Phase II Multicenter Study Of Natalizumab Plus Standard Steroid Treatment For High Risk Acute Graft-Versus-Host Disease
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PHASE II MULTICENTER STUDY OF NATALIZUMAB PLUS STANDARD STEROID TREATMENT FOR HIGH RISK ACUTE GRAFT-VERSUS-HOST DISEASE

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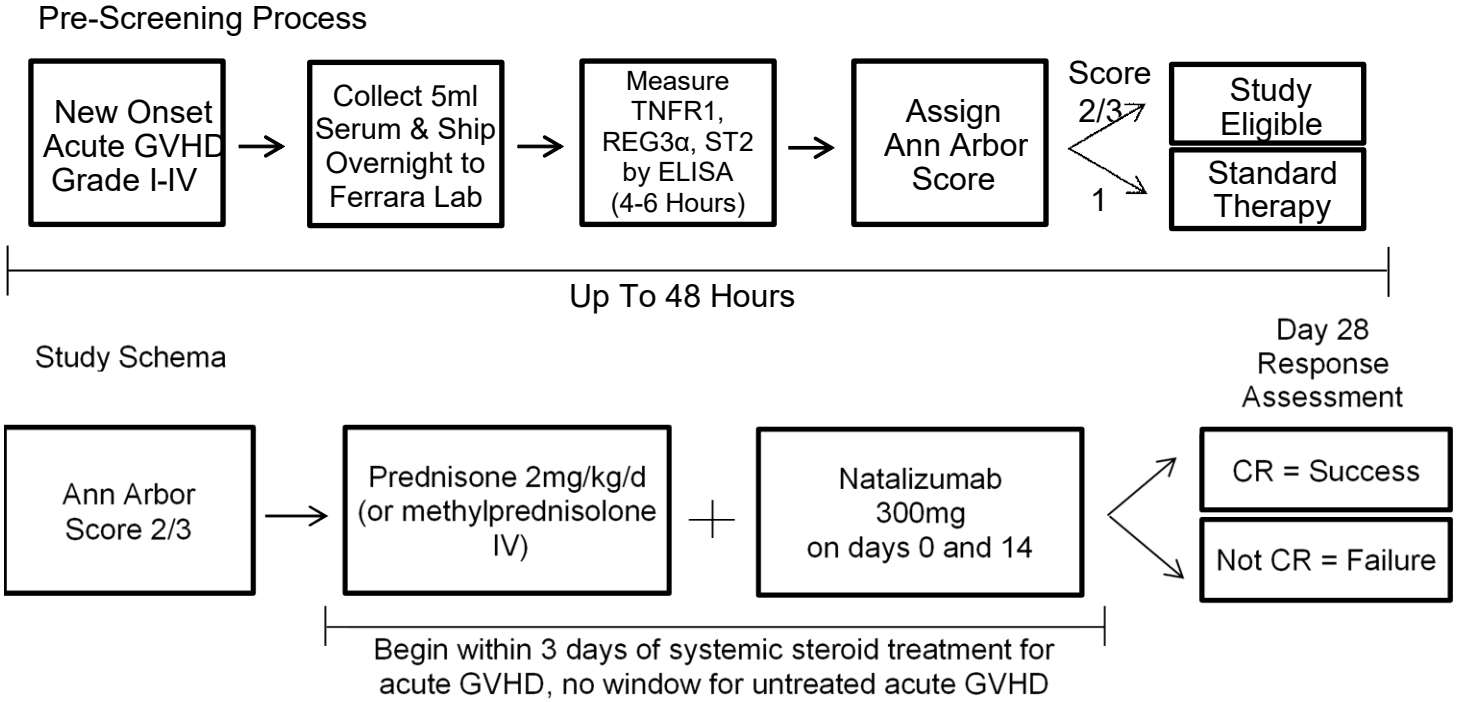
ABBREVIATIONS

Examples Include [the list should be inclusive of the entire protocol]:

6-MP	6-mercaptopurine
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BMT	Bone Marrow Transplant
BMT CTN	Blood and Marrow Transplant Clinical Trials Network
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CD	Crohn's Disease
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CNS	Central Nervous System
Co-PI	Co-Principal Investigator
CR	Complete Response
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
DSMR	Data and Safety Monitoring Report
EBV	Epstein-Barr Virus
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
GI	Gastrointestinal
GVHD	Graft-Versus-Host Disease
HCT	Hematopoietic Stem Cell Transplantation
HHV6	Human Herpes Virus 6
HSV	Herpes Simplex Virus
IND	Investigational New Drug
IL2Rα	Interleukin-2 receptor-alpha
IRB	Institutional Review Board
IV (or iv)	Intravenously
JC	John Cunningham (virus)
MAGIC	Mount Sinai Acute GVHD International Consortium
MS	Multiple Sclerosis
NCI	National Cancer Institute
NOS	Not Otherwise Specified
NR	No Response

NRM	Non-Relapse Mortality
OS	Overall Survival
PE	Physical Exam
PI	Principal Investigator
PJP	*Pneumocystis Jiroveci* Pneumonia
PML	Progressive Multifocal Leukoencephalopathy
PR	Partial Response
PTLD	Post-Transplant Lymphoproliferative Disorder
REG3α	Regenerating islet-derived 3 alpha
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
ST2	Suppressor of tumorigenicity-2
TCI	The Tisch Cancer Institute at the Mount Sinai Health System
TNFR1	Tumor necrosis factor receptor-1
UGI	Upper Gastrointestinal
VZV	Varicella-Zoster Virus

STUDY SCHEMA



STUDY SYNOPSIS

Title	PHASE II MULTICENTER STUDY OF NATALIZUMAB PLUS STANDARD STEROID TREATMENT FOR HIGH RISK ACUTE GRAFT-VERSUS-HOST DISEASE
Phase	Phase II
Methodology	Open label single arm
Study Duration	3 years
Study Center(s)	Multicenter – Icahn School of Medicine at Mount Sinai, City of Hope, Columbia University, Emory University, University of Kansas, Mass General, Mayo Clinic, Memorial Sloan-Kettering, Northwestern University, Ohio State University, University of Pennsylvania, Vanderbilt University
Objectives	1. To improve day 28 GVHD complete response rate for high risk GVHD patients from the historical rate of 42.5% to 57.5% by treatment with natalizumab and high dose systemic corticosteroids (2 mg/kg). 2. To decrease 6-month NRM from the historical rate of 32% to 22% in patients treated on this clinical trial.
Number of Subjects	84 subjects

Inclusion Criteria	1. New onset high risk acute GVHD (Ann Arbor score 2 or 3 as defined in Appendix A) following allogeneic BMT. Any clinical severity (Glucksberg grade I-IV) is eligible. Patients with prior or existing diagnosis of GVHD without any treatment are eligible. Patients given only topical corticosteroids for skin GVHD are eligible. 2. Any donor type (e.g., related, unrelated) or stem cell source (bone marrow, peripheral blood, cord blood). Recipients of non-myeloablative and myeloablative transplants are eligible. 3. No prior systemic treatment for acute GVHD except for a maximum of 3 days of prednisone ≤2 mg/kg/day (or IV methylprednisolone). Topical skin steroid treatment, non-absorbable oral steroid treatment for GI GVHD, and resumption of GVHD prophylaxis agents (e.g., calcineurin inhibitors) are permissible. Patients enrolled in BMT CTN 1501 who randomized to sirolimus are also eligible. Exceptions can be made on a case-by-case basis with Sponsor-Investigator approval. 4. Age 18 years or older. 5. Direct bilirubin must be <2 mg/dL unless the elevation is known to be due to Gilbert syndrome or aGVHD within 3 days of enrollment. 6. ALT/SGPT and AST/SGOT must be <5 x the upper limit of the normal range within 3 days of enrollment, unless the elevation is due to liver GVHD. 7. If the patient is a woman of child-bearing potential, the patient and their sexual partner must agree to practice effective contraception. 8. Written informed consent from patient or legal representative. 9. Biopsy of acute GVHD target organ is strongly recommended but not required. Enrollment should not be delayed for biopsy or pathology results. Patients who do not enroll within 3 days of initiation of systemic steroid treatment for acute GVHD are not permitted to participate.
Exclusion Criteria	1. Patients with malignancy that is suspected or proven to have progressed, relapsed, or be persistent since BMT 2. Uncontrolled active infection 3. Patients with chronic GVHD only. Patients with overlap syndrome are eligible. 4. History of Progressive Multifocal Leukoencephalopathy (PML) 5. Known hypersensitivity to natalizumab 6. Pregnant or nursing (lactating) women 7. Use of other drugs for the treatment of acute GVHD 8. Steroid therapy for indications other than GVHD at doses >0.5 mg/kg/d of methylprednisolone or equivalent within 7 days prior to initiation of GVHD treatment 9. Patients on dialysis 10. Patients requiring ventilator support 11. Investigational agent within 30 days of enrollment without approval from the Sponsor-Investigator
Study Product(s), Dose, Route, Regimen	Natalizumab (Tysabri) 300 mg IV on days 0 and 14 Prednisone (or IV methylprednisolone) 2 mg/kg/d on days 0 to 2 then tapered according to clinical criteria

Duration of Administration	Natalizumab for 2 doses over 14 days, prednisone (or IV methylprednisolone) for 28 days
Reference Therapy	Prednisone (or IV methylprednisolone) 2 mg/kg/d orally day 0 to 2, then tapered according to clinical criteria
Statistical Methodology	We will enroll 84 patients with Ann Arbor score 2 or 3 GVHD which provides 80% power to detect a 15% difference in day 28 CR rates (to 57.5% compared to the historical rate of 42.5%), assuming a Type I error rate of 0.05. Secondary outcomes for efficacy, [e.g., overall response rate (CR+PR), the incidence of steroid refractory GVHD, the incidence of severe GI GVHD (stage 3 or 4), non-relapse mortality, relapse rates, overall survival], and for safety [e.g. serious infections and other adverse events] will be analyzed using descriptive statistics (cumulative incidence curves, Kaplan-Meier, etc.) and compared to historical controls. Outcomes will be analyzed for all patients and separately according to Ann Arbor GVHD score. Historical controls for this study consists of 256 patients who met the criteria for Ann Arbor score 2 or 3 GVHD (see background and rationale) and who were either (1) diagnosed at the University of Michigan or the University of Regensburg or (2) participated in the MAGIC consortium observational study between 2013-2017. Approximately 75% of these patients were diagnosed and treated since 2010, thus the historical control population is comprised primarily of fairly recently transplanted patients.

1.0 BACKGROUND AND RATIONALE

1.1 Acute GVHD

Acute graft-versus-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (HCT) is the major cause of non-relapse mortality (NRM) and ~25% of patients with GVHD will die from this complication, yet it has been graded and treated in the same way for almost 40 years. The GVHD grading system [Przepiorka 1995] uses only clinical symptom severity which imposes major limitations. Maximal GVHD grade, which includes response to treatment, correlates with NRM; however the grade of acute GVHD at diagnosis does not correlate with outcome sufficiently to guide treatment. Thus, treatment is not intensified until primary therapy has failed. This is one reason why the treatment of acute GVHD has not advanced.

Although acute GVHD can develop in the skin, liver or gastrointestinal (GI) tract, it is GI GVHD that is the primary driver of NRM. The vast majority of patients who develop GI GVHD that does not respond to high dose systemic steroids, the only effective treatment, will die [MacMillan 2012]. Unfortunately, the presence of GI GVHD symptoms at diagnosis does not predict well for NRM because the majority of these patients are steroid responsive. Patients with GI GVHD at diagnosis treated in the US and France (n=894, personal communication, Daniel Weisdorf) experienced NRM of 28%, which was not significantly different from patients with an isolated skin GVHD presentation (NRM=25%).

Ann Arbor GVHD Scoring

Given the limitations of the current GVHD clinical staging system, we strategized to develop a GVHD grading system at onset that possessed the following four key attributes: (1) reproducible across different BMT centers and practices, (2) objective, that is, uses biomarkers only, (3) simple, and (4) each score should crisply define a risk group (low, medium, high). GVHD response to steroids strongly correlates to NRM. Most clinical trials, including this one, use day 28 response rates for the primary endpoint as it is a surrogate for GVHD survival [Levine 2010, MacMillan 2010, Saliba 2012]. Use of the standard day 28 response endpoint will facilitate comparisons with other trials of GVHD treatment reported in the literature. However, analysis of multi-center data has shown that grading of clinical GVHD is often inaccurate without the adjudication of expert panels [Weisdorf 2003], which is impractical during the conduct of a clinical trial. The vast majority (>90%, primarily due to steroid refractory GI GVHD) of 6 month NRM is due to GVHD that fails to respond to steroids. We therefore chose to define risk for Ann Arbor scoring according to 6 month NRM instead of GVHD treatment response because they are closely correlated and because NRM is more objective. Furthermore, steroid refractory GI GVHD alone accounts for >80% of 6 month NRM.

Next, we determined that score 1 GVHD should have a 6 month NRM <10%, similar to the 6% NRM seen in patients transplanted at the University of Michigan who do not develop acute GVHD. We then conferred with national GVHD experts to recommend the NRM risk that would be sufficiently high to warrant experimental treatments as primary GVHD therapy. The consensus opinion was that score 3 GVHD should be defined as a 6 month NRM ≥40%. We therefore allowed score 2 GVHD to somewhere in-between score 1 and 3.

Having set the desired attributes of a new GVHD scoring system, which we have named Ann Arbor GVHD Scoring, we measured the concentrations of five validated biomarkers [Paczesny 2009, Paczesny 2010, Ferrara 2011, Vander Lugt 2013] with the strongest individual correlations with NRM (TNFR1, IL2Rα, elafin, REG3α, and ST2) in samples obtained at diagnosis of new onset acute GVHD grades I-IV in 492 patients from either the University of Michigan or the University of Regensburg, Germany. We randomly divided

the samples into a training set (328 patients) and an independent validation set (164 patients). We then used competing risks regression, with relapse after GVHD onset as the competing risk, to model 6 month NRM using combinations of the log-transformed absolute values of the five biomarkers. A model using three biomarkers (TNFR1, REG3α, and ST2) fit the training data well, and the fit was not significantly improved by including either of the other biomarkers (elafin and IL2Rα). Thus, we selected the simplest, three biomarker model, for further development. We then ordered the 328 training patients from lowest to highest according to their predicted probability ($\hat{p}$), of NRM within 6 months of GVHD onset, and selected thresholds that stratified patients into NRM risk groups as defined above (**Fig 1**). Notably, there was more than one threshold that met our defined criteria; therefore we selected thresholds within the middle of the acceptable range. The 6 month NRM rates (score 1, 9%; score 2, 28%; score 3, 47%) were highly statistically different from each other (**Panel A).** When we applied the Ann Arbor scoring system to the independent validation set the results were remarkably similar (score 1, 9%; score 2, 27%; score 3, 45%) (**Panel B**).

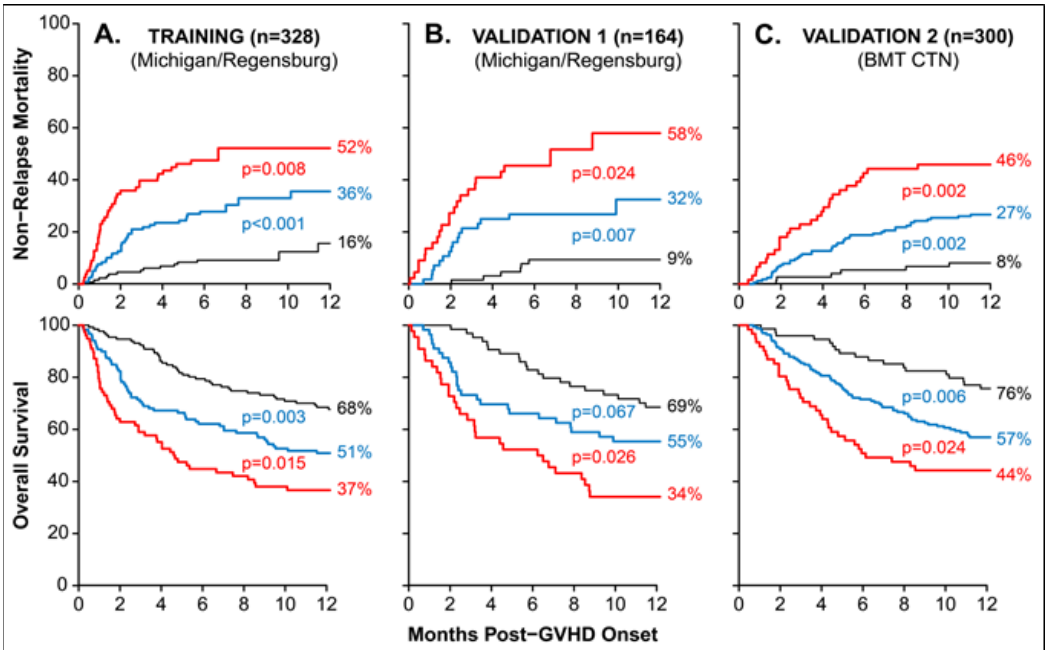


Figure 1. NRM and OS by Ann Arbor score of GVHD. Plasma samples from patients with acute GVHD from the University of Michigan (n=360) and the University of Regensburg (n=132) were analyzed for concentrations of ST2, REG3α, and TNFR1. An algorithm was developed in a training set of 328 patients that stratified patients into 3 grades based on risk of NRM at six months (A). The same algorithm was applied to the remaining 164 patients as the first validation set (B). The algorithm was then applied to a second validation set of 300 patients from two different multi-center BMT CTN trials of primary treatment for acute GVHD (C). P-values denote between scores 1 and 2 (blue) and between scores 2 and 3 (red).

The gold standard for reproducibility of a grading system is its performance in the multi-center setting. Therefore, we analyzed samples from 300 patients enrolled in two multicenter trials of primary treatment for acute GVHD conducted by the BMT CTN (0302 and 0802) [Alousi 2009, Bolanos-Meade 2014]. The eligibility criteria for these studies were new onset acute GVHD grades I-IV. Patients were randomized to prednisone 2 mg/kg/d (or methylprednisolone IV equivalent) and either etanercept, denileukin diftitox, pentostatin, mycophenolate or placebo. There were no significant differences in complete response rates or survival with any of the treatment arms. Panel C shows that in this second independent validation cohort the Ann Arbor scoring system provides reproducible 6 month NRM rates (score 1, 5%; score 2, 19%; score 3, 43%). Importantly, the post-GVHD relapse rate of ~14% was evenly distributed across all 3 Ann Arbor GVHD scores in all cohorts, so the overall survival curves closely mirror the NRM curves. Finally, we confirmed

that Ann Arbor GVHD scores correspond to early treatment response rates, which is important for clinical trial design (**Fig 2**).

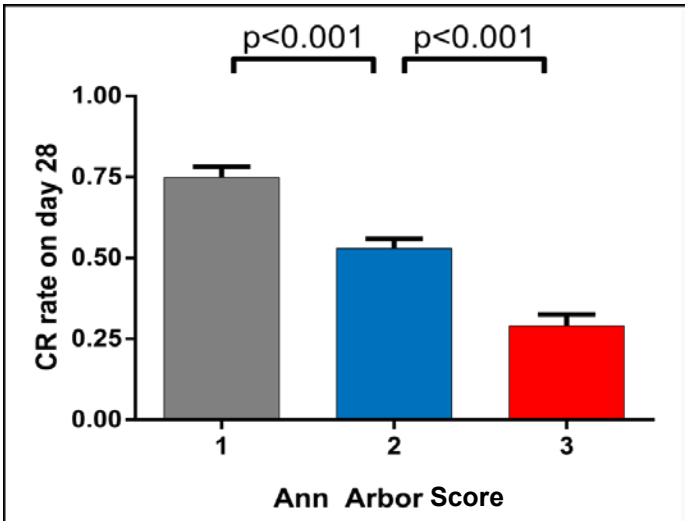


Figure 2. Complete response rates on day 28 (n=792). Score 1 (n=269), score 2 (n=340), score 3 (n=183).

Ann Arbor score 3 identifies steroid refractory GI GVHD.
A major innovation of Ann Arbor scoring is that the risk scores do not depend on GI tract involvement when GVHD is first diagnosed. For example, the NRM of Ann Arbor high risk GVHD is 45%. **The vast majority of these deaths (85%) are from steroid resistant GI GVHD, even though 50% of high risk patients do not have clinical GI symptoms at diagnosis.** This clinical trial will use the day 28 CR rate as the primary endpoint. The trial is powered to detect an increase from the 30% CR rate (in red, **Fig 2**) to 45% [Levine 2015].

1.2 Revised Ann Arbor Scoring Algorithm (April 2017)

A biomarker algorithm that uses only two biomarkers (ST2 and REG3α) to predict lethal GVHD on day 7 after BMT was developed and validated in a large multicenter study population of 1287 patients [Hartwell 2017]. The two biomarker algorithm was then shown to accurately predict outcomes at diagnosis of acute GVHD as well as at other time points post-BMT (such as one week after the initiation of steroid treatment for GVHD) [Major-Monfried 2016]. The two biomarker algorithm has been adapted to produce identical scores to the original Ann Arbor scoring algorithm and replaces the three biomarker algorithm previously used for eligibility for this trial

1.3 Rationale for Inclusion of Ann Arbor 2 GVHD (August 2018)

Outcomes for patients with Ann Arbor 3 GVHD are dismal, with a 45% non-relapse mortality rate within 6 months of diagnosis. Outcomes for patients with Ann Arbor 2 GVHD are also poor with unacceptably high rates of treatment failure (49%) and NRM (27%) as shown in Figures 1 and 2 above. We confirmed that these outcomes have not improved recently by analyzing outcomes of 120 MAGIC patients with Ann Arbor 2 GVHD since 2015. The six month NRM rate in these patients remains stubbornly high at 24%. Patients with Ann Arbor 2 GVHD are therefore in need of better treatments than the current standard of care.

Since this study opened we have screened 229 patients with newly diagnosed GVHD. The proportion of patients with Ann Arbor 3 GVHD is somewhat lower than expected (13%

compared to an expected 20%). Ann Arbor 2 GVHD comprises 21% of all patients screened. While an intervention that improves outcomes for Ann Arbor 3 GVHD would represent a significant advance in the field, an effective intervention for all patients with high risk GVHD (Ann Arbor 2 and 3) is highly desirable. We have therefore redesigned the statistical plan (section 11) such that we can retain the knowledge gained from the patients with Ann Arbor 3 GVHD already enrolled on this trial and expand the eligibility to patients with Ann Arbor 2 GVHD. The expanded eligibility will shorten the time to complete enrollment by 50%.

1.4 Study Agent(s) Background and Associated Known Toxicities

GI GVHD requires T cells to home to the intestinal mucosa [Snider and Liang 2001]. Thus it is unsurprising that blocking T cell trafficking to the intestine abrogates GVHD mortality in animal models. This can be accomplished by interfering with chemokine receptors [Choi 2007] or the integrin-ligand interactions [Murai 2003, Waldman 2006, Ueha 2007] that are necessary for T cells to migrate into the intestinal mucosa. The α4β7-MAdCAM-1 (mucosal vascular addressin) interaction is key in this regard.

The mechanism of action of the monoclonal antibody natalizumab makes it an attractive agent to study for patients at high risk for steroid refractory GI GVHD. Natalizumab binds to the α4 component of the α4β7 integrin and thus inhibits T-cell mediated damage to the GI tract [Yu 2013]. Multiple studies have shown natalizumab to be an effective treatment for active, treatment resistant, Crohn's disease, in which the intestinal tissues are injured, similar to GVHD for >10 years [Gordon 2001, Ghosh 2003, Sandborn 2005, Targan 2007, Kane 2012, Juillerat 2013, Sakuraba 2013]. The most important of these studies was the ENCORE trial which randomized 509 patients with moderately to severely active Crohn's disease to receive natalizumab 300 mg or placebo intravenously at weeks 0, 4, and 8. The primary endpoint was induction of response. Additional efficacy endpoints included the proportion of patients with sustained remission and response or remission over time. Response at Week 8 sustained through Week 12 occurred in 48% of natalizumab-treated patients and 32% of patients receiving placebo (P < .001). Sustained remission occurred in 26% of natalizumab-treated patients and 16% of patients receiving placebo (P = .002). Week 4 response rates were 51% for natalizumab and 37% for placebo (P = .001). Responses remained significantly higher at subsequent assessments (P < .001) in natalizumab-treated patients. Natalizumab-treated patients also had significantly higher remission rates at Weeks 4, 8, and 12 (P < or = .009). The frequency and types of adverse events were similar between treatment groups [Targan 2007]. Natalizumab was FDA-approved in 2008 for use in for the treatment of moderate-to-severe Crohn's disease in patients with evidence of inflammation who have had an inadequate response to, or are unable to tolerate, conventional Crohn's disease therapies.

A recent meta-analysis included 5 randomized clinical trials of 1,771 patients who received natalizumab or placebo in order to induce remission in active Crohn's disease. Failure to achieve remission occurred in 810 (65.4%) of 1,238 patients receiving natalizumab at 2 to 12 weeks, compared with 412 (77.3%) of 533 randomized to placebo. The RR of not achieving remission was reduced with natalizumab (0.88; 95% CI 0.83–0.94, I^2=0%, P=0.72), with no statistically significant funnel plot asymmetry (Egger test, P=0.29) [Ford 2011].

The safety profile of natalizumab has been extensively evaluated with ~100,000 patients treated since FDA approval in 2004 for multiple sclerosis and in 2008 for Crohn's disease. Progressive multifocal encephalopathy (PML) is a serious but rare complication that has been reported in patients treated with natalizumab for either condition. Risk factors include concomitant immunosuppression and seropositivity for JC virus but long duration of treatment (>2 years) is the primary risk factor. It is reassuring that the incidence of PML in patients treated with natalizumab for fewer than 12 months was 4/100,000 [Bloomgren 2012]. The risk of PML in patients treated with immunosuppression is 2/1000 [Biogen

2015]. While unanticipated complications could occur, large clinical trials that included >1400 patients, have shown no other increased risks for natalizumab. However, because natalizumab has not been used to treat GVHD before, this study will be conducted under an IND from the FDA which requires robust safety measures and strict adverse event reporting.

1.5 Safety Data for Natalizumab Generated in this Study (August 2018)

To date, no toxicities observed on this study have been attributed as probably or definitely related to natalizumab. There have been no serious cardiac, pulmonary or liver toxicities. One patient developed renal failure seven months after completion of study treatment in the context of severe chronic GVHD and failure to thrive. This complication was considered unrelated to natalizumab.

Infections have been carefully monitored on this study. Enrollment to this trial was paused once a predetermined threshold of serious infections was observed in order to allow for analysis and safety assessment. The analysis concluded that natalizumab was not contributing to the incidence of serious infections.

Fifteen serious infections were observed in 12 patients (out of 32 study subjects) which include bacterial (n=9), fungal (n=3) and viral (n=3). The most common infection was bacterial sepsis with an enteric organism (Klebsiella, n=5, E coli, n=2; E. faecium, n=1). In 7/8 cases, the bacterial sepsis developed in the context of steroid-refractory stage 4 GI GVHD, a setting in which the intestinal architecture is extensively damaged and bacterial translocation is a well-known complication. One case of Klebsiella sepsis that developed on study day 8 in a patient responding to the combination of high dose steroids and natalizumab and was considered possibly related to natalizumab. The median time to develop a serious bacterial infection was study day 25 (range 2-85 days). The majority of these infections developed after additional immunosuppression had been given for lack of response to GVHD treatment. One presumed aspiration pneumonia that developed 14 days after the last dose of natalizumab was considered unlikely to be related to natalizumab.

The three fungal infections were cases of candida sepsis that developed on study day 14, 37, and 71 in the context of steroid refractory GI GVHD after additional immunosuppression had been initiated for lack of GVHD treatment response.

In two of three viral infections, symptoms were present before the first dose of natalizumab (one case each of EBV PTLD and HHV6 encephalitis). Both of these cases were considered unrelated to natalizumab. One case of CMV colitis that developed on study day 20 in a patient with steroid refractory GI GVHD was considered possibly related to natalizumab.

In summary, the vast majority of serious infections observed on this study have occurred in patients with steroid refractory GVHD who are known to be at very high risk for infections. The types of organisms, the GVHD severity and involvement of the GI tract, the timing relative to natalizumab infusions and the occurrence often after initiation of additional immunosuppression led investigators to attribute these 13/15 infections to causes other than natalizumab. This assessment is consistent with a recent report that GI GVHD treated with steroids is the major risk factor for bacterial and fungal sepsis after allogeneic BMT [Mori 2018]. The two infections that were considered possibly related to do not significantly alter the risk-benefit ratio for this study.

1.6 Other Agents

Prednisone (or methylprednisolone) will be co-administered with natalizumab. Systemic corticosteroid therapy has been the standard treatment for acute GVHD for 40 years and its efficacy and risks are well understood [Martin 2012]. Detailed information on adverse events and potential risks are detailed in section 9.0.

1.7 Rationale

The newly developed Ann Arbor GVHD scoring system risk-stratifies patients at diagnosis. Patients with Ann Arbor score 3 GVHD represent a high risk strata enriched for the development of steroid refractory GI GVHD and early death. The six month NRM for patients with Ann Arbor score 3 GVHD is 1.6 times that of those with GI GVHD at diagnosis (45% vs. 28%). Early identification of patients at high risk for steroid refractory GI GVHD is important as early treatment is more likely to be effective. Furthermore, the low complete response rates (30%) and high NRM rates in patients with Ann Arbor score 3 GVHD warrants experimental treatment. Natalizumab effectively inhibits T-cell trafficking to the GI tract and has shown exceptional efficacy in Crohn's disease with response rates 16% higher than placebo after a single dose. The administration of an agent that inhibits T-cell trafficking to the GI tract to patients with Ann Arbor score 3 GVHD, which includes patients who are at high risk of developing GI GVHD but have not yet done so, is an attractive approach to a highly lethal disease.

The clinical response on day 28 of treatment correlates with long term survival in patients with GVHD [Levine 2010, MacMillan 2010]. Therefore, we chose the day 28 CR rate as the trial's primary endpoint. Because we expect improvements in day 28 CR to translate into better long-term outcomes, 1 year NRM and 1 year survival will be secondary endpoints. Additional secondary endpoints related to efficacy will include the day 28 overall response rate (CR + PR), the proportion of patients who develop treatment-refractory GVHD (defined as those who do not achieve CR or PR by day 28 **or** who receive additional immunosuppression prior to day 28), time to discontinuation of steroid therapy, number of lines of GVHD therapy, and cumulative incidence of chronic GVHD. Secondary endpoints related to safety include 6 month and 1 year relapse rates and incidence of serious infections by 6 months. All patients who receive at least one dose of natalizumab will be considered evaluable for both safety and efficacy.

1.8 Correlative Studies

GI biopsies. We have previously shown that GI pathologic grade [Ferrara 2011] and more recently, that Paneth cell numbers from GI biopsies correlate with GI GVHD outcomes [Levine 2013]. However, major gaps exist in our understanding of how pathologic changes correlate with clinical outcomes such as treatment response and NRM. While not mandated as part of study participation, endoscopic evaluations with biopsies are routinely performed in patients with suspected GVHD and GI symptoms (nausea, vomiting, diarrhea, etc.). When consent is provided, we will analyze small and large bowel biopsy samples obtained from these patients using immunohistochemical staining for cells and proteins with known associations with GI GVHD including Paneth cells, intestinal stem cells, RORγt+ NKp46+ cells producing IL-22, regulatory T cells, α4β7T-cells, and cellular expression of toll-like receptors, NOD-like receptors, α-defensins, and Reg3α.

Furthermore, a recent publication showed that repeating endoscopic evaluations in patients with persistent GI symptoms is helpful [Martinez 2012]. In that retrospective study, histological findings of repeat endoscopic biopsies led to a change in clinical management in 77% of the cases, such as altering the intensity of immunosuppression and/or adding specific treatment for a coexisting infectious disease. From the 84 study patients with Ann Arbor score 2 or 3 GVHD, we estimate that onset biopsies will be available for 90% of the 42 patients with GI symptoms at onset, or 38 patients. We further estimate that

approximately 40% of these patients, or 15, will undergo repeat endoscopic evaluation at day 28 for persistent GI symptoms.

Serial serum biomarkers. GVHD biomarkers measured during treatment correlate with clinical outcomes such as treatment response and survival [Levine 2008, Levine 2012]. For example, a biomarker panel on day 14 of treatment identified patients in CR who were most likely to experience a GVHD flare [Levine 2012]. Therefore, we expect that GVHD biomarker concentrations during treatment measure GVHD alloreactivity independently of clinical symptoms. We will collect and store serum samples during treatment for these future analyses from consenting patients.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 To improve day 28 GVHD complete response rate for Ann Arbor score 2 and 3 GVHD patients from the historical rate of 42.5% to 57.5% by treatment with natalizumab and high dose prednisone (or methylprednisolone) (2 mg/kg).

2.2 Secondary Objectives

2.2.1 To decrease 6-month NRM from the historical rate of 32% to 22% in patients treated on this clinical trial.

2.2.2 To determine the overall survival and NRM rates at 1 year and the cumulative incidence of treatment-refractory GVHD (defined as no improvement or worsening in any target organ by day 28 of treatment or who receive additional immunosuppression prior to day 28), the day 28 overall response rate (CR + PR), time to discontinuation of steroid therapy, number of lines of GVHD therapy, and cumulative incidence of chronic GVHD in patients treated on this clinical trial. Outcomes will be analyzed for all patients and separately for Ann Arbor 2 and 3 GVHD patients.

2.2.3 To determine the cumulative incidence of 6 month and 1 year relapse and the incidence of serious infections by 6 months in patients treated on this clinical trial.

2.2.4 To assess the safety of natalizumab for the treatment of high risk GVHD.

2.2.5 To assess the improvement in day 28 GVHD complete response rate for Ann Arbor score 2 and 3 patients separately in order to evaluate any differences in response to the treatment between two groups.

2.3 Endpoints

The primary endpoint for this clinical study is the proportion of complete response (that is, the percent of patients with skin, liver, and GI GVHD all stage 0) at day 28 of study treatment.

The secondary endpoints are:

1. Overall survival at 1 year
2. Cumulative incidence of NRM at 6 months and 1 year
3. Overall response rate (CR + PR) at day 28. PR is defined as improvement in one or more organs involved with GVHD symptoms without progression in others. For

a response to be scored as PR on day 28, the patient must be in PR on day 28 and have had no intervening non-study therapy for acute GVHD.

4. Cumulative incidence of treatment-refractory GVHD (defined as absence of CR or PR on day 28 of treatment **or** who receive additional immunosuppression prior to day 28)
5. Cumulative incidence of severe GI GVHD stage 3 or 4
6. Time to discontinuation of steroid therapy
7. Number of lines of GVHD therapy (defined as the initiation of a new acute GVHD therapy, regardless of duration)
8. Cumulative incidence of chronic GVHD
9. Number of serious infections (defined as grade 3 by the Blood and Marrow Transplant Clinical Trials Network)

3.0 PATIENT ELIGIBILITY

Subjects must meet all of the inclusion and exclusion criteria to be enrolled to the study. Study treatment may not begin until a subject is enrolled.

3.1 Inclusion Criteria

3.1.1 New onset acute GVHD Ann Arbor score 2 or 3 following allogeneic BMT. Any clinical severity (Glucksberg grade I-IV) is eligible. Patients with prior or existing diagnosis of GVHD without any treatment are eligible. Patients given only topical corticosteroids for skin GVHD are eligible.

3.1.2 Any donor type (e.g., related, unrelated) or stem cell source (bone marrow, peripheral blood, cord blood). Recipients of non-myeloablative and myeloablative transplants are eligible.

3.1.3 No prior systemic treatment for acute GVHD except for a maximum of 3 days of prednisone (or IV methylprednisolone) ≤2 mg/kg/day. Topical skin steroid treatment, non-absorbable oral steroid treatment for GI GVHD, and resumption of GVHD prophylaxis agents (e.g., calcineurin inhibitors) are permissible. Patients enrolled in BMT CTN 1501 who randomized to sirolimus are also eligible. Exceptions can be made on a case-by-case basis with Sponsor-Investigator approval.

3.1.4 Age 18 years or older.

3.1.5 Direct bilirubin must be <2 mg/dL unless the elevation is known to be due to Gilbert syndrome or aGVHD within 3 days of enrollment.

3.1.6 ALT/SGPT and AST/SGOT must be <5 x the upper limit of the normal range within 3 days of enrollment unless the elevation is due to liver GVHD.

3.1.7 If the patient is a woman of child-bearing potential, the patient and their sexual partner must agree to practice effective contraception.

3.1.8 Written informed consent from patient or legal representative.

3.1.9 Biopsy of acute GVHD target organ is strongly recommended but not required. Enrollment should not be delayed for biopsy or pathology results. Patients who do

not enroll within 3 days of initiation of systemic steroid treatment for acute GVHD are not permitted to participate.

3.2 Exclusion Criteria

3.2.1 Patients with malignancy that is suspected or proven to have progressed, relapsed, or be persistent since BMT

3.2.2 Uncontrolled active infection

3.2.3 Patients with chronic GVHD only. Patients diagnosed with overlap syndrome are still eligible.

3.2.4 History of or current diagnosis of progressive multifocal leukoencephalopathy (PML)

3.2.5 Known hypersensitivity to natalizumab

3.2.6 Pregnant or nursing (lactating) women

3.2.7 Use of other drugs for the treatment of acute GVHD

3.2.8 Steroid therapy for indications other than GVHD at doses >0.5 mg/kg/d of methylprednisolone or equivalent within 7 days prior to initiation of GVHD treatment

3.2.9 Patients on dialysis

3.2.10 Patients requiring ventilator support

3.2.11 Investigational agent within 30 days of enrollment without approval from the Sponsor-Investigator (PI)

3.2.12 History of allergic reaction to natalizumab

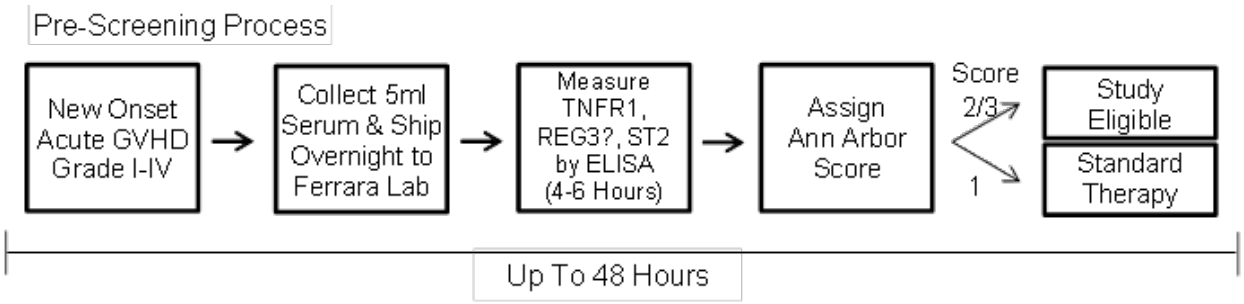
4.0 SUBJECT SCREENING AND REGISTRATION PROCEDURES

PRESCREENING PROCESS

To be eligible for this study, patients must be diagnosed with Ann Arbor score 2 or 3 GVHD. Most patients will be recruited from centers participating in the Mount Sinai Acute GVHD International Consortium (MAGIC) and will already have consented prior to transplant for clinical data and research blood sample submission to the Ferrara Lab at pre-specified time points post-BMT as well as at the onset of acute GVHD. Thus, written informed consent for the pre-screening process to determine eligibility will already have been obtained at each center on a separate, IRB-approved protocol. Patients who initially were not offered or declined to participate in the MAGIC study who consent at the time of onset of acute GVHD can provide the pre-screening research sample needed for Ann Arbor scoring. Because the vast majority of patients consent at time of transplant to the sample collection protocols, this latter option will be infrequently utilized.

The pre-screening process is outlined in the figure below. Pre-screening takes place entirely under the previously provided informed consent detailed above. The process is explained here for completeness. Five mL of serum will be collected from patients with new onset acute GVHD on the day of diagnosis of GVHD (preferable) or up to 48 hours after initiation of steroid treatment for

GVHD. However, first dose of Natalizumab must be initiated within 3 days after the start of steroid treatment for GVHD. Exceptions to this timeline can be made on a case-by-case basis with Sponsor-Investigator approval. Once a sample has been collected, it will be (1) registered in the remote data entry system using the unique natalizumab identification number assigned to the patient at time of registration in the database and (2) shipped priority overnight to the laboratory of Dr. James Ferrara for early AM arrival (**see MAGIC Sample Collection and Storage manual for shipping procedures**). Samples can be received Tuesday through Saturday. Once received in the laboratory, the GVHD biomarkers used to assign the Ann Arbor GVHD score will be measured by ELISA using standard technical procedures (**see Appendix A for details**). Processing, measuring, and confirming the ELISA assay results take 4.5 hours (range 4-6 hours). Once the Ann Arbor score is confirmed by Dr. Ferrara (or Dr. Levine in Dr. Ferrara's absence), the investigator at the participating center will be notified of the Ann Arbor score by telephone with email written confirmation. Only patients with confirmed Ann Arbor score 2 or 3 GVHD will be eligible to enroll onto the clinical trial.



SCREENING AND REGISTRATION PROCESS

Patients must provide informed consent for this clinical trial prior to release of the Ann Arbor Score. It is preferred to obtain informed consent prior to shipping the sample. Enrollment must be complete by the end of the 3 day window; therefore investigators are encouraged to begin the informed consent process at the same time as the pre-screening research serum sample is obtained. Patients who sign informed consent but are not eligible to participate because they do not have Ann Arbor score 2 or 3 GVHD will be considered screen failures as well as any patient who does not meet all the inclusion and exclusion criteria.

Patient registration for this trial will be centrally managed by the MAGIC Data Coordinating Center of the Icahn School of Medicine at Mount Sinai as described below:

A potential study subject who has been screened for the trial and who has signed the Informed Consent document will be initially documented by the participating site on the Screening and Enrollment Log provided by the MAGIC Data Coordinating Center (DCC).

It is the responsibility of the local site investigator to confirm patient eligibility for the clinical trial. Confirmation of Ann Arbor score 2 or 3 GVHD will be provided directly to the identified Primary Site contact from the participating site by Dr. Ferrara, Dr. Levine or designee. All other eligibility criteria will be provided by the participating site. After patient eligibility has been determined, a copy of the **completed** Eligibility form will be submitted by the requesting site to the MAGIC Data Coordinating Center by email to magic@mssm.edu with local site investigator signature and supporting source documentation.

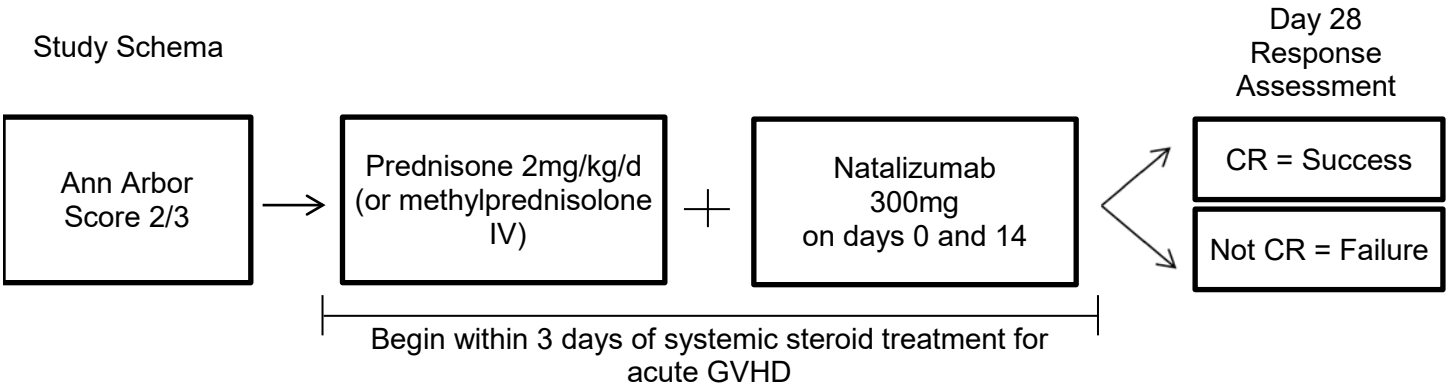
The MAGIC Coordinator, who acts as the registrar, will review the submitted documents and process the registration. Sites should inform the MAGIC Coordinator of a potential registration once a research sample has been submitted for Ann Arbor score assignment given the time sensitivities in this study.

An email will be sent by the registrar to the requesting site registrar to confirm patient registration.

Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in the Screening and Enrollment Log. These patients will not receive study treatment.

5.0 TREATMENT PLAN

Study Schema



5.1 Treatment Dosage and Administration

Protocol treatment must start within **3 days of initiation of systemic steroid treatment for acute GVHD**. For example, a patient diagnosed with acute GVHD who starts systemic steroid treatment on Monday (day 0), who has a research sample shipped to the Ferrara Lab on Tuesday (day 1), will have their Ann Arbor score assigned on Wednesday (day 2). Such a patient should begin study treatment no later than Thursday (day 3). Exceptions to this time line can be made on a case-by-case basis with Sponsor-Investigator approval.

5.1.1 Study treatment will consist of two drugs: Prednisone (or methylprednisolone) which is standard treatment for acute GVHD and natalizumab, the drug under study.

5.1.2 Corticosteroid dosing and taper

All patients enrolled on this trial will receive steroids at a dose of prednisone 2 mg/kg/day orally (or IV methylprednisolone). Centers may use a starting dose of 2 mg/kg of methylprednisolone if that is their institutional practice. The dose of steroids cannot be tapered before study day 2 (day 0 is defined as the day of the first dose of natalizumab), but afterwards local institutional tapering practices can be followed. Steroid therapy can also be given up to 3 days prior to the first dose of natalizumab (day -3 to -1), i.e., during the period from the initiation of systemic steroid treatment for acute GVHD until study therapy begins.

The following is a suggested steroid taper (identical to that used in Blood and Marrow Transplant Clinical Trials Network primary GVHD therapy studies):

> 2 mg/kg/day of steroid divided once or twice/day days 1-6
>
> 1.5 mg/kg/day once daily days 7-13
>
> 1 mg/kg/day once daily days 14-21
>
> 0.5 mg/kg/day once daily days 22-28
>
> Patients should be tapered as tolerated to no less than 0.5 mg/kg/day.
>
> Then taper according to institutional guidelines with a goal to reach ≤0.2 mg/per/kg per day of prednisone or ≤0.16 mg/per/kg per day of methylprednisolone by day 56.

5.1.3 Natalizumab dosing and schedule

Natalizumab will be obtained from Biogen. Each participating site will have a sufficient supply of natalizumab on hand to begin treatment, additional doses will be shipped to the participating site to complete treatment. See section 9.1 for preparation, dispensing, and administration information.

The first dose of natalizumab must be administered within 3 days of start of systemic steroid treatment for acute GVHD. The first day of natalizumab will be day 0 of the study. Exceptions to this time line can be made on a case-by-case basis with Sponsor-Investigator approval.

Natalizumab will be administered at a dose of 300 mg intravenously on days 0 and 14. If, for reasons other than toxicity, the second dose cannot be administered on day 14, the acceptable window for administration is day 12-16.

5.1.4 GVHD Prophylaxis Medications

Medications such as cyclosporine, tacrolimus, sirolimus (if used as GVHD prophylaxis when acute GVHD developed) should be continued at therapeutic doses (according to institutional standards) adjusted as necessary for renal, central nervous system (CNS) or other toxicity using conventional management guidelines.

5.1.5 Topical and Ancillary GVHD therapies

Topical therapy for acute GVHD of the skin and non-absorbable steroids for GI GVHD are allowed.

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

5.1.6 Supportive Care Guidelines

In addition to prescribed study drug plus corticosteroids, all patients should receive the following:

- Transfusion support per institutional practice
- Anti-infective prophylaxis against herpes virus is required but otherwise institutional practice can be followed.
- Anti-infective prophylaxis against *Pneumocystis jiroveci*, bacterial and fungal infections according to standard institutional guidelines.
- Pre-emptive monitoring and treatment for CMV and EBV infections are required but otherwise institutional practice can be followed.

5.2 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events Table (Section 6.4). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

The dose of natalizumab will not be modified on this study for toxicities or co-morbidities that develop between the first and second dose. In the event that a toxicity develops that

the treating physician deems poses an unacceptable risk to administering natalizumab, the dose should not be given and the patient will be removed from study therapy.

The second dose of natalizumab should be administered on day 14 of the study. However, if for reasons other than toxicity the second dose cannot be administered on day 14, the acceptable window is day 12-16.

5.3 Concomitant Medications/Treatments

Concomitant use of other investigational agents is not permitted during the treatment phase of the study.

5.4 Other Modalities or Procedures

Patients who have undergone allogeneic hematopoietic cell transplantation and developed acute GVHD typically are simultaneously being treated for other conditions and transplant-related complications. Such treatments, including the use of steroid therapy as GVHD treatment are considered standard of care and will be considered distinct from the study drug treatment, natalizumab.

5.5 Infection Prophylaxis (August 2017)

This trial originally expected a 24% incidence of severe infections (BMT CTN grade 3) based on an expected distribution of enrollment of 25% grade 1 (mild skin) GVHD, 50% grade II (moderate skin and or mild GI) and 25% grade III/IV (primarily severe GI) GVHD. However, this trial has disproportionately enrolled patients with severe GVHD grade III/IV (12/20, 60%). Patients with severe GI GVHD have considerable damage to the GI mucosa and are at increased risk of translocation of enteric bacteria from the GI lumen into the bloodstream and sepsis. As a result, the incidence of BMT CTN grade 3 infections in the first 20 patients enrolled on this trial (45%) significantly exceeded the expected 24% rate, because more patients at high risk for this complication enrolled than expected. To reduce the risk of this complication, the study DSMC required the protocol include mandatory gram negative antibiotic coverage (fluoroquinolone or institutional preference) from study day 0 to study day 42, which encompasses this high risk period. Antibiotic prophylaxis can be continued beyond day 42 per institutional preference.

5.6 Duration of Therapy

The duration of protocol therapy on this study is 14 days (two doses of natalizumab administered on day 0 and 14). Protocol therapy will end after the second dose of natalizumab has been administered or if any of the following criteria apply:

- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient voluntarily withdraws from treatment **OR**
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

5.7 Off Treatment Criteria

Patients will be removed from protocol therapy when any of the criteria listed in Section 5.6 apply. The reason for ending protocol therapy and the date the patient was removed from treatment will be documented in the study record. All patients who discontinue treatment should comply with protocol specific follow-up procedures as outlined in Section 5.8. The only exception to this requirement is when a subject withdraws consent for all study procedures.

5.8 Duration of Follow-Up

Patients will be followed for 1 year after removal from treatment or until death, whichever occurs first. Patients with acute GVHD are followed closely and frequent clinical evaluations are the norm. The following outlines the minimum frequency of follow-up evaluations, it is anticipated that the majority of patients will be evaluated more frequently.

During the first 6 weeks of participation (i.e., through 4 weeks after the last dose of natalizumab), patients will be evaluated at least weekly for acute toxicity assessment, GVHD staging and management. For the next 6 weeks, patients will be evaluated at least every other week for GVHD staging and management. Thereafter, patients will be evaluated every 3 months (±2 weeks) until 1 year from start of study treatment.

5.9 Off Study Criteria

Patients can be taken off study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation from study will be documented and may include:

5.9.1 Patient withdraws consent (termination of treatment and follow-up);

5.9.2 Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment;

5.9.3 Patient is unable to comply with protocol requirements;

5.9.4 Treating physician judges continuation on the study would not be in the patients best interest;

5.9.5 Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);

5.9.6 Termination of the study;

5.9.7 Patient completes protocol treatment and follow-up criteria.

5.10 Patient Replacement

Patients who enroll in the study but do not receive any study treatment will be replaced. The number of patients and reason(s) for replacement will be recorded and will be used to assess the feasibility of the study design.

6.0 STUDY PROCEDURES

6.1 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

All screening procedures must be performed within 3 days prior to registration unless otherwise stated. The screening procedures include:

6.1.1 ***Informed Consent***

6.1.2 ***Demographics***

Age, gender, race, ethnicity

6.1.3 ***Review subject eligibility criteria***

6.1.4 ***Review concomitant medications***

6.1.5 ***GVHD staging***

6.1.6 ***Physical exam including vital signs, height and weight***

Vital signs (temperature, pulse, respirations, blood pressure), height, weight

6.1.7 ***Adverse event assessment***

Baseline adverse events will be assessed and preexisting conditions will be recorded. See Section 8.0 for Adverse Event monitoring and reporting.

6.1.8 ***Hematology***

Complete blood count and differential

6.1.9 ***Serum chemistries***

Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin.

6.1.10 ***Pregnancy test (only for females of child bearing potential)***

6.2 Procedures During Treatment

6.2.1 **Prior to Each Natalizumab Dose**

- Physical exam, vital signs
- Biomarker serum sample
- Hematology
- Serum chemistries

6.2.2 **GVHD staging, concomitant medication review, toxicity evaluations and adverse event reporting as per time and events table below**

6.2.3 **30 days after treatment termination**

- Toxicity assessment
- Physical exam, vital signs
- Hematology
- Serum chemistries

6.3 Follow-Up Procedures

The minimum frequency of follow-up evaluations is as follows:

During the first 6 weeks of participation (i.e., through 4 weeks after the last dose of natalizumab), patients will be evaluated at least weekly for acute toxicity assessment, GVHD staging and management. For the next 6 weeks, patients will be evaluated at least every other week for GVHD staging and management. Thereafter, patients will be

evaluated every 3 months (±2 weeks) until 1 year from start of study treatment. Follow-up studies are detailed in the Time and Events Table is section 6.4.

6.4 Time and Events Table

	Pre-study	Day 0	Day 7 (±2 days)	Day 14 (±2 days)	Day 21 (±2 days)	Day 28[1] (±1 day)	Day 35, 42 (±3 days)	Week 8, 10, 12 (±6 days)	Month 3, 6, 9, 12 (±2 weeks)
Informed Consent	X								
History and PE	X	X		X					
Pregnancy Test[2]	X								
Contraceptive Counseling[2]	X								
Toxicity Evaluations[3]		X	X	X	X	X	X		X[4]
Progressive Multifocal Leuko-encephalopathy (PML)[5]	Assess for PML at least every 2 weeks from baseline visit to 6 months (assessment can be performed at scheduled visits or by phone)								
Adverse Event Evaluations		Report adverse events from the time of the initial study treatment (Day 0) through 30 days after the last dose of natalizumab. Report serious adverse events as they occur.							
GVHD staging[6]	X	X	X	X	X	X	X	X	
Concomitant Medication Review[7]	X	X	X	X	X	X	X	X	X
Natalizumab Administration		X		X					
LABORATORY STUDIES									
Serum Chemistry	X	X	X	X	X	X	X		
CBC	X	X	X	X	X	X	X		
CORRELATIVE STUDIES									
Serum Sample (5 ml)		X	X	X	X	X			
GI biopsies (if clinically indicated)[8]	Optional: Submit formalin fixed paraffin embedded GI biopsies obtained for standard of care purposes, provided that the tissue is no longer needed for further clinical use.								

[1] Day 28 is the primary response endpoint. GVHD staging should be performed this day, or if needed, within a 1 day window.

[2] Pregnancy test is required only for women of childbearing potential defined as Tanner stage I, post-menopausal for at least 24 consecutive months or post-hysterectomy, salpingotomy, and/or bilateral oophorectomy. Women who are not of reproductive potential are not required to undergo contraceptive counseling. Acceptable documentation includes written or oral documentation by the clinician of one of the following: physical examination indicates Tanner stage I, physician report/letter, operative report or other source documentation in the patient record, discharge summary, or follicle stimulating hormone measurement elevated into the menopausal range.

[3] Toxicity assessment does not replace adverse event reporting, but rather collects the occurrence of any events known to be associated with natalizumab. A baseline toxicity assessment is performed on day 0 **prior** to the first dose of natalizumab. Known or suspected toxicities include hepatoxicity (elevated hepatic enzymes and/or total bilirubin), hematologic abnormalities (increased circulating lymphocytes, monocytes, eosinophils, basophils, nucleated red blood cells), allergic reactions, depression, headache, fatigue, rash, nausea, vomiting, and/or diarrhea.

Patients will be evaluated for these toxicities at each assessment time point up to day 42, including pre-treatment baseline and their presence will be recorded.

[4] At months 3, 6, 9, and 12 report only PML, BMT CTN Grade 3 infections, viral reactivations, and any other toxicity if deemed related to natalizumab by the local investigator. (appendix B for BMT CTN Grade 3 Infection criteria)

[5] Ask patients about difficulty with vision, speech, arm/leg weakness, clumsiness, personality changes, or confusion.

[6] GVHD staging will follow the detailed guidelines provided in the MAGIC Acute GVHD Staging Guidance. Weekly GVHD data will be required for patients co-enrolled on the MAGIC Observational study when patient is still within 3 months of HCT. This data can also be provided for patients not co-enrolled on MAGIC if the patient is seen.

[7] Concomitant medication review will record all other immunosuppressants administered during the reporting period.

[8] Biopsies will be requested and batch shipped after the tissue is no longer needed for further clinical use (minimum six months from biopsy acquisition). See Section 10.3.

7.0 GVHD CLINICAL STAGING

GVHD clinical staging will be according to the established criteria used for Blood and Marrow Transplant Clinical Trials Network GVHD staging (modified Glucksberg criteria). Clinical stage will be used to evaluate patients for the primary endpoint, day 28 CR.

	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Skin	No rash	Rash < 25% BSA	25-50%	> 50% Generalized erythroderma	Plus bullae and desquamation >5% BSA
Liver	Bilirubin ≤ 2 mg/dl	2.1-3 mg/dl	3.1-6mg/dl	6.1-15mg/dl	>15mg/dl
GI tract	Adult: < 500 ml/day	Adult: 500–1000 ml/day	Adult: 1001-1500 ml/day	Adult: >1500 ml/day	Severe abdominal pain +/- ileus, flank blood or melena (regardless of stool volume)
UGI		Severe nausea/vomiting			

- For stage 4 GI GVHD, severe abdominal pain is defined as (1) pain that requires opioid use **and** (2) pain that significantly impacts on performance status as determined by the treating physician
- Comprehensive GVHD staging guidance is provided in the MAGIC GVHD Staging Guidance.

Overall Clinical Grade:

Grade 0	No stage 1-4 of any organ
Grade I	Stage 1-2 skin and no liver or GI involvement
Grade II	Stage 3 skin and/or Stage 1 liver and/or Stage 1 GI
Grade III	Stage 0-3 skin with Stage 2-3 liver and/or Stage 2-3 GI
Grade IV	Stage 4 in any target organ (skin, liver, GI)

7.1 ENDPOINT AND RESPONSE CRITERIA

7.1.1 Definitions

Evaluable for response: All patients will be evaluable for toxicity and response from the time of their first treatment with natalizumab.

Complete Response (CR): All evaluable organs (skin, liver, GI tract) stage 0. For a response to be scored as CR on day 28, the patient must be in CR on that day and have had no intervening additional GVHD therapy.

Partial Response (PR): An improvement in one or more organ involved with GVHD symptoms without worsening in others. For a response to be scored as PR on day 28, the patient must be in PR on that day and have had no intervening additional GVHD therapy.

No response (NR): All responses that are not CR or PR. Patients who receive any GVHD therapy other than steroid therapy, natalizumab, non-absorbable oral steroid therapy, and topical steroids will be scored as NR on day 28 regardless of organ staging.

7.1.2 Proportion of CR and CR+PR

Scoring of CR and PR on day 28 are in comparison to the patient's acute GVHD staging on day 0 of the study.

7.1.3 Treatment refractory GVHD

Patients who are scored as no response on day 28 **or** who receive additional immunosuppression prior to day 28 (i.e., no response) will be considered treatment refractory.

7.1.4 Steroid discontinuation

The date of discontinuation of steroid therapy will be recorded.

7.1.5 Lines of GVHD therapy

Any new acute GVHD therapy will be considered a line of therapy. Resumption or changes in GVHD prophylaxis are not considered new lines of therapy.

7.1.6 Non-Relapse Mortality (NRM)

Any death that occurs after onset of GVHD not attributable to relapse of the underlying disease will be considered a non-relapse death.

7.1.7 Chronic GVHD

The occurrence of chronic GVHD, including date of diagnosis, will be recorded.

7.1.8 Relapse

Relapse, including date of relapse, of the underlying malignancy will be recorded.

7.2 SAFETY/TOXICITY DEFINITIONS

7.2.1 Known or plausible toxicities that may be related to natalizumab will reported. Guidance of determining whether a toxicity is an adverse event attributable to natalizumab is provided in section 8.

7.2.2 Hepatotoxicity

Elevated hepatic enzymes (ALT, AST) and/or total bilirubin > 1.5 times the baseline values on day 0 **or** higher than the upper limit of normal, whichever is higher, will be considered evidence of hepatoxicity.

7.2.3 Hematologic abnormalities

The absolute value of lymphocytes, monocytes, eosinophils, basophils, or nucleated red blood cells will be recorded as a hematologic abnormality if the value is >1.5 times the upper limit of normal.

7.2.4 Systemic Infections

Infections are common in patients with acute GVHD. Any grade 3 infection as defined by the Blood and Marrow Transplant Clinical Trials Network will be reported.

Grade 3 Bacterial Infections:

a. Bacteremia with deep organ involvement
b. Severe sepsis with bacteremia
c. Fasciitis requiring debridement
d. Pneumonia requiring intubation
e. Brain abscess or meningitis without bacteremia
f. Clostridium difficile toxin positive stool with toxic dilatation or renal insufficiency with/without diarrhea.

Grade 3 Fungal Infections:

a. Fungemia, including candidemia
b. Proven or probable invasive fungal infections (e.g. Aspergillus, Mucor, Fusarium, Scedosporium)
c. Disseminated fungal infections
d. Pneumocystis jiroveci pneumonia

Grade 3 Viral Infections:

a. Severe VZV infection with either associated coagulopathy or organ involvement
b. CMV end organ involvement
c. EBV Post-transplant lymphoproliferative disorder (PTLD)
d. Adenovirus with end organ involvement (except adenoviral conjunctivitis or upper respiratory tract disease)
e. All lower respiratory tract viruses
f. Viral encephalitis or meningitis

7.2.5 Viral Reactivations

Because viral reactivations often require treatment in the BMT population, even in the absence of end organ disease, the following viral infections/reactivations will be reported:

The date, anatomical site or body fluid (e.g., blood, nasopharyngeal swab, stool, etc.), and method of detection for CMV, EBV, HHV6, VZV, HSV and adenovirus will be reported.

7.2.6 Progressive multifocal leukoencephalopathy (PML)

The occurrence of PML will be reported.

7.3 Safety/Tolerability

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 4.0 for reporting of adverse events (http://ctep.cancer.gov/reporting/ctc.html).

8.0 ADVERSE EVENTS

8.1 NATALIZUMAB

For the most recent safety update, please refer to the current Investigator's Brochure or Study Agent Prescribing Information.

8.1.1 Contraindications

Patients who have or have had PML
Patients who have had a hypersensitivity reaction to natalizumab

8.1.2 Special Warnings and Precautions for Use

- Herpes encephalitis and meningitis: Life-threatening and fatal cases have occurred.
- Hepatotoxicity: Significant liver injury, including liver failure requiring transplant, has occurred.
- Hypersensitivity reactions: Serious hypersensitivity reactions (e.g., anaphylaxis) have occurred.
- Immunosuppression/Infections: Natalizumab may increase the risk for certain infections.

8.1.3 Interaction with other medications

The use of concomitant immunosuppressants or inhibitors of TNFα increase the risk of PML and other infections.

8.1.4 Adverse Reactions

The most serious adverse reactions with natalizumab are:
• Progressive Multifocal Leukoencephalopathy (PML)
• Hypersensitivity
• Immunosuppression/Infections

The most common adverse reactions (incidence ≥ 10%) were headache and fatigue in both multiple sclerosis (MS) and Crohn's disease (CD) studies. Other common adverse reactions (incidence ≥ 10%) in the MS population were arthralgia, urinary tract infection, lower respiratory tract infection, gastroenteritis, vaginitis, depression, pain in extremity, abdominal discomfort, diarrhea NOS, and rash. Other common adverse reactions (incidence ≥ 10%) in the CD population were upper respiratory tract infections and nausea.

The most frequently reported adverse reactions resulting in clinical intervention (i.e., discontinuation of natalizumab), in the MS studies were urticaria (1%) and other hypersensitivity reactions (1%), and in the CD studies were the exacerbation of Crohn's disease (4.2%) and acute hypersensitivity reactions (1.5%).

Infections

Progressive Multifocal Leukoencephalopathy (PML) occurred in three patients who received natalizumab in clinical trials. Two cases of PML were observed in the 1869 patients with multiple sclerosis who were treated for a median of 120 weeks. These two patients had received natalizumab in addition to interferon beta-1a. The third case occurred after eight doses in one of the 1043 patients with Crohn's disease who were evaluated for PML. In the post marketing setting, additional cases of PML have been reported in natalizumab-treated multiple sclerosis and Crohn's disease patients who were not receiving concomitant immunomodulatory therapy.

In multiple sclerosis studies, the rate of any type of infection was approximately 1.5 per patient-year in both natalizumab-treated patients and placebo-treated patients. The infections were predominately upper respiratory tract infections, influenza, and urinary tract infections. In one multiple sclerosis study, the incidence of serious infection was approximately 3% in both natalizumab-treated patients and placebo-treated patients. Most patients did not interrupt treatment with natalizumab during infections. The only opportunistic infection in the multiple sclerosis clinical trials was a case of cryptosporidial gastroenteritis with a prolonged course.

In Crohn's disease studies, the rate of any type of infection was 1.7 per patient-year in natalizumab-treated patients and 1.4 per patient-year in placebo-treated patients. The most common infections were nasopharyngitis, upper respiratory tract infection, and influenza. The majority of patients did not interrupt natalizumab therapy during infections and recovery occurred with appropriate treatment. Concurrent use of natalizumab in CD clinical trials with chronic steroids and/or methotrexate, 6-MP, and azathioprine did not result in an increase in overall infections compared to natalizumab alone; however, the concomitant use of such agents could lead to an increased risk of serious infections.

In two CD studies, the incidence of serious infection was approximately 2.1% in both natalizumab-treated patients and placebo-treated patients. In a third CD study, the incidence of serious infection was approximately 3.3% in natalizumab-treated patients and approximately 2.8% in placebo-treated patients. In clinical studies for CD, opportunistic infections (*Pneumocystis carinii* pneumonia, pulmonary *Mycobacterium avium intracellulare*, bronchopulmonary aspergillosis, and *Burkholderia cepacia*) have been observed in <1% of natalizumab-treated patients; some of these patients were receiving concurrent immunosuppressants. Two serious nonbacterial meningitides occurred in natalizumab-treated patients compared to none in placebo treated patients.

Infusion-related Reactions

An infusion-related reaction was defined in clinical trials as any adverse event occurring within two hours of the start of an infusion. Observe subjects for one hour after end of infusion. In MS clinical trials, approximately 24% of natalizumab-treated multiple sclerosis patients experienced an infusion-related reaction, compared to 18% of placebo-treated patients. In the controlled CD clinical trials, infusion-related reactions occurred in approximately 11% of patients treated with natalizumab compared to 7% of placebo-treated patients. Reactions more common in the natalizumab-treated MS patients compared to the placebo-treated MS patients included headache, dizziness, fatigue, urticaria, pruritus, and rigors.

Acute urticaria was observed in approximately 2% of patients. Other hypersensitivity reactions were observed in 1% of patients receiving natalizumab. Serious systemic hypersensitivity infusion reactions occurred in <1% of patients. All patients recovered with treatment and/or discontinuation of the infusion.

Infusion-related reactions more common in CD patients receiving natalizumab than those receiving placebo included headache, nausea, urticaria, pruritus, and flushing. Serious infusion reactions occurred in CD studies at an incidence of <1% in natalizumab-treated patients.

MS and CD patients who became persistently positive for antibodies to natalizumab were more likely to have an infusion-related reaction than those who were antibody-negative.

8.2 Adverse Event Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial and is done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Data on adverse events will be collected from the time of the initial study treatment (day 0) through 30 days after the last dose of natalizumab. Any serious adverse event that occurs more than 30 days after the last natalizumab dose and is considered related to the study treatment must also be reported. Serious Adverse Events (SAEs) will continue to be followed until:

- Resolution or the symptoms or signs that constitute the serious adverse event return to baseline;
- There is satisfactory explanation other than the study treatment for the changes observed; or
- Death.

The investigator is responsible for the detection, documentation, grading and assignment of attribution of events meeting the criteria and definition of an AE or SAE. The definitions of AEs and SAEs are given below. It is the responsibility of the principal investigator to ensure that all staff involved in the trial is familiar with the content of this section.

Any medical condition or laboratory abnormality with an onset date before initial study treatment administration is considered to be pre-existing in nature. Any known pre-existing conditions that are ongoing at time of study entry should be considered medical history.

All events meeting the criteria and definition of an AE (CTCAE grade 3 or higher) or SAE, as defined in Section 8.3, occurring from the initial study treatment administration through 30 days following the last dose of the study treatment or study intervention must be recorded as an adverse event in the patient's source documents and on the CRF regardless of frequency, or assessed relationship to the study treatment.

In addition to new events, any increase in the frequency or severity (i.e., toxicity grade) of a pre-existing condition that occurs after the patient begins study treatment is also considered an adverse event and must be reported if the severity is CTCAE grade 3 or higher.

8.3 Definitions

8.3.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

- Diagnostic and therapeutic non-invasive and invasive (i.e., surgical) procedures will not be reported as adverse events. However, the medical condition for which the procedure was performed must be reported if it meets the definition of an adverse event unless it is a pre-existing (prior to protocol treatment) condition.

- Event reporting for GVHD treatment protocols can be complicated and confusing for investigators, data managers, and regulatory oversight bodies because patients typically develop numerous complications as part of the typical treatment course not related to study therapy. Furthermore, transplant-related complications often occur both simultaneously and in series, as one

complication leads to a series of downstream events. Therefore, a well-conceived event reporting plan separates background transplant and GVHD noise as might be seen with any transplant where GVHD develops from study related events that are relevant to patient safety. On this study, we will not report CTCAE grade 1 and 2 adverse events (which make up the majority of events) unless the investigator determines the event should be reported to protect subject safety.

- Symptoms of the original or targeted disease are not to be considered adverse events for this study. The following symptoms are indicative of the underlying disease, GVHD, and will not be reported as adverse events (unless the event is considered serious or deemed by the investigator to be a natalizumab infusion reaction):

    - Nausea, vomiting, diarrhea, anorexia
    - Skin rash
    - Fever

- Abnormal laboratory values or test results constitute adverse events if they induce clinical signs or symptoms or require therapy. They are to be captured under the signs, symptoms or diagnoses associated with them.

8.3.2 **Serious Adverse Event**

An adverse event is considered “serious” if, in the view of the investigator, it results in any of the following outcomes:

- Death
  If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

- A life-threatening adverse event
  An adverse event is considered ‘life-threatening’ if, in the view of either the investigator, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization for $\geq$ 24 hours.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- A congenital anomaly/birth defect

- Important medical event
  Any event 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 and may require medical or surgical intervention to prevent one of the outcomes listed in this definition of “Serious Adverse Event”. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that do not result in inpatient hospitalization or the development of drug dependency or drug abuse.

Previously planned (prior to signing the informed consent form) surgeries should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study. Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (for example, for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. However, if the preexisting condition worsened during the course of the study, it should be reported as an SAE.

8.3.3 Expected Adverse Events

An adverse event (AE) is considered "expected" if:

- For approved and marketed drugs or devices, those adverse events are described in the approved Package Insert (Label).
- For investigational new drugs or devices, those adverse events are described in the FDA Investigator's Brochure.
- In clinical research studies, information on expected adverse events is also summarized in the protocol and in the consent document. See section 9.1 for the list of expected adverse events related to the drug under study.

8.3.4 Unexpected Adverse Event

An adverse event (AE) is considered "unexpected" if it is not described in the Package Insert, Investigator's Brochure, in published medical literature, in the protocol, or in the informed consent document.

8.4 Adverse Event Characteristics

8.4.1 CTCAE Term

(AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be down loaded from the CTEP web site. (http://ctep.cancer.gov)

8.4.2 Attribution of the AE

The investigator or co-investigator is responsible for assignment of attribution.
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.

8.5 Serious Adverse Event Reporting Guidelines

The Sponsor Investigator will report SAEs to regulatory bodies and to the participating sites in the following manner:

Sponsor SAE Reporting

<table>
<tr><th rowspan="2">Event occurring <u>within 30 Days</u> post last Natalizumab infusion</th><th colspan="2">Report to:</th><th rowspan="2">Event occurring <u>after 30 Days</u> post last Natalizumab infusion</th><th colspan="2">Report to:</th></tr>
<tr><th>FDA / IRB</th><th>Consortium</th><th>FDA / IRB</th><th>Consortium</th></tr>
<tr><td rowspan="2">All SAEs;
✓ Expected or Unexpected
✓ Unrelated, Unlikely, Possible, Probable, or Definite</td><td rowspan="2">5 Days from knowledge</td><td rowspan="2">Monthly</td><td>SAEs –
✓ Expected or Unexpected
✓ Possible, Probable, or Definite</td><td>5 Days from knowledge</td><td>Monthly</td></tr>
<tr><td>SAEs –
✓ Expected
✓ Unrelated, Unlikely, Possible, Probable, or Definite</td><td>Annually</td><td>Monthly</td></tr>
</table>

8.5.2 The Sponsor Investigator must be notified within 5 business day of study team's knowledge of any event meeting the criteria and definition of a serious adverse event, regardless of attribution, occurring during the study or within 30 days of the last administration of the natalizumab. PML must be reported within 24 hours to the sponsor investigator.

8.5.3 The investigator must report all events meeting the criteria and definition of a serious adverse event that are <u>unexpected</u> and <u>possibly related</u> (definite, probable or possible to study treatment administration) to the local IRB as per local IRB policy.

8.5.4 All Serious Adverse Events whether <u>expected</u> or <u>unexpected</u> and <u>possibly related</u> (definite, probable or possible) to study treatment administration will be reported using the Serious Adverse Event form within 5 days of first awareness of the event to the MAGIC Data Coordinating Center. A copy of the form should be sent to the MAGIC Coordinator via email to **magic@mssm.edu**.

The MAGIC Data Coordinating Center will disseminate information regarding serious adverse events to the participating sites within 5 days of review of the information by Drs. Ferrara or Levine only in the case that the event(s) is believed to be related (i.e. probably, or definitely) to the study medication. All other Serious Adverse Events will be discussed on monthly webinars held with all participating centers (see section 12).

The Sponsor Investigator will be responsible for reporting of events to the FDA and supporters, as appropriate and defined in the regulations under 21 CFR 312.32.

8.6 Routine Reporting

All other adverse events that are not SAEs as defined in section 8.3—such as those that are expected, or are unlikely or definitely not related to the study participation—are to be reported annually as part of regular data submission and as per the local IRB policies.

8.7 Reporting of Unanticipated Problems

Upon becoming aware of any incident, experience, or outcome (not related to an adverse event) that may represent an unanticipated problem involving risks to subjects or others, the investigator should assess whether the incident, experience, or outcome represents an unanticipated problem. The incident, experience or outcomes are considered unanticipated if it meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency);
2. Related or possibly related to participation in the research; and
3. Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the investigator determines that the incident, experience, or outcome represents an unanticipated problem, the investigator must report it to the MAGIC Data Coordinating Center within 5 days of first awareness of the events, and to the local IRB as per local IRB policy.

8.8 Stopping Rules

Natalizumab has not previously been studied as a treatment of acute GVHD, therefore this study will use strict stopping rules to mitigate risks to study subjects. These stopping rules were designed keeping in mind the high rates of treatment failure with standard treatment and 6 month non-relapse mortality (32% in AA2 and AA3 patients combined) experienced by the high risk GVHD population under study.

The following risks were considered in the development of the stopping rules. Progressive multifocal leukoencephalopathy is a serious, potentially lethal complication and is listed as a black box warning on the prescribing information brochure for natalizumab. Other serious infections, including herpes encephalitis and meningitis, have been reported with natalizumab. Patients with GVHD and on immunosuppression are already at increased risk for development of serious infections. The degree to which natalizumab may further increase that risk is unknown. Therefore we will follow the below stopping rules designed to minimize any increased risk by adding natalizumab to GVHD standard therapy.

STOPPING RULE #1: PML

The clinical study will be stopped if one case of PML develops. Notification from the site to the Sponsor Investigator must occur within 24 hours of event. A webinar will be scheduled within 3 days of Sponsor knowledge for the participating sites to discuss the case. In the event of one case, the informed consent will be revised to inform potential subjects that a case of PML was observed on this clinical trial. The trial will only be re-opened to accrual with approval by the Tisch Cancer Institute (TCI) DSMC, the external advisory board, and the FDA after review of all safety data available and after any additional safety measures are implemented. If a second case of PML develops, the study will be closed to accrual and no further doses of natalizumab will be administered to any patient currently undergoing treatment.

STOPPING RULE #2: EXCESS NON-RELAPSE MORTALITY

Although we do not expect natalizumab to increase the incidence of fatal infections or contribute to increased mortality, for safety reasons we will continuously monitor the incidence of non-relapse mortality throughout the study. If natalizumab adversely affects the risk of non-relapse mortality we expect that effect to manifest itself during the early follow-up period after administration of the drug.

We will continuously monitor non-relapse mortality occurring within 100 days of study administration. If the incidence of NRM occurring within 100 days of study administration is greater than the associated boundary value b_k listed in the table below, among the k patients enrolled in the trial, then accrual will be halted for safety considerations. We

estimated the stopping boundaries based on the crude non-relapse mortality rates of the updated historical controls (13% for AA2 and 41% for AA3).

Maximum # of AA2 Patients, k	1-15	16-30	31-42
Boundary, b_k	2	3	4

Maximum # of AA3 Patients, k	20	21- 25	26-30	31- 35	36-40	41- 42
Boundary, b_k	9	10	12	14	15	16

Specifically, given different risks for NRM by Ann Arbor score, we will monitor NRM for patients with Ann Arbor 2 and Ann Arbor 3 GVHD separately. For patients with Ann Arbor 2 GVHD, if more than 2 out of the first 15 patients, 3 out of the first 30, or 4 out of the first 42 patients, experience NRM within 100 days of study drug administration, the trial will be halted for safety reasons. We have already enrolled 32 patients with Ann Arbor 3 GVHD out of a planned 42 patients and more than 10 patients completed their follow-up for NRM. For patients with Ann Arbor 3 GVHD, if more than 9 of the first 20 patients, 10 out of the first 25 patients, 12 out of the first 30 patients,14 of the first 35 patients, 15 out of the first 40 patients or 16 of the first 42 patients experience NRM within 100 days of study drug administration, the trial will be halted for safety considerations

The operating characteristics of this stopping rule are as follows:

AA2 patients	**True Toxicity Rate**			
	5%	10%	13%	15%
Probability of Early Stopping	0.09	0.44	0.68	0.78

AA3 patients	**True Toxicity Rate**			
	27%	30%	41%	45%
Probability of Early Stopping	0.08	0.16	0.68	0.82

Using these boundaries, for the patients with Ann Arbor 2 GVHD, if the true toxicity rate is 5%, 10%, 13% or 15%, the probability of stopping the trial early is 0.09, 0.44, 0.68, and 0.78 respectively. For the patients with Ann Arbor 3 GVHD, if the true toxicity rate is 27%, 30%, 41% or 45%, the probability of stopping the trial early is 0.08, 0.16, 0.68, and 0.82 respectively. This stopping rule was computed using the toxbdry function in R and calculations of this function as published [Jennison and Turnbull 2000, Ivanova 2005].

An enrollment rule to prevent an excessive number of toxicities as described in Song and Ivanova will be used to inform us of the number of additional patients we can recruit when the current patient has not yet completed follow-up[Song and Ivanova 2015]. Formally, the trial can enroll m new patients such that $r + x + m \leq b_{n+m} + M$, $r + x + m - 1 < b_{n+m-1} + M$, and $n + m \leq K$ where r is defined as the number of patients that have not completed follow-up and are still being followed for toxicity; x is the number of patients that have experienced toxicity n is the total number of patients enrolled to date and M is the fixed design parameter. To be able to complete the study in a timely manner, we allow M to be 26.

9.0 DRUG INFORMATION

9.1 NATALIZUMAB

- Other names for the drug: TYSABRI

- Description: Natalizumab is supplied as 300 mg natalizumab in 15 mL (20 mg/mL) in a sterile, single-use vial free of preservatives.

- Classification - type of agent: Immunomodulator

- Mode of action: Natalizumab binds to the α4-subunit of α4β1 and α4β7 integrins expressed on the surface of all leukocytes except neutrophils, and inhibits the α4-mediated adhesion of leukocytes to their counter-receptor(s).

- Pharmacokinetics: In patients with CD, following the repeat intravenous administration of a 300 mg dose of natalizumab, the mean ± SD maximum observed serum concentration was 101 ± 34 mcg/mL. The mean ± SD average steady-state trough concentration was 10 ± 9 mcg/mL. The estimated time to steady-state was approximately 16 to 24 weeks after every four weeks of dosing. The mean ± SD half-life, volume of distribution, and clearance of natalizumab were 10 ± 7 days, 5.2 ± 2.8 L, and 22 ± 22 mL/hour, respectively. The effects of total body weight, age, gender, race, selected hematology and serum chemistry measures, co-administered medications (infliximab, immunosuppressants, or steroids), and the presence of anti-natalizumab antibodies were investigated in a population pharmacokinetic analysis (n=1156). The presence of anti-natalizumab antibodies was observed to increase natalizumab clearance. Pharmacokinetics of natalizumab in patients with renal or hepatic insufficiency have not been studied.

- Side effects: See section 8.1 of this document for complete listing of adverse effects.

- Drug Interactions: Natalizumab interacts with immunosuppressant agents and increases the risk of infection.

- Storage and stability: TYSABRI single-use vials must be refrigerated between 2 to 8°C (36° to 46°F). Do not use beyond the expiration date stamped on the carton and vial label. DO NOT SHAKE OR FREEZE. Protect from light. Natalizumab must be stored in a secure, limited access area.

- Preparation and Dispensing:

    1. Use aseptic technique when preparing natalizumab solution for intravenous infusion. Each vial is intended for single use only.
    2. Natalizumab is a colorless, clear to slightly opalescent concentrate. Inspect the natalizumab vial for particulate material and discoloration prior to dilution and administration. If visible particulates are observed and/or the liquid in the vial is discolored, the vial must not be used.
    3. To prepare the solution, withdraw 15 mL of natalizumab concentrate from the vial using a sterile needle and syringe. Inject the concentrate into 100 mL 0.9% Sodium Chloride Injection, USP. No other IV diluents may be used to prepare the natalizumab solution.
    4. Gently invert the natalizumab solution to mix completely. Do not shake. Inspect the solution visually for particulate material prior to administration.
    5. The final dosage solution has a concentration of 2.6 mg/mL.
    6. Following dilution, infuse natalizumab solution immediately, or refrigerate solution at 2 to 8°C, and use within 8 hours. If stored at 2 to 8°C, allow the solution to warm to room temperature prior to infusion. DO NOT FREEZE.

- Administration:
  1. Infuse natalizumab 300 mg in 100 mL 0.9% Sodium Chloride Injection, USP, over approximately one hour (infusion rate approximately 5 mg per minute). Do not administer natalizumab as an intravenous push or bolus injection. After the infusion is complete, flush with 0.9% Sodium Chloride Injection, USP.
  2. Observe patients during the infusion and for one hour after the infusion is complete. Promptly discontinue the infusion upon the first observation of any signs or symptoms consistent with a hypersensitivity-type reaction
  3. Use of filtration devices during administration has not been evaluated. Other medications should not be injected into infusion set side ports or mixed with natalizumab.

- Availability: Provided by Biogen Idec, Inc.

- Return and Retention of Study Drug:

  Any remaining/expired/used is to be destroyed on site according to the institution standard operating procedure for drug destruction and documented on the drug accountability logs.

- Drug Accountability:
  The principal investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the investigational drug, natalizumab. The drug accountability records will capture drug receipt, drug dispensing, drug return and final disposition.

9.2 CORTICOSTEROIDS (Prednisone and Methylprednisolone)

Glucocorticoids produce widespread and diverse physiologic effects on carbohydrate, protein, and lipid metabolism, electrolyte and water balance, functions of the cardiovascular system, kidney, skeletal muscle, and the nervous systems. Glucocorticoids reduce the concentration of thymus- dependent lymphocytes (T-lymphocytes), monocytes, and eosinophils. Glucocorticoids selectively bind to the cortisol receptors on human lymphoid cells. They also decrease binding of immunoglobulin to cell surface receptors and inhibit the synthesis and/or release of interleukins, thereby decreasing T-lymphocyte blastogenesis and reducing expansion of the primary immune response. The specific cellular mechanisms that act to halt DNA synthesis are thought to be related to inhibition of glucose transport or phosphorylation, retardation of mitosis, and inhibition of protein synthesis.

Toxicities/Side Effects of Corticosteroids

	Common (>20%)	**Occasional** 5-20%	**Rare** <5%
Immediate: Within 1-2 days of receiving drug	Insomnia, Hyperphagia	Gastritis	Hyperuricemia
Prompt: Within 2-3 weeks,	Immunosuppression, personality changes (mood swings, euphoria, anxiety, depression), pituitary-adrenal axis suppression, acne	Hyperglycemia, facial erythema, poor wound healing, infections (bacterial, fungal, parasitic, viral), edema	Pancreatitis (L), increased intraocular pressure (L), hypertension, psychosis, vertigo; headache

Delayed: Any time later during therapy	Cushing's syndrome (moon facies, truncal obesity)	Striae and thinning of the skin, easy bruising, muscle weakness, osteopenia	Spontaneous fractures (L), growth suppression, Peptic ulcer and GI bleeding, pseudotumor cerebri (increased intracranial pressure with papilledema, headache), aseptic necrosis of the femoral and humeral heads (L)
Late: Any time after completion of treatment		Cataracts	

(L) Toxicity may also occur later.

Formulation, Stability, Guidelines for administration: Numerous preparations of commercially available corticosteroids are available. This study permits any commercially available form of prednisone or methylprednisolone to be used. The package insert should be referenced for further information.

10.0 CORRELATIVES/SPECIAL STUDIES

The goal of the planned laboratory correlative studies is to improve our understanding of the biological processes that drive GVHD and its clinical outcomes.

10.1 Sample Collection Guidelines

The correlative sample collection schedule is detailed in section 6.4 above. Serum will be collected in no additive, silicone coated glass or plastic tubes containing no anticoagulant (red or gold top tube). Samples will be processed at the participating center and batch shipped to Ferrara Laboratory quarterly for storage. Sample processing details are found in Appendix A. Instructions for quarterly batch shipping are found in the MAGIC Sample Collection and Storage Manual.

10.2 Assay Methodology

See Appendix A.

10.3 Specimen Banking

Patient samples collected for this study will be retained at the Icahn School of Medicine at Mount Sinai. Specimens will be stored indefinitely or until they are used up. If future use is denied or withdrawn by the patient, best efforts will be made to stop any additional studies and to destroy the specimens.

When consent is provided, we will analyze small and large bowel biopsy samples obtained from these patients using immunohistochemical staining for cells and proteins with known associations with GI GVHD. Sites will be asked to submit formalin fixed paraffin embedded GI biopsies that were obtained for standard of care purposes, provided that this tissue is no longer needed for further clinical use. These biopsies will be requested and batch shipped after the tissue is no longer needed for further clinical use (minimum six months from biopsy acquisition).

Drs. Ferrara and Levine will be responsible for reviewing and approving requests for clinical specimen from potential research collaborators from within the MAGIC consortium.

The specimens, DNA, and their derivatives may have significant therapeutic or commercial value. The Informed Consent form contains this information and informs the subject that

there is the potential for financial gain by the Icahn School of Medicine at Mount Sinai, the investigator or a collaborating researcher or entity.

The following information obtained from the subject's medical record may be provided to research collaborators when specimens are made available:

- Diagnosis
- Collection time in relation to study treatment
- Clinical outcome – if available
- Demographic data

11.0 STATISTICAL CONSIDERATIONS

11.1 Study Design/Study Endpoints

This is a phase II open label multicenter clinical trial. The primary study endpoint is day 28 CR rate was chosen due to its correlation with long term survival in patients with GVHD.

Because we expect improvements in day 28 CR to translate into better long-term outcomes, secondary endpoints include 1 year NRM and 1 year survival. Additional secondary endpoints related to efficacy will include the day 28 overall response rate (CR + PR), the proportion of patients who develop treatment-refractory GVHD (defined as not CR/PR by day 28 of treatment or who receive additional immunosuppression prior to day 28), time to discontinuation of steroid therapy, number of lines of GVHD therapy, and cumulative incidence of chronic GVHD. Secondary endpoints related to safety include 6 month and 1 year relapse rates and incidence of serious infections by 6 months.

Because natalizumab has not been studied as a treatment for acute GVHD, we have developed stopping rules to maximize patient safety protection. A single case of PML will cause suspension of study enrollment until (1) a safety review has been conducted and (2) the informed consent is modified to include that a case of PML developed on this study. A second case of PML will cause the study to terminate.

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Because we are changing the design of the study and enrolling another cohort in addition to the patients currently enrolled, the planned interim analysis will not be applicable. We therefore calculated the conditional power, i.e., the probability that the study results will be statistically significant given the data observed thus far. We have so far enrolled 32 patients with Ann Arbor 3 GVHD and 28 have sufficient follow-up for the primary endpoint assessment (28 day response). We observed 12 CRs, 6 PRs and 10 NRs. Given these data, the conditional power of the study based on the previous design (90 patients with Ann Arbor 3 GVHD to be enrolled and 37 CRs to be observed in order to reject the null hypothesis) is 0.81 using an exact binomial test. Therefore, our conditional power analysis indicates that the new study design is sufficiently powered.

The historical controls for this study consists of 256 patients who met the criteria for Ann Arbor score 2 (n=142) or 3 (n=114) GVHD and who were either (1) diagnosed at the University of Michigan or the University of Regensburg or (2) participated in the MAGIC consortium observational study between 2013-2017. Approximately 75% of these patients were diagnosed and treated since 2010, thus the historical control population is comprised primarily of fairly recently transplanted patients.

11.2 Sample Size and Accrual

We revised the study design to allow us to include patients with Ann Arbor 2 GVHD without losing the data already generated for patients with Ann Arbor 3 GVHD. We have fixed the

numbers of patients with Ann Arbor 2 and Ann Arbor 3 GVHD to provide a final study ratio of 1:1. This allows us to weight the two GVHD scores equally for the calculation of the predicted magnitude of improvement and the accompanying power and sample size. eWe maintained a conservative estimate of 15% for the magnitude of improvement in weighted day 28 CR (from 42.5% to 57.5%), similar to the 14% effect size seen within 4 weeks (i.e. after one dose of natalizumab) in active Crohn's disease. We chose our sample size based on both feasibility and power requirements. A sample size of 84 (42 AA2 and 42 AA3 patients) will provide 80% power to detect a 15% difference in CR rates, with a Type I error rate of 0.05. Finally, we have re-estimated the historical day 28 CR rate using the most up-to-date data available (256 patients transplanted up until 7/2017).

11.3 Data Analyses Plans

The primary endpoint is day 28 CR rate. Death, lack of CR at day 28, or initiation of additional immunosuppressive therapy will be considered failures for this endpoint. The day 28 CR rate in the study patients will be compared to the historical control rate of 42.5%.

Secondary outcomes such as the overall response rate (CR+PR), the incidence of steroid refractory GVHD, the incidence of severe GI GVHD (stage 3 or 4), non-relapse mortality, relapse rates, overall survival, will be analyzed using descriptive statistics (cumulative incidence curves, Kaplan-Meier, etc.) and compared to historical controls. Outcomes will be analyzed for all patients and separately according to Ann Arbor GVHD score.

12.0 DATA AND SAFETY MONITORING

The safety of subjects is paramount and supersedes all other concerns. This study will employ several layers of oversight to ensure that patient safety is protected. These layers (described in more detail further below) are:

1. The local Data and Safety Monitoring Committee (DSMC) at each site which will be responsible for monthly reviews of patient data at each site
2. The Protocol Data and Safety Monitoring Committee (DSMC), composed of the individual site PI's which will review all facets of study conduct at all sites on monthly webinars
3. The External Advisory Board (see below for membership), which is composed of national experts in BMT and GVHD who will be responsible to (1) review and advise on the interim analysis when performed and (2) recommend whether the study can reopen whenever a stopping rule is triggered (see section 8.8). A meeting of the EAB will be scheduled in a timely fashion as needed in order to accomplish these review functions and subsequently these recommendations will be provided to the TCI DSMC and to each IRB.
4. The Tisch Cancer Institute Data and Safety Monitoring Committee (TCI DSMC) of the Mount Sinai Health System is the DSMB of record for this study. The DSMB will be compliant with the NIH approved DSMP Charter. This committee will be responsible for monitoring the safety and data integrity of the trial. It is a DSMB entirely composed of members with no connection to this clinical trial. The DSMB will have final authority on interpreting the interim analysis and whether the study can reopen whenever a stopping is triggered.
5. The IRBs at each participating site and the Mount Sinai Health System.
6. The FDA which will receive safety reporting as required by IND regulations (21 CFR 312.3).

Thus, the investigators conducting the trial will review the trial conduct at their own site as well as each other's sites on a monthly basis, thereby providing a high level of attention to any safety concerns that may develop. Additionally, authority is vested in three independent bodies (the External Advisory Board, the TCI DSMC, and the IRBs) to monitor, and if safety concerns warrant, close the clinical trial. Lastly, this trial will be conducted in a manner compliant with the Federal regulations pertaining to clinical trials conducted under an IND.

Local DSMC: Each participating site is required to have its own Data and Safety Monitoring Committee (DSMC) for the study. This committee will be composed of the local site principal investigator, site co-investigator(s), site data manager or study coordinator and other members of the study staff involved in the conduct of the trial.

During the committee's monthly meeting, the principal investigator will discuss matters related to:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data
- Retention of study participants

These meetings are to be documented by the site data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the site principal investigator. Each site is required to submit the completed DSMR to the MAGIC Coordinator at the Icahn School of Medicine at Mount Sinai on a monthly basis together with other pertinent documents.

Similarly, protocol deviations are to be documented using the Notice of Protocol Deviation Form and requires the signatures of both the site's data manager or study coordinator and the site principal investigator. These reports are to be sent to the MAGIC Coordinator at the Icahn School of Medicine at Mount Sinai within 7 calendar days of awareness of the event and on a monthly basis with the Protocol Specific Data and Safety Monitoring Report.

Protocol DSMB: The centers participating in this study are collaborating centers in MAGIC (Mount Sinai Acute GVHD International Consortium) which has been holding monthly webinars to review GVHD clinical data from each of these sites since January 2013. The local site principal investigator, data manager, and study coordinator participates in these monthly webinars. While this study is open, in addition to the local DSMC meetings, the monthly MAGIC webinars will provide an additional forum for discussion of the above matters, which will keep all participating centers current on all data and safety issues that arise, thereby providing an additional layer of safety oversight.

The MAGIC Data Coordinating Center is responsible for collating all the Data and Safety Monitoring Reports from all the participating sites, and providing the information to the TCI Data Safety Monitoring Committee.

External Advisory Board: The scientific advisory board is composed of national experts in BMT and GVHD. The members are Chair -; Joseph Antin, MD, Chief, Adult Oncology Hematopoietic Stem Cell Transplantation Program, Dana-Farber Cancer Institute, Nelson Chao, MD, Chief, Division of Hematologic Malignancies and Cellular Therapy/BMT, Duke Cancer Institute and Steven Burakoff, MD, Dean for Cancer Innovation at the Icahn School of Medicine at Mount Sinai.

TCI DSMC: The TCI DSMC serves as the data and safety monitoring board (DSMB) for investigator initiated studies conducted under the auspices of the Tisch Cancer Institute (TCI) at the Mount Sinai Health System. It is compliant with the National Institutes of Health and National Cancer Institute charter for DSMBs. This board will be responsible for monitoring the safety and data integrity of the trial. It is a DSMB entirely composed of members with no connection to this clinical trial.

12.1 Multisite Clinical Monitoring Procedures

This clinical study will be coordinated by the MAGIC Data Coordinating Center (DCC) of the Icahn School of Medicine at Mount Sinai. As such it will be conducted in accordance

with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements.

This study will be monitored by a representative of the MAGIC Data Coordinating Center. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a participating site will undergo site initiation meeting to be conducted by the DCC. This will be done as an actual site visit, teleconference, videoconference, or web-based meeting after the site has been given access to the study database and assembled a study reference binder. The site's principal investigator and his study staff should make every effort in attending the site initiation meeting. Study-related questions or issues identified during the site initiation meeting will be followed-up by the appropriate DCC personnel until they have been answered and resolved.

Monitoring of this study will include both 'Centralized Monitoring', the review of source documents at the DCC and 'On-site Monitoring', an actual site visit. The first 'Centralized' visit will occur after the first subject enrolled completes treatment (i.e., two doses of natalizumab). The study site will send the de-identified source documents to the DCC for monitoring. 'Centralized' monitoring may be requested by the DCC if an amendment requires changes to the protocol procedures. The site will send in pertinent de-identified source documents, as defined by the DCC for monitoring.

The first annual 'On-site' monitoring visit should occur after the first five study participants are enrolled or twelve months after a study opens, whichever occurs first. The annual visit may be conducted as a 'Centralized' visit if less than three subjects have enrolled at the study site. The type of visit is at the discretion of the DCC. At a minimum, a routine monitoring visit will be done at least once a year, or once during the course of the study if the study duration is less than 12 months. The purpose of these visits is to verify:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Proper storage, dispensing and inventory of study medication
- Compliance with regulations

During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the DCC representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. While most patient cases will be selected from patients accrued since the previous monitoring visit, any patient case has the potential for review. At least one or more unannounced cases will be reviewed, if the total accruals warrant selection of unannounced cases.

The MAGIC Data Coordinating Center (DCC) expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner. For review of study-related documents at the DCC, the site will be required to ship or fax documents to be reviewed.

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site Investigator is aware of his/her ongoing responsibilities. In general, a site close-out is conducted during a site visit; however, site close-out can occur without a site visit if all of the following apply:

- No patient has signed the Informed Consent Form and has enrolled into the study
- Investigational agent has not been dispensed

- All investigational agent and materials have been returned as defined for the study or destroyed and accounted for properly.

13.0 QUALITY ASSURANCE AND AUDITS

The Data Safety Monitoring Board can request a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues. A "for cause" audit would be conducted by the Project Manager of the MAGIC Data Coordinating Center.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the MAGIC Data Coordinating Center that such a request has been made.

14.0 REFERENCES

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15.0 APPENDICES

Appendix A: Ann Arbor Scoring Manual
Appendix B: BMT CTN Infection Severity Grading guide