observed with the omission of ATG and engraftment was unchanged.[42]

There is thus accumulating evidence to favor the elimination of ATG from CBT protocols with no apparent detriment to cord engraftment and no increase in the debilitating toxicity of chronic GVHD.

2.6 ATG in Haplo-Cord Transplantation

The success of haplo-cord transplantation depends on early haplo-donor engraftment that serves as a myeloid bridge until durable cord blood engraftment can take place. We have to date used ATG as part of our conditioning regimen for haplo-cord transplantation as is common practice in centers using this transplant platform. In addition to protecting against graft rejection and GVHD, in this unique scenario where two competing grafts are infused, ATG may also serve to support the transient engraftment of CD34+ selected haplo-identical stem cells by preventing their rapid destruction by cord lymphocytes – a 'graft versus graft' effect. In our experience, its use leads to robust haplo-donor-derived neutrophil and platelet engraftment occurring at a median of 11 days and 19 days post-transplant respectively.

In an effort to improve immune reconstitution, two groups have examined the effects of removing ATG from haplo-cord conditioning protocols. Lindemans et. al. reported on 5 patients at their center in Utrecht who received haplo-cord transplants without ATG – 1 pediatric patient with MDS and an active aspergillus infection whose haplo-graft was CD34-selected and 4 adult patients with poor risk malignant disease, whose haplo-products were CD19/ αβ-T-Cell depleted.[43] None of the 5 patients had a successful haplo-myeloid engraftment. This group was compared to 14 patients who underwent haplo-cord transplantation using ATG with CD34-selection of the haplo-donation. Early myeloid engraftment was seen in 8 of these 14 patients. ATG exposure appeared to be the only predictor of successful engraftment. 87% of haplo-graft failures went on to ultimately lose their cord graft also and as a result transplant related mortality was correlated to haplo-engraftment (70% +/- 16% with no myeloid bridge versus 12% +/-12% with successful bridge).[43]

A group from Memorial Sloan Kettering Cancer Center (MSKCC) reported on a series of patients who received double cord blood transplants combined with CD34+ selected haplo-identical peripheral blood stem cells. After myeloablative conditioning, patients received cyclosporine and mycophenolate mofetil but no ATG. In this cohort, 51% of patients had a successful haplo-myeloid bridge to durable cord engraftment with neutrophil recovery occurring at a median of 12 days post-transplant and no recurrent neutropenia. A further 14% had transient early haplo-derived neutrophil recovery but with a second leucocyte nadir prior to cord engraftment. 32% had no haplo-engraftment.[44] Of note, 77% of patients in this study developed a pre-engraftment syndrome at a median onset of post-transplant day 10, characterized by fever not attributable to infection, rash and capillary leak. This was seen in 61% of patients who had a robust myeloid bridge but observed in 94% of patients with rapid haplo-rejection.[44]

2.7 Acute Graft versus Host Disease

Graft versus host disease (GVHD) occurs as a result immunocompetent donor-derived T cells recognizing recipient tissues as foreign or 'non-self'. Occurring to some degree in up to 50% of allogeneic HSCT recipients, it remains a major cause of morbidity and transplant-related mortality. GVHD can be divided into two phases – acute and chronic, distinguishable by their timing of onset and clinic-pathologic manifestations.

Acute GVHD typically affects the skin, GI tract and liver with clinical signs including a maculopapular rash, hyperbilirubinemia with jaundice, nausea, vomiting, anorexia and watery diarrhea with crampy abdominal pain. By classic definition, this develops within the first 100 days post-transplant. The severity can be graded according to the extent of organ involvement – Grade 1 being considered mild, Grade 2 moderate, Grade 3 severe and Grade 4 very severe.[45]

Inflammatory triggers released as a consequence of conditioning chemotherapy or infection initiate the early phase of acute GVHD. Damage associated molecular pattern (DAMP) molecules released into the extracellular space from dying cells and pathogen associated molecular pattern (PAMP) molecules derived from invading intestinal micro-organisms, stimulate the innate immune system - setting off a cascade of cytokine and chemokine signaling events that attract and prime donor T-cells for activation and expansion.[45] Key cytokines released by this tissue damage include pro-inflammatory IL-6, TNFa, IFN-y and IL-1 **(Figure 2).**

Specific T helper cell subsets are stimulated that mediate later cytotoxic tissue damage. Th1 cells release IFN-y, which may be important for the development of gut GVHD.[46] Th2 cells secrete the anti-inflammatory cytokines IL-4, IL-10 and IL-13 but may polarize macrophages towards a pro-inflammatory IL-6 secreting phenotype.[47] Some studies have suggested an association between Th2 polarization and pulmonary and skin GVHD.[48] Th17 cells secrete IL-17, thought to mediate GVHD severity.[49] Conversely, regulatory T cells (Tregs) - which secrete anti-inflammatory IL-10, TGF-B and IL-35, are suppressed during acute GVHD.[50]

Ultimately, cytotoxic CD8+ T cells attracted into this inflammatory milieu mediate tissue damage through FAS-ligand signaling and the secretion of perforin and granzyme. Tissue damage is further augmented by macrophages releasing inflammatory cytokines IL-6 and TNF that have a directly toxic effect.[51]

Figure 2. A brief overview of acute GVHD pathogenesis. GVHD, graft-versus-host disease; DAMPS, danger-associated molecular patterns; PAMPS, pathogen-associated molecular patterns; APC, antigen-presenting cell; MΦ: macrophage.[50]

2.8 IL-6 Biology

In murine studies, systemic IL-6 levels increase after allogeneic HSCT and gradually return to baseline. Chen et. al. showed that levels remain high in mice that develop GVHD, with increased expression particularly noted in the gut and liver. Selective knockout of IL-6 in either recipient or donor cells was able to protect from GVHD development.[52] A separate study showed that selective IL-6 knockout in donor T cells led to a reduction in severe GVHD and prolonged survival.[53] In humans, IL-6 levels

increase after chemotherapy, reaching a peak within 2 weeks and then falling toward baseline. Single nucleotide polymorphisms (SNPs) in the IL-6 gene or the gene encoding the IL-6 receptor (IL6R) can result in higher serum levels of these proteins. Patients who receive grafts from donors that are either hetero- or homozygous for a particular SNP - 174 G<C in the IL-6 promoter region, are at significantly increased risk of developing acute GVHD (Odds ratio 3.3).[54]

Interleukin-6 is a cytokine with complex effects. It is involved in both acute and chronic inflammation but also has anti-inflammatory effects important for immunoregulation and tissue regeneration. IL-6 is present at baseline concentrations in healthy human plasma at under 14 pg/mL. Levels can dramatically increase during inflammation. A variety of cells can secrete IL-6 including fibroblasts, myocytes and endothelial cells. During acute inflammation however, monocytes and macrophages appear to be the primary source. A large proportion of CD4+ and CD8+ T cells circulating in the early post-HSCT period release high quantities of IL-6.[50]

IL-6 signaling takes place through at least two distinct mechanisms. Classical signaling takes place through the membrane-bound IL-6 receptor (IL6R). This receptor is expressed on limited cell types, including hepatocyteJAKs, intestinal epithelial cells, neutrophils, macrophages and naïve T-cells. It has a short intracellular domain and so complexes with another cell membrane protein to exert its intracellular signaling effects – the ubiquitously expressed gp130. Classic IL6R signaling is linked to tissue regeneration and repair – particularly in the liver, gastro-intestinal system and muscle tissue.[50] The membrane-bound IL6R can be cleaved to a soluble form by a disintegrin and metalloproteinase (ADAM) proteases. The soluble IL6R can complex with circulating IL-6 and subsequently bind to gp130 on cells that do not usually express the membrane-bound IL6R. This is known as 'trans-signaling' and may be the critical mediator of the pro-inflammatory effects of IL-6.[50] Upon complexing with IL-6 and IL6R, the intracellular domain of gp130 prompts the auto-phosphorylation of the janus kinases (JAK). This allows the phosphorylation and activation of STAT3 which translocates to the nucleus to act as a transcription factor for target genes. Most IL-6 effects appear to be STAT3-mediated but signaling also can occur through RAS/MAPK/ERK and PI3K/AKT channels.[55] IL-6 is a primary driver of the acute phase response – the increase in serum proteins C-reactive protein, serum amyloid P, ferritin and fibrinogen due to their increased production and release from the liver. Elevation of these parameters in cancer is partially attributable to IL6 release from tumor cells.

The overall effect of IL-6 on immunocompetent cells is to promote the polarization of pro-inflammatory Th2 and Th17 T-cell subsets while suppressing Tregs. It also regulates the survival and maturation of B-lymphocytes and their differentiation into plasma cells.

2.9 Tocilizumab

Tocilizumab is a humanized monoclonal IL-6 receptor antibody that is currently FDA approved for the treatment of rheumatoid arthritis, juvenile arthritis, giant cell arteritis and cytokine release syndrome (CRS). Activity has also been reported in multicentric Castleman's disease and inflammatory bowel disease. It inhibits membrane-bound and soluble forms of the IL-6 receptor, therefore inhibiting both classical and trans-signaling pathways. Pharmacokinetic studies report a long half-life of 6 to over 9 days after a single dose of the drug. Clearance may be faster in the setting of active CRS.[56]

The bulk of experience with tocilizumab comes from the rheumatology sphere – where the standard dosing schedule is 8 mg/kg administered intravenously (IV) over 60 minutes every 2-4 weeks. There is also a subcutaneous formulation available for selected indications. Treatment in this setting is

generally well tolerated, with low rates of discontinuation and overall low rates of adverse events. The common side effects reported with continued use are skin and soft tissue infections, dyslipidemia, increased liver transaminases and transient cytopenias.[57] Gastro-intestinal perforations, primarily in patients with diverticulitis, were reported at a rate of 0.26 events per 100 patient-years of intravenous tocilizumab therapy. Most of these patients were using concomitant non-steroidal anti-inflammatory therapy, steroids or methotrexate and the relative contribution of these concomitant medications versus tocilizumab is unknown. Genentech has advised caution in administering tocilizumab to patients with a history of diverticulitis or bowel perforation.

In treating CRS, tocilizumab is administered at a stat dose of 8 mg/kg IV. If no clinical improvement is observed, this dose can be repeated every 8 hours to a maximum of four doses. The FDA approval for this indication was based on a retrospective analysis of patients treated with tocilizumab for severe or life-threatening CRS after treatment with CTL019 or KTE-C19 chimeric antigen receptor T-cell (CAR-T) products on prospective trials. In treating 60 patients with a median of 1-2 doses of tocilizumab, no adverse events attributable to the drug were observed.[56]

2.10 Tocilizumab in the Treatment and Prevention of GVHD

The identification of IL-6 as an inflammatory mediator of GVHD has led investigators to test the use of tocilizumab in treating steroid-refractory (SR) GVHD. The first case was reported by our center in 2010. A 22 year old male with severe acute gut GVHD that was refractory to steroid and multiple secondary agents was treated with tocilizumab 8 mg/kg fortnightly for 8 doses. Clinical improvement was noted after the first dose and continued with successive treatments. Eight months after completing his course of therapy he remained free of diarrhea and abdominal cramping.[58] Following on from this, four small studies have reported on the use of tocilizumab in SR GVHD with complete responses ranging between 40 – 62.5%.[59-62] Treatment has maximal benefit in gut and skin GVHD but has no activity against liver GVHD. Responses may be more durable if tocilizumab is administered as first-line therapy after steroid failure.[60,62]

Two published studies to date have examined the use of tocilizumab in addition to tacrolimus and methotrexate as a prophylactic strategy for acute GVHD. Kennedy et. al. administered a single dose of tocilizumab at a dose of 8mg/kg on transplant day -1 with either myelo-ablative or reduced intensity conditioning to patients receiving HLA-matched sibling or unrelated grafts.[63] The cumulative incidence of grade II-IV acute GVHD at day 100 was 12%, with only 3% of patients suffering grade III-IV disease. Combined upper and lower gut GVHD occurred in 8% of patients.[63] Using the same GVHD prophylactic regimen and administration schedule, Drobyski et. al. studied outcomes in an older patient cohort (median age of 66 versus 48 in the Kennedy cohort) receiving matched related or unrelated grafts after myelo-ablative or reduced intensity busulfan-containing conditioning.[64] The day 100 cumulative incidence of grade II-IV and III-IV acute GVHD in this study were 14% and 3% respectively. Strikingly, no lower gut GVHD was observed in the first 100 days post-transplant.[64] The authors of both studies showed that IL-6 signaling blockade did not impede immune reconstitution.[63,64] In both studies, tocilizumab was found to be safe, with adverse events largely confined to transient elevations in liver enzymes and infections that occurred at similar rates in patients receiving standard prophylactic regimens.[63,64]

Tocilizumab has also been used as GVHD prophylaxis in place of ATG serotherapy for pediatric patients undergoing haplo-identical HSCT for chemorefractory acute myeloid leukemia following treosulfan-based conditioning. All grafts in this study were αβ-T-cell depleted and in addition to tocilizumab

8mg/kg on day -1, patients received bortezomib with or without abatacept as additional GVHD prophylaxis.[65] Patients achieving a complete remission after transplant received CD45RA-depleted donor lymphocyte infusions with or without hypomethylation therapy. The cumulative incidence of grade II-IV acute GVHD was 18%. While there were several non-conventional elements to this study, the authors concluded that the substitution of tocilizumab for ATG was not associated with increased graft failure or severe GVHD.[65]

Finally, two further studies evaluating tocilizumab as GVHD prophylaxis are currently recruiting patients – NCT03434730 at MSKCC is treating patients undergoing double cord blood transplantation with tocilizumab 8 mg/kg on transplant day -1 in addition to standard GVHD prophylaxis. NCT03699631 at the Medical College of Wisconsin is treating HSCT patients with tocilizumab 8 mg/kg on transplant day -1 to prevent acute GVHD and with a repeated dose on day +100 as chronic GVHD prophylaxis.

2.11 Rationale

Given the concern for an adverse effect of ATG on cord blood immune reconstitution, we wish to minimize and ultimately eliminate its use in our haplo-cord transplant platform. In doing this, we aim to reduce the incidences of infection, viral reactivation and relapse of malignant disease while minimizing the risk of graft rejection and preserving the low rates of GVHD we currently enjoy.

The co-infusion of a T-cell replete cord blood unit with a T-cell depleted haplo-identical graft however presents the added potential immune complication of graft versus graft rejection – where haplo-progenitors suffer early destruction before they can engraft and provide the transient myeloid bridge to the more durable but delayed cord engraftment. Previous efforts at haplo-cord transplantation without ATG have been limited by this complication. Rejection of the haplo-graft appears to be heralded by a ‘pre-engraftment syndrome’, occurring between day +7 and day +14. This syndrome is characterized by fever and capillary leak bearing much similarity to clinical CRS and may in part be driven by IL-6.

The IL-6 receptor antibody tocilizumab is approved for the treatment of CRS occurring after CAR T-cell therapy. In addition, there is growing evidence supporting its efficacy in the treatment and prophylaxis of GVHD in adult hematopoietic stem cell transplantation. It has proven to date to be safe and has no negative impact on immune reconstitution after transplant or on T-cell proliferation after CAR T-cell therapy.

We plan to administer a single dose of tocilizumab at day -1 to patients receiving haplo-cord transplantation for hematologic malignancy. We hypothesize that the inhibition of IL-6 signaling for the first 3-4 weeks post-transplant will facilitate the transient engraftment of the haplo-graft without a prolonged neutropenia or second nadir prior to cord engraftment. Used as an adjunct to tacrolimus and mycophenolate mofetil, it will also act as prophylaxis for acute GVHD. To ensure patient safety, we will initially treat patient with 3 doses of ATG 1.5 mg/kg as per our established protocol. In the absence of safety signals, we will gradually remove 1 dose of ATG in sequential patient cohorts until it has been fully eliminated.

Weill Cornell is one of the leading centers internationally for haplo-cord transplantation and our experience can be extremely useful to the transplant community.

2.12 Correlative Studies Background

Our collaborator Prof. Boelens at MSKCC has developed and validated a pharmacokinetic model to measure ATG exposure. We will collect and freeze EDTA plasma on days 0, +7, +14, +21 and +28, from patients in study groups I – III receiving ATG. These samples will be analyzed at MSKCC to calculate individual patient's ATG exposure for correlation with clinical outcomes.

Paralleling our clinical study, we will evaluate patterns of immune reconstitution and changes in the cytokine milieu after haplo-cord transplantation utilizing the established expertise of the Guzman laboratory.

Peripheral blood (PB) will be collected from all patients at 1,2,3,6 and 12 month post-transplant in two separate tubes - one in a Transfix tube (contains a fixative that stabilizes cell surface antigens and prevents cellular degradation for up to 14 days) allowing the analysis of unperturbed cells after collection, and another heparinized sample which will processed by ficoll gradient and mononuclear cells will be cryopreserved for future studies. The cellular immune-profile will be assessed in real time on cells collected in the Transfix tubes using flow cytometry. Two multi-parameter antibody panels designed to broadly screen for changes in PB leukocyte compartments will be initially utilized, allowing us to track the progressive recovery of monocytes, NK cells, DCs, B-lymphocytes and T-lymphocytes (which include cytotoxic, helper and regulatory T cells). A portion of the fixed cells will also be preserved for the later examination of specific cell populations or intracellular signaling pathways as guided by our initial survey. We expect that the gradual reduction of ATG in our successive patient cohorts will hasten CB CD4+ T-lymphocyte recovery and hypothesize that suppression of IL-6 signaling with tocilizumab will increase the development of Tregs while suppressing Th2 and Th17 effector phenotypes.

Pro-inflammatory cytokines invoked by pre-transplant conditioning chemotherapy are known to mediate acute GVHD. They are likely also to drive the peri-engraftment syndrome previously reported between days 7 and 14 in unsuccessful haplo-cord transplants performed without ATG. As we modify our preparative regimen to substitute tocilizumab for ATG, we will be particularly interested in observing changes in the cytokine milieu. To this end, serum will be collected and stored prior to the start of conditioning chemotherapy and then at days 0, +7, +14, +21 and +28; weeks 6,8 and 10; and at months 3,6 and 12. Samples will be tested using a bead-based multiplex immunoassay that will assess 13 cytokines simultaneously including IL-6 as well as IFNy, IL4, IL17 and TGFb - cytokines secreted by Th1, Th2, Th17 and Treg helper T-phenotypes respectively. We predict that serum IL-6 levels will increase in the 4 weeks following tocilizumab administration due to delayed clearance, while we expect a decrease in other pro-inflammatory cytokines mirroring the lymphocyte changes described above. In addition, bone marrow aspirate will be collected at 1,3,6 and 12 months and cell compartments analyzed using flow cytometry in a similar fashion to PB. MNCs will be also cryopreserved for later study and separated serum stored for cytokine/proteomic analysis. We acknowledge that with flow cytometry we are unable to determine the contribution of each graft to blood and marrow compartments, however we will be able to infer from chimerism analyses. Data can be later validated using single cell RNA seq in sex-mismatched grafts.

This initial study will provide us with a global understanding of immune and cytokine profiles after haplo-cord transplantation. Building from this, with future funding we will use our bio-banked material to avail of advanced research technologies available at Weill Cornell to characterize post-transplant immune reconstitution at a deeper level and try to unravel which and whose cells are responsible for desired graft versus tumor effect. We expect that by reducing ATG we will reduce the incidence of relapse and thus we will investigate the effects of our modification on BM MRD using NGS technology. Mass Cytometry by Time of Flight (CyTOF) would facilitate immune-monitoring at high-resolution that may be of particular interest in select groups of patients developing transplant complications such as disease relapse, GVHD or viral reactivations. Given the size and success of our haplo-cord program at Weill Cornell and our established academic core, we are uniquely placed to answer these exciting questions that will make a significant impact in transplant medicine.

3. Study Design

3.1 Overall Design

This study is a prospective phase II non-inferiority study investigating tocilizumab as a potential alternative to ATG in haplo-cord transplantation. It is a single-center study based at Weill Cornell Medicine/New York Presbyterian Hospital.

Our hypothesis is that tocilizumab is a safe and effective alternative to ATG in haplo-cord transplantation, facilitating transient engraftment of the haplo-identical stem cell graft without prolonged neutropenia or second nadir prior to durable cord engraftment while also preventing GVHD.

This study plans to enroll patients with hematologic malignancies in need of alternate donor transplant. All subjects will be conditioned with fludarabine, melphalan and TBI, followed by a single dose of tocilizumab 8 mg/kg on day -1. We will enroll patients into 4 successive cohorts, initially administering our current standard 3 doses of ATG 1.5 mg/kg (Total dose 4.5 mg/kg). In the absence of safety signals, we will drop one dose of ATG in successive cohorts until the drug ultimately has been eliminated (See Section 7.2 for further detail on treatment plan).

The primary endpoint of the study is successful haplo-derived neutrophil engraftment. Treatment will only be of interest if there is evidence that this rate is greater than 60%. We use a single arm, Bayesian study design. Each dose group will accrue up to 10 subjects. If there are 4 or fewer successes, that dose group will be deemed unacceptable and the next higher ATG dose for which there were 5 or more successes will be expanded. In other words, there will be 10 subjects initially enrolled in the first group (total ATG dose of 4.5 mg/kg). At the point of 5 or more successes are observed, subjects will be enrolled at dose group II (even if less than 10 subjects were accrued to group I). This will continue until there is a point where there are 4 or fewer successes out of 10 subjects. At this point, the next lower group (higher dose) will be expanded to a total of 40 subjects with interim analyses done after each 10 subjects for futility. An expansion cohort sample size of 40 has 80.9% power of declaring the regimen active if the true success probability is 80% (See Section 12.1 for further detail on statistical plan).

Paralleling our clinical study, extensive correlative study is planned to evaluate patterns of immune reconstitution and changes in the cytokine milieu after haplo-cord transplantation. (See Section 2.5 for further information on planned correlative studies).

3.2 Scientific Rationale for Study Design

Given concern for an adverse effect of ATG on cord blood immune reconstitution, we wish to minimize and ultimately eliminate its use in our haplo-cord transplant platform. In doing this we aim to reduce the incidences of infection, viral reactivation and relapse of malignant disease while minimizing the risk of graft rejection and preserving the low rates of GVHD we currently enjoy.

However, the co-infusion of a T-cell replete cord blood unit with a T-cell depleted haplo-identical graft presents the potential immune complication of graft versus graft rejection – where haplo-progenitors suffer early destruction before they can engraft and provide the transient myeloid bridge to the more durable but delayed cord engraftment. Previous efforts at haplo-cord transplantation without ATG have been limited by this complication. Rejection of the haplo-graft appears to be heralded by a 'pre-engraftment syndrome', occurring between day +7 and day +14. This syndrome is characterized by fever and capillary leak bearing much similarity to clinical CRS and may in part be driven by IL-6.

The IL-6 receptor antibody tocilizumab is approved for the treatment of CRS occurring after CAR T-cell therapy. In addition, there is growing evidence supporting its efficacy in the treatment and prophylaxis of GVHD in adult hematopoietic stem cell transplantation. It has proven to date to be safe and has no negative impact on immune reconstitution after transplant or on T-cell proliferation after CAR T-cell therapy.

We hypothesize that the inhibition of IL-6 signaling for the first 3-4 weeks post-transplant will facilitate the transient engraftment of the haplo-graft. Used as an adjunct to tacrolimus and mycophenolate mofetil, it should also act as prophylaxis for acute GVHD. The study incorporates a staged reduction of ATG to ensure patient safety. In the absence of safety signals, we will gradually remove 1 dose of ATG in sequential patient cohorts until it has been fully eliminated.

Extensive background and rationale for the study is provided in section 2.

3.3 Justification for Dose

Tocilizumab will be administered as a single dose of 8 mg/kg intravenously on day -1 of the haplo-cord transplant conditioning regimen. Patients over 100kg receive a capped dose of 800 mg. This is the recommended intravenous dose of tocilizumab for treatment of its licensed indications, including cytokine release syndrome (CRS). Prior studies evaluating tocilizumab as GVHD prophylaxis in stem cell transplantation also used this dose and administration schedule.

3.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last study visit and last scheduled procedure in the Schedule of Assessments (SOA), Section 6.1. This final assessment takes place at 1 year post-transplant. The end of the study is defined as

completion of the last visit or procedure shown in the SOA in the trial globally. Upon completion of the study, subjects will continue to be followed for long-term outcomes including survival, disease progression, chronic GVHD and secondary malignancy.

4. Subject Selection

4.1 Study Population

Subjects will be eligible for this study if they have a malignant hematological disease that can be potentially cured with allogeneic stem cell transplantation.

4.2 Inclusion Criteria

1. Subject must have a confirmed diagnosis of one of the following:
    a. Relapsed or refractory acute leukemia (myeloid or lymphoid)
    b. Acute leukemia in first remission at high-risk for recurrence
    c. Chronic myelogenous leukemia in chronic, accelerated phase or blast-crisis
    d. Myelodysplastic syndromes
    e. Chronic myeloproliferative disease
    f. Recurrent, refractory or high-risk malignant lymphoma
    g. Chronic lymphocytic leukemia, relapsed or with poor prognostic features
    h. Multiple myeloma
    i. Other hematological disorder in need of allogeneic transplant (e.g. blastoid dendritic cell neoplasm)
2. Age ≥ 18 years.
3. Likely to benefit from allogeneic transplant in the opinion of the transplant physician.
4. An HLA-identical related or unrelated donor cannot be identified within an appropriate time frame.
5. Karnofsky Performance Status (KPS) of ≥ 70%.
6. Acceptable organ function as defined below:
    a. Serum bilirubin: <2.0 mg/dL
    b. ALT (SGPT) <3x upper limit of normal (ULN)
    c. Creatinine Clearance: >50 mL/min/1.73m^2 (eGFR as estimated by the modified MDRD equation)
    d. Left ventricular ejection fraction >40%
    e. Pulmonary diffusion capacity >40% predicted

    Note: Patients whose organ function or KPS do not fulfill these criteria may still be enrolled if considered appropriate transplant candidates and after discussion in transplant conference.

7. Ability to understand and the willingness to sign a written informed consent document.

4.3 Exclusion Criteria

1. Life expectancy is severely limited by concomitant illness or uncontrolled infection.
2. Evidence of chronic active hepatitis or cirrhosis
3. Uncontrolled HIV disease.
4. Pregnancy or lactation
5. History of complicated diverticulitis, including fistulae, abscess formation or gastrointestinal perforation.
6. History of allergic reactions attributed to compounds of similar chemical or biologic composition as tocilizumab, including known allergies to Chinese hamster ovary cell products or other recombinant human or humanized antibodies.

4.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of inadequate organ function may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5. Registration Procedures

5.1 Subject Registration

Subjects will be registered within the WRG-CT as per the standard operating procedure for Subject Registration.

6. Study Procedures

6.1 Schedule of Assessments

See Table 1 on page 24.

Table 1. Schedule of Assessments

	Baseline	0	3	7	14	21	28	42	56	70	100	180	365
Informed Consent	X												
Physical exam, height, weight, and KPS	X	X		X	X	X	X	X	X	X	X	X	X
GVHD and other morbidity assessments					X	X	X	X	X	X	X	X	X
Toxicity assessments	X	X		X	X	X	X	X	X	X	X	X	X
Infectious disease titers[4]	X												
Chest CT or x-ray	X												
LVEF, or shortening fraction	X												
Pulmonary Function Test	X												
HLA typing[5]	X												
B-HCG serum pregnancy test[9]	X												
Dental/Social Work review	X												
Hematology[1], blood chemistries[2] and inflammatory markers[3]	X	X	X	X	X	X	X	X	X	X	X	X	X
Fasting lipids	X										X		X
Disease restaging	X										X	X	X
Bone marrow examination	X						X[8]				X[7]	X[7]	X[7]
Lumbar Puncture[10]	X												
Chimerism	X				X		X		X		X	X	X
Lymphocyte Subsets & Ig levels	X				X		X		X		X	X	X
Pneumococcal antibody levels	X											X	X
HLA Antibodies	X						X[7]				X[7]		X[7]
Sample for correlative assays[6,7]	X	X	X	X	X	X	X	X	X	X	X	X	X

Notes:

1. Hematology includes CBC with differential and platelet count. CBC performed at least three times a week from Day 0 until ANC >500 cells/microL for three days after nadir. CBC performed twice weekly until Day 28. CBC performed approximately weekly after Day 28 until 12 weeks post-transplant.
2. Blood chemistries include: serum creatinine, bilirubin, alkaline phosphatase, AST, and ALT, LDH, sodium, magnesium, potassium, chloride, and thyroid panel (where standard of care should be according to institutional guidelines). Blood chemistries performed twice weekly if possible until Day 28. Blood chemistries performed weekly if possible after Day 28 until day 100 post-transplant.
3. Inflammatory markers include C-reactive protein, ferritin and fibrinogen.
4. Infectious disease titers to be done as per institutional guidelines.
5. If not already performed.
6. Correlative assays may include pharmacokinetic assays of tocilizumab and rATG, measurement of novel biomarkers of graft versus host disease, assessment of minimal residual disease (MRD), cytokine analysis and assays of immune reconstitution.
7. Recommended, not required.
8. Bone Marrow aspirate and biopsy for pathology post-transplant will be performed as per the treating physician's medical-related recommendation. Cytogenetic and molecular analysis as directed by clinical team.
9. Females of childbearing potential only; to be performed within 4 weeks of conditioning regimen.
10. As clinically indicated.

6.1.1 Pre-Transplant Evaluation

(See also **Table 1**, page 24)

Subjects will be admitted to the hospital for a period of three to six weeks, sometimes more. In the first week, subjects will receive the conditioning regimen.

The following observations are considered standard evaluations for transplant eligibility and should be determined as close to conditioning as possible and at a reasonable interval from transplant usually < 12 weeks before initiation of conditioning therapy. More remote tests may be utilized upon approval of the patient's physician.

These tests may be adjusted as warranted by clinical circumstances and evolving transplant policy. Please also refer to institutional transplant work up guidelines.

1. Medical history, physical examination, vital signs, height and weight.
2. KPS (Karnofsky Performance Score)
3. Complete blood count (CBC) with differential and platelet count, serum creatinine, bilirubin, alkaline phosphatase, ALT, and AST.
4. Serum c-reactive protein, ferritin and fibrinogen
5. Fasting lipid profile including serum cholesterol and triglyceride levels
6. Infectious disease titers, as per institutional guidelines.
7. Immunoglobulin levels
8. Beta -HCG serum pregnancy test for females of childbearing potential within 4 weeks of conditioning.
9. High resolution HLA typing, if not already performed.
10. Pre-transplant chimerism testing.

11. Left ventricular ejection fraction (LVEF) or shortening fraction.
12. Pulmonary Function Test
13. Chest CT or chest x-ray as clinically indicated.
14. Dental / Social Work review.
15. Bone marrow evaluation with aspirate and/or biopsy. Cytogenetic and molecular analysis as clinically indicated.
16. Diagnostic lumbar puncture as clinically indicated (particularly ALL and High-grade lymphoma).

6.1.2 Post-Transplant Evaluation

The follow-up schedule for scheduled study visits is outlined in Table 2 below. The study visit assessments are outlined in Table 1.

These tests may be adjusted as warranted by clinical circumstances and evolving transplant policy. Please also refer to institutional transplant work up guidelines.

The exact day of the tests is approximate. Tests can be scheduled several days before or after. The window is up to five days before and after in the first four weeks, up to seven days before and after until day +100 and up to one month before and after at subsequent time points.

Study Visit	**Target Day Post-Transplant**
1 week	7 ± 2 days
2 weeks	14 ± 5 days
3 weeks	21 ± 5 days
4 weeks	28 ± 5 days
6 weeks	42± 7 days
8 weeks	56 ± 7 days
10 weeks	70 ± 7 days
100 day	100 ± 7 days
6 months	180 ± 28 days
12 months	365 ± 28 days

Table 2: Follow-up schedule for scheduled study visits.

6.1.3 Follow-up Phase

After 1 year, subjects will be followed for up to 5 years for long-term outcomes including: survival, disease relapse, chronic GVHD and secondary malignancy. The frequency of follow-up will be at the discretion of the treating physician.

7. Study Intervention

7.1 Stem Cell Source and Cell Dose

7.1.1 Umbilical Cord Blood Unit

The umbilical cord blood (UCB) unit must supply a minimum cell dose of 1.2 x 107 total nucleated cells per kilogram of recipient weight (TNC/kg).

The unit must match with the recipient at a minimum of 4 of 6 at HLA-A, -B, -DRB1 loci. This may include 0-2 antigen mismatches at each A or B (at the antigen level) or DRB1 (at the allele level) loci. The best-matched unit fulfilling the cell dose requirements should be utilized. All typing will be done using molecular typing. Though molecular level typing will be available, a match is defined at intermediate resolution for HLA-A and –B and at high resolution for –DRB1.

All recipients will be tested for class I and class II HLA antibodies. This should be done within 100 days of the planned transplant. If antibodies are present UCB should be chosen that are not targeted. This may require DQ and DP testing of the umbilical cord blood.

When available, the cord blood and maternal HLA types will be reviewed by WCMC or the Cord Blood Bank to identify units with shared IPA targets with the recipient, or HLA-mismatched but non-inherited maternal antigen (NIMA) matched units. These cord blood units will be preferred as grafts for the present study assuming a similar HLA match.
HLA-C matching at the antigen level will also be considered after the above selection criteria. Thus, for units equal on all other accounts, an HLA-C match will be considered.

One back-up unit will also be identified per subject following similar selection criteria.

7.1.2 Haplo-identical Donor

The preferred 3rd party donor will be a young HLA haplo-identical relative. The use of pediatric donors is restricted to donors who are over the age of 14 and weigh more than 50 kg.

After appropriate evaluation as per transplant program criteria, the donor will receive G-CSF (filgrastim) 5 mcg/kg SQ BID or 10 mcg/kg SQ daily for four consecutive days (doses rounded to the nearest vial size). Apheresis will start on the morning of the fifth day and proceed until sufficient cells have been collected. If a second day of collection is required, G-CSF will be administered on day 5 after collection.

The apheresis procedure will be conducted as per transplant program policy. Typically, four total blood volumes (TBV) will be collected or less if a high CD34 yield is expected based on a high

number of circulating CD34+ cells in the blood sample drawn immediately prior to apheresis.

After collection and prior to cryopreservation, cells will be T-cell depleted using the Miltenyi Clinimax® depletion device. This procedure will be performed, if possible, in the WCMC stem cell laboratory. We also have obtained FDA approval to use contract services provided by progenitor cell tech (Hackensack, NJ). The target will be to obtain a product containing less than 1x104 CD3+ cells per kg of recipient body weight and approximately 3x106/kg CD34 positive cells.

If donor-specific HLA-antibodies are present in the recipient an effort should be made to choose a third-party donor who is not targeted. This may require DQ and DP testing of the third-party donor.

Occasionally the use of an unrelated haplo-identical CD34-selected peripheral blood stem cell (PBSC) graft will be permissible, such as in cases where no haplo-identical relative can be identified, or when the recipient has donor specific antibodies directed against all haplo-identical relatives.

7.1.3 Infusion of Cells

The infusion of UCB and the haplo-identical units will be separated by at least 2 hours and preferably they will occur on successive days (Day 0 and Day 1). The order of infusion is not specified.

Several lines of evidence indicate that post-thaw cord viability affects engraftment. If an umbilical cord blood product turns out to have less than 70% CD34 cell viability upon thawing, a second UCB product will be rapidly ordered and infused as soon as possible.

7.2 Treatment Plan

7.2.1 Conditioning Regimen

<u>Conditioning Regimen</u>: Age <60yrs

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1 or day 0
Fludarabine	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2			Haplo Infusion	UCB Infusion
Melphalan						140 mg/m^2			
Tocilizumab							8 mg/kg		
TBI				2Gray	2Gray				
ATG Group I			1.5 mg/kg		1.5 mg/kg		1.5 mg/kg		
ATG Group II			1.5 mg/kg		1.5 mg/kg		_		
ATG Group III			1.5 mg/kg		_		_		
ATG Group IV			_		_		_		

Conditioning Regimen: Age >60yrs

	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day 1 or day 0
Fludarabine	30 mg/m^2	30 mg/m^2	30 mg/m^2			Haplo Infusion	UCB Infusion
Melphalan				140 mg/m^2			
Tocilizumab					8 mg/kg		
TBI		2Gray	2Gray				
ATG Group I	1.5 mg/kg		1.5 mg/kg		1.5 mg/kg		
ATG Group II	1.5 mg/kg		1.5 mg/kg		_		
ATG Group III	1.5 mg/kg		_		_		
ATG Group IV	_		_		_		

Fludarabine:
Age <60yrs: 30 mg/m2/day IV x 5 days (Day -7 to Day -3) to a total dose of 150 mg/m2.
Age >60yrs: 30 mg/m2/day IV x 3 days (Day -5 to Day -3) to a total dose of 90 mg/m2.
Fludarabine will be dosed according to actual body weight.

Melphalan: 140 mg/m2 intravenously x 1 day on Day -2 for all patients.
Melphalan will be dosed according to actual body weight. Cryotherapy with ice chips will be administered to prevent mucositis.

Tocilizumab: 8 mg/kg/day IV x 1 day on Day -1 for all patients.
Tocilizumab will be dosed according to actual body weight. Patients >100kg will receive a capped maximum dose of 800 mg.

TBI: Conditioning will be intensified with 2 doses of TBI (4 Gy) for all patients. This serves to decrease graft rejection and reduce the risk of relapse of malignant disease.

Rabbit ATG (rATG)*: In the absence of safety signals, dosing will be reduced as the study proceeds as follows –

- Cohort I: 1.5 mg/kg/day IV x 3 days on Days -5, -3 and -1 (Total dose 4.5 mg/kg).
- Cohort II: 1.5 mg/kg/day IV x 2 days on Days -5 and -3 (Total dose 3 mg/kg).
- Cohort III: 1.5mg/kg/day IV x 1 day on Day -5 only (Total dose 1.5 mg/kg).
- Cohort IV: No rATG to be administered.

ATG will be dosed according to actual body weight. The first dose will be infused over at least six hours, and any subsequent doses over at least 4 hours. Pre-medications include acetaminophen 650 mg PO, diphenhydramine 25-50 mg PO/IV, and methylprednisolone 2 mg/kg (1 mg/ kg at the initiation and 1 mg/kg half-way through ATG administration). Circumstances may require minor changes in scheduling of chemotherapy. Variations of up to 24 hours in scheduling will be acceptable.

7.2.2 Post-Transplant Course (GVHD Prophylaxis)

Subjects will receive either tacrolimus (Prograf ®) or cyclosporine (Neoral ®, Gengraf ®) and another immunosuppressant - mycophenolate mofetil (Cellcept ®), starting before transplant to reduce the risks of GVHD and to promote the growth of the graft.

Tacrolimus: 0.03 mg/kg/day IV continuous infusion (CI) over 24 hr from 4 PM Day -2 until engraftment or when subject is able to take PO, then tacrolimus approximately 0.09 mg/kg PO in 2 divided doses. Tacrolimus should be given at full dose to maintain levels of 5-15 ng/mL through day 180, tapered by 20% every week thereafter. PO tacrolimus can be used when IV access for CI tacrolimus is unavailable.

Cyclosporine: May be used as an alternative to Tacrolimus. 3 mg/kg/day IV over 24 hr from 9 PM Day -2 until engraftment or when subject is able to take PO, then cyclosporine 3.5 mg/kg PO twice a day. Cyclosporine should be given at full dose to maintain levels of 150-400 ng/mL through day 180 and tapered by 20% every week thereafter. PO cyclosporine can be used when IV access for infusion is unavailable.

Infection, toxicity or other clinical circumstances may prompt earlier discontinuation or adjustment of tacrolimus/cyclosporine doses. In the presence of GVHD, a clinical decision by the attending physician will determine if tacrolimus/cyclosporine can be tapered or should be continued.

Mycophenolate Mofetil (MMF): will be started on Day -2 and given at a dose of 1000 mg every 8 hours until day 28. MMF can be given PO or IV. Infection, toxicity, very low patient weight (<50kg) may prompt earlier discontinuation or adjustment of doses.

Note: Patient specific circumstances may mandate minor variations in conditioning medications and/or GVHD prophylaxis, such as reduction of some of the doses of medications, changes in schedule or – rarely - substitution of a particular drug. These changes will be noted and justified, but will not require IRB approval.

7.2.3 Supportive Care

CMV Prophylaxis

- Prophylaxis will follow institutional unit policy and will be guided by the recipient and donor CMV serostatus tested during pre-transplant screening.

Cytomegalovirus (CMV) monitoring:

- CMV viral load monitoring by PCR at least weekly until Day 100 and at least monthly until Day 210, regardless of donor/recipient CMV status.
- Pre-emptive CMV treatment should be strongly considered for any positive result on by DNA testing (i.e, viral load) within the first 100 days of transplant.

PTLD Prophylaxis

- **Rituximab:** All patients with prior EBV exposure not previously exposed to rituximab or who have not received rituximab in the six months prior to transplant, will receive one dose of rituximab 375 mg/m2 prior to or upon admission.

Epstein-Barr virus (EBV) monitoring:

- EBV viral load monitoring by PCR at least weekly until Day 100 and at least monthly until Day 365 is strongly recommended.
- Rising EBV titers should warrant investigation for EBV post-transplant lymphoproliferative disorder (PTLD) - including CT imaging and PET scanning for positive CT findings. A bone marrow evaluation is recommended if any evidence of PTLD is found on imaging or peripheral blood analysis.
- Evidence of PTLD or consecutive increases in EBV PCR should lead to treatment with rituximab at a dose and schedule per institutional policy.

A prophylactic broad-spectrum antifungal with anti-mold activity is strongly recommended.

Other infection prophylaxis and supportive care will be as per institutional unit policy.

In some cases, a drug called filgrastim (G-CSF) may be administered to hasten the recovery of blood counts.[1-3] Administration will follow institutional guidelines. Generic formulations of filgrastim may be utilized as required by insurance or pharmacy.

Blood transfusion policy should follow institutional policy.

8. Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

8.1 Discontinuation of Study Intervention

Tocilizumab administration and subsequent transplantation are one-time treatments. There is no indication for subjects to be taken off treatment except those mentioned below. Even if the subject suffers near fatal toxicity, it would not affect his/her ability to stay active on the study.

A subject's follow-up in the study will end after one of the following applies:

- Disease progression,
- Subject decides to withdraw from the study, or
- Subject lost to follow-up
- Subject death
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator.

8.1.1 Disease Progression or Disease Persistence

Relapsing subjects (i.e. those with pathologically proven disease progression) will be removed from protocol therapy and followed for survival and secondary malignancy.

8.1.2 Extraordinary Medical Circumstances

If, at any time, the constraints of this protocol are detrimental to the subject's health and/or the subject no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair.
- Document the reason(s) for discontinuation of therapy in subject records.
- Follow the subject for survival, progression, relapse, and secondary malignancies.

8.2 Participant Discontinuation/Withdrawal from the Study

The reason for participant discontinuation or withdrawal from the study will be recorded in the subject medical record. The study chair will be notified. Subjects will continue to be followed for survival, progression and secondary malignancy.

Subjects who sign the informed consent form but do not undergo transplantation may be replaced. Subject who sign the informed consent form, receive a haplo-cord transplant and die before evaluation of the primary study endpoint at Day +21 may be replaced. Subjects who sign the informed consent form, receive transplantation, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

9. Criteria for Study Evaluation

Neutrophil engraftment
Neutrophil engraftment will be defined as the first day in which the ANC is > 500 cells/microL for three consecutive days. Chimerism studies will be used to determine the percentage cord vs. haplo-identical donor engraftment.

Platelet engraftment
Platelet engraftment will be defined as the first day the platelet count is > 20,000/microL without transfusion support for seven consecutive days. Chimerism studies will be used to determine the percentage cord vs. haplo-identical donor engraftment.

Failure of the haplo-graft
Failure of the haplo-graft will be defined as the absence of neutrophil engraftment by Day +21 or a drop in the ANC to < 0.3 cells/microL for five consecutive days occurring after initial neutrophil engraftment within the first 3 weeks post-transplantation (second nadir)

Progression Free Survival
Relapse will be recorded by the day of initial detection of malignant cells if these cells were on subsequent testing confirmed to be increasing in number or by unequivocal radiological progression. The molecular detection of matched related donor will not be taken into account for the definition of clinical recurrence. The diagnosis of disease recurrence will be based on clinical and pathological criteria.

Treatment Related Mortality
Treatment related mortality is considered any death that cannot be explained by persistence, relapse or progression of the underlying malignancy once the preparative regimen starts.

Acute GVHD
Acute GVHD will be scored according to the Glucksberg system and the IBMTR Severity Index.[66] Whenever possible, histologic biopsies will be taken to confirm diagnosis of GVHD.

Chronic GVHD
Chronic GVHD will be scored according to the NIH Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Report.[67] Whenever possible, histologic biopsies will be taken to confirm diagnosis of GVHD.

10. Data Reporting / Regulatory Considerations

10.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled subjects.

10.1.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group-based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

10.2 Regulatory Considerations

10.2.1 Institutional Review Board/Ethics Committee Approval

As required by local regulations, the Investigator will ensure all legal aspects are covered, and approval of the appropriate regulatory bodies obtained, before study initiation.

Before initiation of the study at each study center, the protocol, the ICF, other written material given to the patients, and any other relevant study documentation will be submitted to the appropriate Ethics Committee. Written approval of the study and all relevant study information

must be obtained before the study center can be initiated or the IP is released to the Investigator. Any necessary extensions or renewals of IRB approval must be obtained for changes to the study, such as amendments to the protocol, the ICF, or other study documentation. The written approval of the IRB together with the approved ICF must be filed in the study files.

The Investigator will report promptly to the IRB any new information that may adversely affect the safety of the patients or the conduct of the study. The Investigator will submit written summaries of the study status to the IRB as required. On completion of the study, the IRB will be notified that the study has ended.

All agreed protocol amendments will be clearly recorded on a protocol amendment form and will be signed and dated by the original protocol approving signatories. All protocol amendments will be submitted to the relevant institutional IRB for approval before implementation, as required by local regulations. The only exception will be when the amendment is necessary to eliminate an immediate hazard to the trial participants. In this case, the necessary action will be taken first, with the relevant protocol amendment following shortly thereafter.

Once protocol amendments or consent form modifications are implemented at the lead site, Weill Cornell Medicine, updated documents will be provided to participating sites, as applicable. Weill Cornell Medicine must approve all consent form changes prior to local IRB submission.

Relevant study documentation will be submitted to the regulatory authorities of the participating countries, according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

10.2.2 Ethical Conduct of the Study

The Investigators and all parties involved should conduct this study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines and the applicable national and local laws and regulatory requirements.

This study will be conducted under a protocol reviewed and approved by the applicable ethics committees and investigations will be undertaken by scientifically and medically qualified persons, where the benefits of the study are in proportion to the risks.

10.2.3 Informed Consent

The investigator or qualified designee must obtain documented consent according to ICH-GCP and local regulations, as applicable, from each potential subject or each subject's legally authorized representative prior to participating in the research study. Subjects who agree to participate will sign the approved informed consent form and will be provided a copy of the signed document.

The initial ICF, any subsequent revised written ICF and any written information provided to the subject must approved by IRB prior to use. The ICF will adhere to IRB requirements, applicable laws and regulations.

10.2.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor-Investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10.2.5 Record Retention

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file. Essential documents should be retained for 2 years after the final marketing approval in an ICH region or for at least 2 years since the discontinuation of clinical development of the IP. In addition, all subjects medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

11 Statistical Considerations

11.1 Study Design/Endpoints

Primary endpoint: The primary endpoint is the percentage of successful haplo-derived neutrophil engraftment. This is defined as (1) an ANC of >500 cells/microL for three consecutive days with the first on or before Day+21, AND (2) absence of a second nadir—a drop in the ANC to < 0.3 cells/microL for five consecutive days occurring after initial neutrophil engraftment within the first 3 weeks post-transplantation.

There will be up to four different ATG doses tested:

Study Group#	Total ATG Dose
1	4.5 mg/kg
2	3 mg/kg
3	1.5 mg/kg
4	0 mg/kg

We will define the lowest tolerated dose of ATG as the lowest dose that has no evidence that successful haplo-derived neutrophil engraftment at Day +21 is less than 60% of subjects. An analysis of a historic cohort demonstrates success haplo-engraftment in 80% of subjects and this treatment would not be of interest if there is evidence at this time that the rate is below 60%.

11.2 Sample Size/Accrual Rate

Sample size/ power: We will enroll 10 evaluable subjects in each study group consecutively starting with #1. Accrual will continue at the same ATG dose level until at least 10 subjects are evaluable or if

futility is established before 10 patients have been enrolled. Subjects who die or relapse before the Day +21 assessment are not considered evaluable, and will be replaced. Assuming that there will be no more than 5% non-evaluable patients, the smallest sample size for the trial is 10 patients if the first dose level is deemed unacceptable due to the first futility analysis and the largest would be 70 evaluable patients for a possible accrual of 74 (= 70 x 1.05) if the last dose group fully accrues. It is anticipated that approximately 35 patients will be accrued per year.

We use a single arm, Bayesian design. Each dose group will accrue up to 10 patients. If there are 4 or fewer successes, that dose group will be deemed unacceptable and the next higher dose for which there were 5 or more successes will be expanded. In other words, there will be 10 patients initially enrolled in the first group (total ATG dose of 4.5 mg/kg). At the point 5 or more successes are observed, patients will be enrolled at dose group #2 (even if less than 10 patients were accrued to group #1). This will continue until there is a point where there are 4 or fewer successes out of 10 patients. At this point, the next lower group (higher dose) will be expanded to a total of 40 patients with interim analyses done after each 10 patients for futility. The interim analyses will be performed after each cohort of 10 subjects have passed the Day +21 mark and accrual will not be suspended for the interim analyses during the expansion phase. The interim analysis is for futility only. If futility is reached prior to accruing 40 total patients in the expansion phase, consideration will be given to expanding the next higher dose level.

Trial criteria: Initially, cohort sizes will be of 10 until futility is met. The primary endpoint is the successful haplo-derived neutrophil engraftment. It is assumed that the treatment will only be of interest if there is evidence that this rate is greater than 60%.

Evidence of futility: $Pr[Pr(RR > 60\%) \geq 0.90] < 0.05$

We will stop a dose level for futility when the probability that the success rate is greater than 60% being 0.90 or greater is less than 0.05. For the initial cohorts of 10, futility will be indicated if there are 4 or fewer successes. If there are five successes observed, accrual will proceed on the next dose group, even if less than 10 patients were accrued in the current group. Once futility is met at a dose level (or the last dose level is reached without any prior level indicating futility), the previous dose group tested (next higher dose level) without hitting futility will be expanded to 40 total patients (or the last dose level if it was reached). For the expansion cohort, interim analyses will be performed after every 10 patients have been followed for the endpoint. Accrual will not stop for the completion of an interim analysis. The trial futility stopping rules for the expanded cohort are below:

Trial stopping rules:

# patients	Stop for futility
10	$\leq$ 4 successes
20	$\leq$ 11 successes
30	$\leq$ 18 successes
40 (maximum sample size)	$\leq$ 27 successes

Trial operating characteristics for expansion cohort: A sample size of 40 has a type I error of approximately 0.12, meaning that there is a 0.12 chance of declaring the regimen is acceptable when the true success proportion is 0.60. This sample size has 80.9% power of declaring the regimen active if the true success probability is 80%.

Early stopping criteria: Trial accrual will be suspended for evaluation of subject safety if:

- Grade III-IV acute GVHD exceeds 25% after 20 total patients have been accrued.
- Liver toxicity > Grade 2 as per CTCAE v. 5.0 within the first 40 days of transplant unrelated to known GVHD, infection, VOD or clearly-defined drug effects in 20% or more patients after 15 or more total patients have been accrued.

If there is unacceptable toxicity as described above, the study team will evaluate the adverse event profile and decide, in conjunction with the DSMB, to terminate the trial, modify the treatment protocol, or reopen it for accrual.

11.3 Analysis of Endpoints

11.3.1 Analysis of Primary Endpoints

The trial will go through each dose level sequentially. For each dose level, we will report the proportion of success using a binomial point estimate and corresponding 95% exact binomial confidence interval.

11.3.2 Analysis of Secondary Endpoints

Secondary endpoints: The secondary endpoints include progression-free survival, overall survival, transplant-related mortality, proportion of platelet engraftment success, proportion of failure of the haplo-graft, proportion of acute GVHD, and proportion of chronic GVHD. These are defined in Section 10.

Analysis of secondary endpoints: All proportions will be estimated with a binomial point estimate and corresponding 95% exact binomial confidence interval. This will be done separately for each dose group and for all dose groups combined. The time to event endpoints will be summarized with a Kaplan-Meier estimator. We will generate the corresponding survival curves with 95% confidence intervals. Again, this will be done for each dose group and all groups combined. In addition, we will generate the median values with the corresponding 95% confidence interval for each dose group and all patient combined.

Additional secondary analyses will be a competing risk cumulative incidence analysis. The endpoints of interest are: acute GVHD, chronic GVHD, liver toxicity, infection, viral reactivation, and PTLD. For each endpoint, the competing risk will be all other endpoints and death. Analysis will be done for each dose group as well as all dose groups combined.

Finally, we will also model the data. For time to event endpoints, we will use a Cox proportional hazards model that will include the dose group as an explanatory variable. For binomial endpoints, we will use logistic regression with dose group as an explanatory variable.

11.4 Reporting and Exclusions

11.4.1 Evaluation of Toxicity

Subjects are evaluable for toxicity from the start of their transplant conditioning regimen until they reach post-transplant Day +100.

11.4.2 Evaluation of Response

Subjects who die or relapse before the Day +21 assessment are not considered evaluable for the primary study endpoint and will be replaced.

12. Adverse Event Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug or device under investigation. Safety will be monitored by evaluation of adverse events reported by subjects or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.

12.1 Adverse Event Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

12.1.1 Investigational Agent or Device Risks (Expected Adverse Events)

Stem cell transplant is a complex procedure with prolonged initial admission and numerous immediate and delayed complications as well as frequent readmission. Expected adverse events are those listed in the consent form and include regimen-related toxicities, myelosuppression, opportunistic infections and GVHD.

12.2.2 Adverse Event Characteristics and Related Attributions

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

12.1.3 Recording of Adverse Events

- Stem cell transplant is a complex procedure with prolonged initial admission and numerous immediate and delayed complications as well as frequent readmissions.

- Expected adverse events are those listed in the consent form and include regimen-related toxicities, myelosuppression, opportunistic infections and GVHD. Most expected adverse events of Grade III and higher CTCAE severity will be captured in the transplant database and reported to the IRB upon continuing review.

- **Adverse events that are judged to be unexpected and at least possibly related to the investigational procedure will be reported to the study chairman within 48 hours. Such events will be reported to the local IRB within the institution's prescribed time period.**

- **All fatal adverse events will also be reported to the study chairman within 48 hours and reported to the local IRB within the institution's prescribed time period.**

12.1.4 Reporting of AE to WCM IRB

All AEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link:
http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

12.1.5 Events of Special Interest

Non-fatal gastro-intestinal (GI) perforation has been reported in subjects treated with tocilizumab on randomized control studies for rheumatoid arthritis. In these trials tocilizumab was administered either intravenously or subcutaneously as a regular therapy every 4 weeks. The overall rate of GI perforation was 0.26 events per 100 patient years. These events were primarily reported as complications of diverticulitis. Most patients who developed this complication were taking concomitant non-steroidal anti-inflammatory medications, corticosteroids or methotrexate. In comparison, the GI perforation rate in rheumatoid arthritis patients treated with corticosteroid alone is 039 per 100 patient years.

In published case series where tocilizumab has been used as prevention or treatment of GVHD, no episodes of GI perforation were observed. Subjects with a past history of complicated diverticulitis, including fistulae, abscess formation or perforation are excluded from this study.

Any events of GI perforation will be reported immediately to the study PI, the IRB and the DSMB.

12.2 Definition of SAE

SAEs include death, life threatening adverse experiences, hospitalization or prolongation of hospitalization, disability or incapacitation, overdose, congenital anomalies and any other serious events that may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition.

12.2.1 Reporting of SAE to IRB

All SAEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link:
http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

12.2.2 Reporting of SAE to FDA

IND application sponsor must report any suspected adverse reaction or adverse reaction to study treatment that is both serious and unexpected. Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and must be reported to FDA as soon as possible but no later than 7 calendar days following the sponsor's initial receipt of the information.

i. death,
ii. a life-threatening adverse event,
iii. in-patient hospitalization or prolongation of existing hospitalization,
iv. a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
v. a congenital anomaly or birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or research subject and may require medical or surgical intervention to prevent one of the outcomes listed as serious

CDER-only Biologic INDs:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biologic Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

12.3 AE/SAE Follow Up

All SAEs and AEs reported during this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized and no more follow-up is required. This requirement indicates that follow-up may be required for some events after the subject discontinues participation from the study.

13. Unanticipated Problems Involving Risks to Subjects or Others

13.1 Definition of Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSO)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

13.1.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPIRTSOs) to the reviewing Institutional Review Board (IRB). The UPIRTSO report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UPIRTSO;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UPIRTSO.

To satisfy the requirement for prompt reporting, UPIRTSOs will be reported using the following timeline:

- UPIRTSOs that are serious adverse events (SAEs) will be reported to the IRB within 24 hours of the investigator becoming aware of the event.
- Any other UPIRTSO will be reported to the IRB within 7 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), Food and Drug Administration (FDA), and the Office for Human Research Protections (OHRP) within 7 days of the IRB's receipt of the report of the problem from the investigator.

14. Data and Safety Monitoring Plan (DSMP)

The Weill Cornell Medical College Data Safety Monitoring Board (DSMB) is being requested to review safety data and to make recommendations regarding continuation, termination, or modification to the study. The study will be reviewed by the DSMB on a semi-annual basis.

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Appendix A: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B: Background Drug Information

CYCLOSPORINE (Neoral ®, Gengraf ®, Sandimmune ®)

AVAILABILITY
There are two formulations of cyclosporine. Modified cyclosporine (Neoral ®, Gengraf ®) has increased bioavailability compared with non-modified cyclosporine (Sandimmune ®). The modified and non-modified formulations are not bioequivalent. The only formulation available for intravenous use is unmodified and is commercially available in solution at 50 mg/mL concentration (5 mL). Modified cyclosporine is available for oral use as capsules (25 mg, 50mg, 100 mg) or solution (100 mg/mL).

STORAGE & STABILITY
Cyclosporine solution for intravenous use should be stored below 30 oC /86 oF and protected from light. Infusions should be prepared in glass containers. If PVC bags are used, the solution must be used immediately to minimize patient exposure to di(2-ethylhexyl)phthalate (DEHP) which is leached into cyclosporine solutions. Cyclosporine capsules and oral solutions should be stored at 20-25 oC /68-77 oF. Liquid must not be administered from Styrofoam cups. Not suitable for freezing.

PREPARATION FOR IV USE
Dilute the concentrate for injection by adding 1 mL (50 mg) of cyclosporine to 20—100 mL of 0.9% Sodium Chloride injection or 5% Dextrose injection.

ADMINISTRATION
Oral therapy should be started as soon as possible as per protocol and 8 to 12 hours after stopping intravenous therapy. The conversion from IV to oral therapy should use a 1:3 ratio with dose rounding to the nearest 25 mg and administered as a divided dose every 12 hours. Concomitant medications (such as azoles) should be taken into account.

TOXICITY
The most frequent adverse events reported with cyclosporine use are:

- Cardiovascular – hypertension (8-53%), edema (5-14%)
- Central nervous system – headache (2-25%), paresthesia (1-11%), confusion, posterior reversible encephalopathy syndrome (PRES)
- Dermatologic – hypertrichosis (5-19%)
- Endocrine/metabolic – hirsutism (21-45%), hypertriglyceridemia (15%), hyperglycemia, hypomagnesemia, hyperkalemia, hyperuricemia
- Gastrointestinal – nausea (2-23%), diarrhea (3-13%), gingival hyperplasia (2-16%), abdominal distress (<1%-15%), dyspepsia (2-12%), hepatotoxicity
- Infection – increased susceptibility to infection (3-25%)
- Neuromuscular/skeletal – tremor (7-55%), leg cramps (2-12%)
- Renal – increased serum creatinine (16-≥50%), renal insufficiency (10-38%), thrombotic microangiopathy (TMA)

DRUG INTERACTIONS
Cyclosporine is extensively metabolized by CYP 3A isoenzymes, in particular CYP3A4, and is a substrate of the multidrug efflux transporter P-glycoprotein. Agents that induce or inhibit these proteins may alter plasma concentrations of cyclosporine. Of particular importance are azole antifungals (increase cyclosporine levels), letermovir (May increase the serum concentration of cyclosporine; decrease dose of letermovir to 240 mg PO once daily), mycophenolate mofetil (cyclosporine may decrease the serum concentration of mycophenolate) and statins (avoid the use of atorvastatin, lovastatin, pitavastatin, and simvastatin in combination with cyclosporine. Rosuvastatin (max dose 5 mg/day) and pravastatin (max dose 20 mg/day) are preferred.)

FILGRASTIM (G-CSF: Granulocyte Colony Stimulating Factor, Neupogen®)

AVAILABILITY
G-CSF is commercially available in 1.0 and 1.6 mL vials containing 300 mcg and 480 mcg G-CSF, and in prefilled syringes containing 300 mcg/0.5 mL and 480 mcg/0.8 mL.

STORAGE & STABILITY
Intact vials and prefilled syringes should be stored under refrigeration. Do not allow the drug to freeze.

ADMINISTRATION
The daily dose of G-CSF should be injected subcutaneously in one or two sites. The dose following peripheral blood stem cell infusion is 5 mcg/kg/day. The dose of G-CSF may be rounded up to the nearest vial size.

TOXICITY
The most common side effect associated with G-CSF is bone pain. Bone pain is usually reported as mild or moderate and, if necessary, may be treated with non-opioid or opioid analgesics.

FLUDARABINE (Fludara®)

AVAILABILITY
Fludarabine is commercially available as a white, lyophilized powder. Each vial contains 50 mg of fludarabine, 50 mg of mannitol and sodium hydroxide to adjust pH.

STORAGE & STABILITY
Intact vials should be stored under refrigeration. Reconstituted vials are stable for 16 days at room temperature or under refrigeration. Solutions diluted in D5W or NS are stable for 48 hours at room temperature or under refrigeration.

PREPARATION
Fludarabine should be reconstituted with Sterile Water for Injection, USP or normal saline per institutional pharmacy guidelines.

ADMINISTRATION
Fludarabine will be administered as an IV infusion over 30 minutes.

TOXICITY
Myelosuppression, (dose-limiting toxicity), fever, mild nausea and/or vomiting, diarrhea, stomatitis, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia (may be life-threatening), peripheral neuropathy, and pulmonary toxicity. (Both pneumonia and hypersensitivity reactions have been reported. Fatal pulmonary toxicity has been described, especially when fludarabine was used in combination with pentostatin. Severe, fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status was encountered almost exclusively after very high doses of fludarabine. Such toxicity has only been rarely demonstrated at the 25-30 mg /m2 dosage of fludarabine. Very rarely described complications include transfusion-associated graft versus host disease. Tumor lysis syndrome has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed.

MELPHALAN (Alkeran®)

AVAILABILITY
Melphalan for IV use is commercially available in sterile 50 mg vials. The product is a lyophilized powder with 20 mg povidone per vial. Also provided is 10 mL of special diluent for use in reconstituting the product. The special diluent has 0.20 g sodium citrate, 6 mL propylene glycol, 0.5 mL 95% ethanol, and sterile water.

STORAGE & STABILITY
Intact vials should be stored at room temperature (15oC-30oC) and protected from light. Reconstituted solutions are chemically and physically stable for at least 90 minutes at room temperature. Solutions further diluted in 0.9% sodium chloride to a concentration of 0.1 mg/mL to 0.45 mg/mL are stable for at least 60 minutes. Solutions diluted to 1 mg/mL are reported to be physically stable for at least 4 hours at room temperature-chemical stability of this dilution is not known. Because of the relative instability of melphalan solutions, it is recommended that administration of the diluted solution be completed within 60 minutes of reconstitution. Reconstituted solutions should not be refrigerated.

PREPARATION
Melphalan should be prepared immediately before intended use. Each vial is reconstituted with 10 mL of the special diluent to yield a concentration of 5 mg/mL. The reconstituted solution may be diluted with 0.9% sodium chloride to a concentration of 0.1 mg/mL to 0.45 mg/mL.

ADMINISTRATION
The total dose of melphalan will be administered by short IV infusion over 30-60 minutes, as per institutional pharmacy guidelines.

TOXICITY
The major toxicity of melphalan is bone marrow suppression, usually lasting four to eight weeks. Other toxicities include nausea, vomiting, diarrhea, and mucositis. Less common toxicities include pulmonary fibrosis, interstitial pneumonitis, vasculitis, alopecia, hemolytic anemia, and allergic reactions. Transient rises in BUN and creatinine have occurred with high dose melphalan and also acute renal failure. Tissue necrosis may result if infiltration occurs.

MYCOPHENOLATE MOFETIL (Cellcept®; Myfortic®; MMF)

AVAILABILITY
Mycophenolate mofetil is available as a Capsule, as mofetil: CellCept®: 250 mg;
as Injection, powder for reconstitution, as mofetil hydrochloride: CellCept®: 500 mg [contains polysorbate 80]; as Powder for oral suspension, as mofetil: CellCept®: 200 mg/mL (225 mL) [provides 175 mL suspension following reconstitution; contains phenylalanine 0.56 mg/mL; mixed fruit flavor]; as a Tablet, as mofetil: CellCept®: 500 mg [may contain ethyl alcohol]; and as a Tablet, delayed release, as mycophenolic acid: Myfortic®: 180 mg, 360 mg [formulated as a sodium salt].

STORAGE & STABILITY
Intact vials should be stored at room temperature 15ºC to 30ºC (59ºF to 86ºF). Store solutions at 15ºC to 30ºC (59ºF to 86ºF) and begin infusion within 4 hours of reconstitution. Store capsules at room temperature of 15ºC to 39ºC (59ºF to 86ºF). Tablets should be stored at room temperature of 15ºC to 39ºC (59ºF to 86ºF) and protected from light. Store powder for oral suspension at room temperature of 15ºC to 39ºC (59ºF to 86ºF). Once reconstituted, the oral solution may be stored at room temperature or under refrigeration. Do not freeze. The mixed suspension is stable for 60 days.

PREPARATION
Mycophenolate mofetil is stable in D5W should be reconstituted per institutional pharmacy guidelines.

ADMINISTRATION
Intravenous solutions of mycophenolate mofetil should be administered over at least 2 hours (either peripheral or central vein); do not administer intravenous solution by rapid or bolus injection. Oral dosage formulations (tablet, capsule, suspension) should be administered on an empty stomach to avoid variability in MPA absorption. The oral solution may be administered via a nasogastric tube (minimum 8 French, 1.7 mm interior diameter); oral suspension should not be mixed with other medications. Delayed release tablets should not be crushed, cut, or chewed.

TOXICITY
Pain, abdominal pain, fever, headache, infection, sepsis, asthenia, chest pain, back pain, hypertension, tremor, insomnia, dizziness, acne, rash, diarrhea, constipation, mild N/V, oral monoliasis, anemia, leukopenia, thrombocytopenia, hypochromic anemia, leukocytosis, peripheral edema, hypercholesterolemia, hypophosphatemia, edema, hypo or hyperkalemia, hyperglycemia, infection, dyspnea, cough increase, pharyngitis, bronchitis, pneumonia, UTI, hematuria, kidney tubular necrosis, urinary tract disorder.

RABBIT ANTITHYMOCYTE GLOBULIN (Thymoglobulin®, rATG)

AVAILABILITY
Antithymocyte globulin is commercially available. Each package contains two vials: the first vial contains 25 mg antithymocyte globulin, and the second vial contains > 5 mL SWFI diluent.

STORAGE & STABILITY
Ampules must be refrigerated (2oC-8oC/ 36oF-46oF),). Do not freeze.

PREPARATION
Reconstitute 25 mg vial with diluent provided by manufacturer (SWFI > 5 mL). Roll vial gently to dissolve powder. Use contents of vial within 4 hours of reconstitution. Dilute dosage to a final concentration of 0.5 mg/mL in 0.9% sodium chloride injection or 5% dextrose injection. Gently invert admixture 1-2 times to mix solution. Use admixture solution immediately. Final concentration must be 0.5 mg/mL.

ADMINISTRATION
Infuse the first dose over at least six hours, and subsequent doses over at least 4 hours. Infuse through a 0.22 micron in-line filter into a high-flow vein. Premedications include acetaminophen 650 mg PO, diphenhydramine 25-50 mg PO/IV, and methylprednisolone 1 mg/kg (at the initiation and half-way through antithymocyte globulin administration).

TOXICITY
Infusion-related toxicities, including fevers, chills, rash, dyspnea, cardiovascular (hypo- or hypertension, tachycardia, edema, chest pain). In rare cases, anaphylaxis has been reported in which case the infusion should be terminated immediately, and emergency treatment with epinephrine and other resuscitative measures should be instituted. rATG should not be administered again to this patient. Immunosuppression is a common feature of rATG and can result in severe infections including sepsis, CMV, and urinary tract infections. Serum sickness, neutropenia (57%), thrombocytopenia (37%), leucopenia (57%), pain (46%), headache (40%), nausea and diarrhea (37%), peripheral edema (34%), systemic infection, malaise, pain, stomatitis, GI bleed, swelling or redness at injection site, myalgia, back pain, development of human anti-rabbit antibodies (HARA).

TACROLIMUS (Prograf®, FK506)

AVAILABILITY
Tacrolimus is commercially available as an injection (5 mg/mL; 1 mL ampules) and as oral capsules (0.5 mg, 1 mg, and 5 mg).

STORAGE & STABILITY
Store tacrolimus capsules and injection at controlled room temperature, 15oC-30oC (59oF-86oF).

PREPARATION – FOR IV USE
Tacrolimus injection must be diluted prior to IV infusion with 0.9% sodium chloride or 5% dextrose injection to a concentration of 4-20 mcg/mL. Solutions should be prepared in non-PVC plastic or glass. Tacrolimus injection and diluted solutions of the drug should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

ADMINISTRATION
Oral therapy should be started as soon as possible as per protocol and 8 to 12 hours after stopping intravenous therapy. Oral doses will be administered twice a day. The conversion from IV to oral therapy should take into account concomitant medications (such as voriconazole).

TOXICITY
In patients receiving tacrolimus, 5% to 47% experienced anemia, 8% to 32% experienced leukocytosis, and 14% to 24% experienced thrombocytopenia. Rare cases of microangiopathic hemolytic anemia have been reported. Chest pain was reported in 19%. Mild to moderate hypertension is a common adverse effect associated with tacrolimus therapy. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache (37% to 64%), tremors (48% to 56%), insomnia (32% to 64%), paresthesia (17% to 40%), and dizziness (19%). Tremor and headache may respond to dosage reduction. Visual changes, agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams have been reported in 3% to 15%. Hyperkalemia (13% to 45%), hypokalemia (13% to 29%) hypophosphatemia (49%) and hypomagnesemia (16% to 48%) have been associated with tacrolimus therapy. Hyperuricemia has been reported in >3%. Gastrointestinal adverse effects included nausea (32% to 46%), vomiting (14% to 29%), anorexia (7% to 34%), constipation (23% to 35%), and diarrhea (37% to 72%). Nephrotoxicity was reported in 38% to 52% of liver and kidney transplant patients, respectively. Hematuria has been reported in greater than 3%. Abnormal liver function tests have been reported in 6% to 36% of patients; ascites in 7% to 27%.

Other effects reported in clinical trials include pain, fever, asthenia, back pain, and peripheral edema. The incidence of hyperglycemia was 17% and may require therapy with insulin. Other less frequently occurring effects include abscess, chills, peritonitis, and photosensitivity reactions. Anaphylaxis has been reported in a few patients receiving intravenous tacrolimus. Tacrolimus injection contains cremophor which in other drugs has been associated with anaphylaxis. Because tacrolimus is an immunosuppressant, the risk of opportunistic infections is increased.

DRUG INTERACTIONS
Tacrolimus is metabolized by cytochrome P450 3A4. Drugs that are inhibitors (e.g. itraconazole) of inducers (e.g. phenytoin) of 3A4 might be expected to increase or decrease tacrolimus concentrations, respectively, possibly resulting in increased or decreased effects.

TOCILIZUMAB (Actemra®)

AVAILABILITY
Tocilizumab is commercially available in single-dose vials for further dilution prior to intravenous infusion (20 mg/mL in 4 mL, 10 mL and 20 mL ampules) and for subcutaneous injection in a single-dose prefilled syringe or single-dose prefilled autoinjector (162 mg/0.9 mL).

STORAGE & STABILITY
Tocilizumab must be refrigerated at 2 oC to 8 oC (36 oF to 46 oF) and cannot be frozen. Vials, syringes and autoinjectors must be protected from light by storage in the original package until time of use.

PREPARATION

For patients over 30 kg dilute tocilizumab to 100 mL in 0.9% or 0.45% Sodium Chloride Injection, USP for intravenous infusion using aseptic technique. For patients less than 30 kg dilute tocilizumab to 50 mL in 0.9% or 0.45% Sodium Chloride Injection, USP using aseptic technique.

ADMINISTRATION
Administer as a single intravenous drip infusion over 1 hour. Tocilizumab is not to be given as a bolus or push.

TOXICITY
Toxicity data has been gathered from the use of tocilizumab on a continuous basis for rheumatologic indications, where many patients were also taking steroid therapy or other biologic agents such as methotrexate. The most common serious adverse reactions were infections including pneumonia, urinary tract infection, cellulitis, herpes zoster, gastroenteritis, sepsis and bacterial arthritis. Events of gastrointestinal perforation have been reported in clinical trials, primarily as complications of diverticulitis. Therapy is associated with increases in total cholesterol, triglycerides, LDL, and/or HDL in 20% of subjects. Monitoring should be implemented. Increased serum alanine aminotransferase is seen in up to 36% of patients and increased serum aspartate aminotransferase in up to 22%. These elevations did not result in permanent of clinically evident hepatic injury in clinical trials. Treatment can lead to neutropenia and thrombocytopenia. Infusion related reactions have been noted in 4-20%. Anaphylactic was reported in 0.1%. Headache, hypertension, dizziness and rash have been reported in <10% of patients.

DRUG INTERACTIONS
Cytochrome P450s in the liver are down-regulated by infection and inflammation stimuli including the cytokine IL-6. Inhibition of IL-6 signaling may restore CYP450 activities to higher levels than those see in the absence of tocilizumab leading to increased metabolism of drugs that are CYP450 substrates. In vitro studies showed that tocilizumab has the potential to affect expression of multiple CYP enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Caution advised when administering tocilizumab with CYP3A4 substrate drugs where a decrease in effectiveness is undesirable. Therapeutic monitoring of effect or drug concentration may be required.