Study Protocol and Statistical Analysis Plan

Title: Phase II Trial of Natalizumab (Tysabri®) plus Steroids for Initial Therapy of Acute Graft Versus Host Disease (aGVHD) of the Gastrointestinal Tract

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1. OBJECTIVES

1.1 Study Design

This is a phase II study of natalizumab in combination with standard of care steroids for the treatment of acute graft versus host disease (aGVHD) of the gastrointestinal (GI) tract.

1.2 Primary Objectives

- GVHD-free survival rate at day 56 after the first dose of Natalizumab is administered.

1.3 Secondary Objectives

- Proportions of complete response (CR), very good partial response (VGPR), partial response (PR), mixed response (MR), no response (NR) and progression of aGVHD at day 28 and day 56 after the first dose of natalizumab is administered.

- Proportions of complete response (CR), very good partial response (VGPR), partial response (PR), mixed response (MR), no response (NR) and progression of GI aGVHD at day 28 and day 56 after the first dose of natalizumab is administered.

- Incidence of aGVHD flares requiring therapy after initial CR or PR by day 28 after the first dose of natalizumab is administered.

- Steroid dose at day 28, day 56, day 100, day 180 and one year after the first dose of Natalizumab is administered.

- Incidence of systemic infections at day 56 and day 180 after the first dose of Natalizumab is administered.

- Overall and GVHD free survival at 100 days, 180 days and one year after the first dose of Natalizumab is administered.

2. BACKGROUND

2.1 Study Agent

2.1.1 Natalizumab

Natalizumab (Tysabri®, Biogen Inc.) is a recombinant humanized IgG4 monoclonal antibody. The human region of this product functions as IgG4, considered to be the least immune activating of the IgG immunoglobulins as it does not have high affinity to most Fc gamma receptors and does not activate complement. Natalizumab is engineered by incorporating murine complementarity-determining regions of the murine antibody that binds to the α4 chain of α4β7 integrin, onto human IgG4 (*Rudick 2004*). Natalizumab is

therefore a selective adhesion molecule inhibitor, acting directly against the α4 subunit of α4 integrins. One integrin, α4β1, is found on activated lymphocytes and helps bind lymphocytes to ligands such as vascular cell adhesion molecule-1 (VCAM-1) in areas of inflammation. Another integrin, α4β7, interacts with mucosal addressin-cell adhesion molecule and mediates homing of lymphocytes to the gastrointestinal (GI) tract.

VCAM-1 is found in high concentrations in plaques found in multiple sclerosis (MS). Natalizumab as an inhibitor of the interaction between lymphocytes and VCAM-1 was quickly identified to be effective in MS. There are 3 distinct mechanisms of action that are believed to occur in MS. Firstly, natalizumab blocks the interaction between endothelial cells and extracellular matrix proteins. Secondly, it blocks the interaction of monocytes and microglial cells to ligands of α4β1 integrin including osteopontin and VCAM-1. Lastly, the blockade of the interaction of lymphocytes and extracellular matrix proteins is believed to induce apoptosis of these cells, further suppressing the immune activity at disease sites. (*Rudick 2004*)

In the 1990s the idea that inhibition of α4β1 integrin could modulate inflammatory diseases was tested in animal models. Anti-α4β1 integrin was found to prevent experimental autoimmune encephalomyelitis, an animal model that resembles MS (*Yednock 1992*). Further animal models indicated that molecules directed against α4β1 integrin decreased MRI lesions in experimental autoimmune encephalomyelitis (*Kent 1995 – 2 articles*).

In a phase I study of 28 patients with relapsing-remitting MS (RRMS) or secondary progressive MS, natalizumab was found to be safe and effective (*Sheremata 1999*). A phase II trial was conducted comparing placebo, 3.0mg/kg and 6.0mg/kg of natalizumab in RRMS or secondary progressive MS (*Miller 2003*). Marked reductions in both relapse rates and number of new or enlarging lesions were seen with both doses when compared to placebo at 6 months after treatment. There was no significant difference in adverse events reported in the 3 treatment groups.

Two pivotal phase III studies examining the impact of natalizumab on clinical outcomes in MS have led to the approval of this medication for the treatment of RRMS by the Food and Drug Administration. In 2006, Rudick et al. reported the results of a large randomized study comparing interferon beta-1a with and without the addition of natalizumab (*Rudick 2006*). Combination therapy proved to decrease the annual rate of relapse over a two-year period (0.34 vs. 0.75, $P<0.001$). The second randomized trial was the AFFIRM study by Polman et al., which randomized patients to receive natalizumab versus placebo for RRMS (*Polman 2006*). Again a significant reduction in rate of relapse at one year (by 83% with natalizumab) was observed as well as a significant decrease in the number of new or enlarging T2 hyperdense lesions on MRI.

α4β7 is increased in disease sites of inflammatory bowel disease, providing another potential target for natalizumab. Pilot studies have shown that a single dose of natalizumab could induce remission and significantly improve disease activity in Crohn's disease and ulcerative colitis (*Gordon 2001 and 2002*). Large randomized data from

Europe have further shown a benefit to natalizumab at a dose of 3 mg/kg when compared to placebo (*Ghosh 2003*) in Crohn's disease. In this study, at 6 weeks, 71% of patients who received 2 infusions of 3mg/kg had response as compared to 38% who received placebo alone. This significant benefit with natalizumab was sustained at 12 weeks after treatment. Although the ENACT clinical trials demonstrated no significant early benefit to natalizumab in patients with Crohn's disease, it did provide some benefit to maintaining response in patients treated with ongoing monthly infusions if they had an initial response (*Sandborn 2005*).

2.1.2 Treatment-related Effects of Natalizumab

Natalizumab is generally safe, causing only occasional hypersensitivity reactions. (*Polman 2006*) The five level dose escalation study by Sheramata *et al*. showed very minimal adverse events, almost all of which were reported at the 3.0mg/kg dose. Of the six patients receiving the 3.0mg/kg dose, one experienced headache, two experienced nausea and one had shakiness. One patient receiving 1.0mg/kg reported urticaria. Serum natalizumab concentrations rapidly decreased at doses of 0.1 mg/kg and 0.3mg/kg, but were detectable for one week when 0.3mg/kg was administered. In contrast, patients receiving 1.0 and 3.0 mg/kg had detectable concentrations for 3 to 8 weeks. Of note anti-idiotypic antibodies were identified in 3 patients who received the 3.0mg/kg dose and persisted for 8 weeks after drug administration.

The most concerning toxicity observed with natalizumab remains progressive multifocal leukoencephalopathy (PML), an opportunistic brain infection caused by JC virus. After the incidence of 0.3% was reported by the SENTINEL trial (*Rudick 2006),* natalizumab was removed from the market voluntarily in 2005. At that time, a large retrospective review of patients who had received natalizumab was embarked upon to determine the overall incidence of PML in a larger cohort of patients (*Bloomgren 2012*). Two hundred and twelve cases of PML were identified amongst 99,571 patients treated with natalizumab (2.1 cases per 1000 patients). The lowest dose of natalizumab that has been associated with PML is 8 doses, indicating that long-term administration is the major risk attributable to developing PML. Other risk factors associated with the development of PML included positive anti-JC virus antibodies prior to treatment and the use of immunosuppressants prior to natalizumab administration.

2.2 Study Disease – Acute GVHD

Graft versus host disease (GVHD) is caused by allorecognition of host antigens by donor lymphocytes and is still a major cause of morbidity and mortality in patients receiving allogeneic stem cell transplantation (*Ferrara 1991*). The condition occurs in 30 to 50% of patients receiving HLA matched transplants from a related donor and in 50 to 70% of those receiving transplants from an unrelated donor (*Johnston 2008*). Classically, acute GVHD occurs during the first few months after transplant and usually involves the skin, liver, and intestinal tract, although other organs can also be involved (*Deeg 2006*). The affected host organs are already compromised by intense conditioning regimens prior to transplant. This injury which allows for the entry of microbial agents as well as the subsequent cytokine

activation is thought to be the initial trigger for acute GVHD. This consequently leads to the recruitment and activation of alloreactive naïve donor T cells in secondary lymphoid tissue (*Goker 2001*). Target organ damage is subsequently mediated by the direct cytotoxic actions of activated donor effector T cells, which migrate to extralymphoid target sites, as well as the actions of local cytokines.

Acute GVHD involving the gastrointestinal system usually manifests as anorexia, nausea, and voluminous diarrhea (>500ml/day) which can be hemorrhagic. These symptoms are also seen in other post-transplant complications including toxicity from conditioning, medications and infections. This can make the diagnosis of acute GVHD of the gut difficult for the clinician. Although other organs can be affected by acute GVHD, the role of the gastrointestinal system is unique in that damage to this system appears to further propagate acute GVHD. The loss of an intact gastrointestinal tract during transplant not only allows for local invasion of microbial agents, but also disrupts the normal gut flora. This subsequently leads to the production of lipopolysaccharide, a potent stimulator of TNFα, IL-1 and IL-12 (*Hill 2000*). This therefore allows for the further activation of cytokines which leads to progression of acute GVHD.

The mainstay of acute GVHD treatment remains corticosteroid therapy. The starting dose is usually 1-2mg/kg daily of prednisone or the equivalent and is continued for 1-2 weeks prior to a steroid taper. The response rate to single-agent steroid therapy when analyzed in large retrospective reviews is approximately 50% (*MacMillan 2002, Martin 1990*). Unfortunately, even with an initial response to steroids, the durability of this response remains low at 24% and 40% for matched unrelated and related donor transplantation, respectively (*Roy 1992, Weisdorf 1990*).

The suboptimal results observed historically with steroids alone as treatment of acute GVHD warrants the study of other compounds which may provide more benefit than steroids alone. Monoclonal antibody therapies directed against CD3, IL-2 and TNF-α have been used in conjunction with steroids to try to improve the response rates and length of response (*Goker 2001, Arai 2000, Bruner 2003, Tanaka 2000, Couriel 2009*). The main drawback to these treatments remains the same, the broad immunosuppression leads to both secondary infectious complications as well as disease progression. The Blood and Marrow Transplant Clinical Trials Network completed a multi-center randomized, four-arm, phase II trial designed to identify the most promising agent for combination with steroids as initial therapy for the treatment of GVHD (*Alousi 2009*). Day 28 complete response rates were 26% with etanercept, 60% with mycophenolate mofetil (MMF), 53% with denileukin diftitox, and 38% with pentostatin. Overall survival at 9 months was also highest with MMF (64%). Unfortunately in phase III study of MMF versus placebo for the treatment of acute GVHD, there was no difference in GVHD free survival at 8 weeks, incidence of infection or overall survival at 6 months. (*Logan 2013*)

Multiple prospective and retrospective studies have been done adding agents like those described above to treat acute GVHD. As reported by the consensus guidelines of the American Society of Bone Marrow Transplantation, none of these results have been robust enough to show that combination first line therapy is better than steroids alone (*Martin*

2012). For this reason, further investigation into new agents is needed to improve the response rates of acute GVHD to first line treatment. Targeting α4β7 integrin for acute GVHD is a novel approach which may be effective and yield less overall immune suppression.

2.3 α4β7 integrin and Acute GVHD

GVHD is mediated by the activation of donor lymphocytes against host antigens. This is mediated by interactions between donor T cells and antigen presenting cells (APCs). Multiple adhesion molecules, including intercellular molecule 1 (ICAM-1), lymphocyte function–associated antigen 1 (LFA-1) and vascular cell adhesion molecule 1 (VCAM-1), have been identified in the activation of T cells by APCs (*Kobayashi 2000*). Once donor T lymphocytes interact with APCs, they differentiate into effector and memory T cells that target various organs implicated in acute GVHD. It is now recognized that the development of these cytotoxic T lymphocytes occurs in secondary lymphoid tissue, most notably in gut-associated secondary lymphoid tissue (GALT) which includes the Peyer's patches of the small intestine (*Murai 2003*), mesenteric lymph nodes and the appendix. This further explains why the gastrointestinal tract acts not only as a target site but a vital amplifier of GVHD.

Once activated in GALT, T cells recirculate in the bloodstream and home to specific organs based on specific adhesion molecules expressed on the T cell. Molecules expressed on T cells that lead to lymphocyte trafficking back to the gastrointestinal tract include L-selectin (*Dutt 2005*), MAdCAM-1 (*Ueha 2007*) and LPAM (α4ß7 integrin) (*Petrovic 2004*). T cells that home to the intestine have been shown in mouse models to induce crypt loss, loss of mucin-containing goblet cells within the crypts, increased apoptosis of epithelial cells at the crypt bases, and a severe infiltrate of inflammatory cells between the crypt walls and in the lamina propria. (*Dutt 2005*) This results in loss of the normal architecture of the intestine, which leads to severe symptoms of gastrointestinal GVHD, including prolonged diarrhea and gut dysfunction. These adhesion molecules represent a new target for both treatment and monitoring of acute GVHD.

We have previously analyzed by flow cytometry the expression of α4ß7 integrin on lymphocytes from 59 hematopoietic stem cell transplant recipients and found α4β7 integrin to be significantly up-regulated on both naïve and memory T cell subsets in patients who subsequently developed intestinal aGVHD compared with those who developed primarily cutaneous aGVHD or did not develop aGVHD (*Chen 2009 and 2013*). These preliminary studies demonstrate that α4ß7 integrin can act as a potential target to decrease GVHD of the gastrointestinal tract while maintaining the beneficial graft versus tumour effect.

2.4 Rationale for α4ß7 Integrin Inhibition in Gastrointestinal Acute GVHD

Based on the preliminary data provided above that demonstrates the expression of α4β7 integrin to be associated with gastrointestinal GVHD, it is reasonable to test natalizumab for

the treatment of patients with gastrointestinal GVHD. As indicated above, once T cells have migrated to the gut, they can cause severe damage to the crypt architecture, which can be difficult to regenerate once damage has occurred. For this reason, most treatments have been unsuccessful at treating gut GVHD as the initiation of treatment is often after this damage has occurred. In this study, the initiation of natalizumab at the onset of symptoms, in addition to steroids, will provide the best chance of preventing crypt necrosis by shutting down the homing of T cells to the gut as early as possible.

It is hoped that given its specific targeting of α4β7 integrin, natalizumab can be successful in treating acute GVHD, yet be less globally immunosuppressive, the main concern with current available treatments of acute GVHD. Retinoic acid, known to increase the expression of α4β7 integrin, has been shown to increase the homing of T cells to the gut, worsening acute GVHD of the gut. In this mouse model, retinoic acid did not demonstrate an upregulation of T cells to the spleen, liver, and lung. This suggests that inhibiting the α4β7 integrin by natalizumab provides a specific targeted treatment of gut GVHD, without impacting other organ systems. (*Chen 2013*)

Treatments for acute GVHD are not only immunoscuppressive, but also impair the graft versus tumor effect that is desired from donor T cells targeting malignant cells in the recipient. Ceacam1, an adhesion molecule found on donor T cells, is thought to control the proliferation, activation and trafficking of T lymphocytes in the pathogenesis of GVHD, partially mediated through α4β7 integrin. Absence of Ceacam1 leads to increased rates of gut GVHD while maintaining the desired graft versus tumor effect. (*Lu 2011*) The absence of α4β7 integrin on hematologic malignancies provides natalizumab with the unique ability to abrogate gut GVHD without effecting the graft versus tumor effect.

There have been studies as outlined above that have examined the maximal tolerated and efficacious doses of natalizumab in treating patients with autoimmune diseases including MS and Crohn's disease. In this phase II study, we plan to use much lower doses of natalizumab to determine if there is a possible treatment effect on acute gastrointestinal GVHD.

2.5 Rationale for proposed starting doses and regimen chosen

The planned treatment for patients presenting with acute GVHD with GI involvement will be steroids with natalizumab. In a phase I study of natalizumab, doses of 0.03 and 0.1mg/kg resulted in drug levels that were undetectable after completion of the infusion. (*Sheramata 1999*) The estimated half life of the 3.0mg/kg dose in this study was 4-5 days. Approximately 90% of patients will achieve over 80% saturation of α4 receptors after receiving a dose of 3.0mg/kg or higher. Receptor saturation is maintained for 3-4 weeks with the 3.0mg/kg dose.(*Rudick 2004*) Standard monthly dosing of 300mg IV has been successfully used in patients with MS and Crohn's disease.

The treatment of acute GVHD, unlike MS or Crohn's disease, is an acute process that should require short courses of treatment to demonstrate a response. An initial standard dose of 300mg IV will be used at confirmation of acute gut GVHD. If at 4 weeks, there has been a response, but it is less than a complete response, patients can be treated with a second dose of

300mg IV of natalizumab if he/she did not have a positive JC viral load or any toxicity to the first dose of natalizumab. If patients have no response or progression after one dose, they will not be given a second dose. Patients who receive a second dose of natalizumab will be evaluated for GVHD-free survival and response at day 56 after first treatment dose was administered.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Participants must have acute GVHD of the lower gastrointestinal tract as defined by the clinical impression of the treating physician, requiring systemic treatment. Minimum criteria for GI GVHD includes diarrhea of greater than 500 mL/day. Biopsy of the GI tract is required for study entry and must confirm the diagnosis of acute GVHD. Stool samples to rule out infectious causes of diarrhea, including norovirus, Clostridium difficile and other clinically indicated infections must also be negative. Eligibility includes:

3.1.1.1 Acute GVHD developing after allogeneic hematopoietic stem cell transplantation (HSCT) using bone marrow, peripheral blood stem cells, or umbilical cord blood. Recipients of non-myeloablative, reduced intensity and myeloablative transplants are eligible.

3.1.1.2 Patients who develop acute GVHD after donor lymphocyte infusion (DLI) are eligible.

3.1.1.3 There is no specified time window after day 0 of transplant as acute GVHD is only defined by clinical manifestations.

3.1.1.4 Patients must have experienced neutrophil engraftment after HSCT as defined by absolute neutrophil counts ≥ 500 / μL × 3 consecutive measurements. Absolute neutrophil count (ANC) should be calculated using the standard formula (Neut + Bands)(WBC × 10^{1}).

3.1.1.5 Platelets $\geq 10{,}000$ / μL (platelet transfusions are allowed on the same day)

3.1.1.6 The presence of hepatic, upper GI and/or cutaneous acute GVHD is permitted.

3.1.1.7 Steroids can be started up to 7 days prior to the administration of natalizumab.

3.1.2 Age ≥ 18

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

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study:

3.2.1 Patients with the entity of Acute/Chronic GVHD overlap syndromes.

3.2.2 Requiring mechanical ventilation

3.2.3 Vasopressor requirement

3.2.4 Concurrent hepatic veno-occlusive disease (VOD) based on clinical examination

3.2.5 Karnofsky performance status < 30

3.2.6 Participants may not be receiving any other study agents for at least 7 days prior to enrollment

3.2.7 Prior use of natalizumab for any reason is not allowed

3.2.8 Pregnant women are excluded from this study because of the potential teratogenic effects of natalizumab. Because natalizumab enters breast milk, and the effect is unknown in infants, breastfeeding should be discontinued if the mother is treated with natalizumab.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

There is no evidence to suggest that the outcome will differ by gender or ethnicity. Given the small size of our study there is insufficient power to detect small effects in these groups. There is no plan to exclude women, minorities, or underrepresented populations and they will be able to participate if eligibility criteria are met.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

4.3 General Guidelines for Other Participating Institutions

N/A

4.4 Registration Process for Other Participating Institutions

N/A

5. TREATMENT PLAN

Treatment will be administered on an inpatient and outpatient basis depending on the participant's clinical condition. Expected toxicities and potential risks as well as dose modifications for natalizumab are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's acute graft versus host disease (GVHD).

5.1 Pre-treatment Criteria

5.1.1 For all cycles of natalizumab:

- ANC ≥ 500 / μL (G-CSF support is allowed)
- Platelets ≥ 10,000 / μL (platelet transfusions are allowed on the same day)

5.2 Agent Administration

5.2.1 Natalizumab

- Administration - Natalizumab at a dose of 300 mg should be infused in 100 mL 0.9% Sodium Chloride Injection, USP over approximately one hour. After the infusion is complete, flush with 0.9% Sodium Chloride Injection, USP.

- Dosing and Dosing schedule – Patients enrolled in the trial will receive 300mg of natalizumab as described above in administration of the drug. For patients who have a less than complete response after the first dose of natalizumab, a second dose of 300mg will be administered in the same fashion as the first dose. If however a patient has a complete response, no response, or progression of aGVHD, experiences a serious hypersensitivity reaction, or has a grade 3 or higher toxicity related to natalizumab, then a second dose of natalizumab will not be administered at 28 days after the first dose is given.

- Drug, Tubing and Filtration – No special IV tubing or filtration is required.

- Hydrations – No hydration is required.

- Special Equipment – Standard IV infusion pump is required.

- Observation – Participants will be observed for 2 hours following completion of the infusion of natalizumab for the first infusion. Patients will be monitored for development of hypersensitivity symptoms, including urticaria, dizziness, fever, rash, rigors, pruritus, nausea, flushing, hypotension, dyspnea, and chest pain. If

there are no reactions to the first infusion, observation for one hour is required for the second infusion.

- Vital signs – Vital signs (temperature, heart rate, blood pressure, and respiratory rate) should be monitored approximately every 10 minutes during the infusion of natalizumab.

- Infusion reactions – participants will be monitored closely for possible infusion-related reactions from the administration of natalizumab. Symptoms include lightheadedness, fever, rash, sweats, sensation of throat closing, shortness of breath, throat/lip swelling, headache, and pruritis. If an infusion-related reaction is suspected, the infusion should be immediately stopped and treatment with supportive measures such as intravenous fluids, corticosteroids, and diphenhydramine should be given at the discretion of the investigator. Patients who experience a serious hypersensitivity reaction should not be re-treated with natalizumab.

5.3 Definition of Dose-Limiting Toxicity

N/A

5.4 General Concomitant Medication and Supportive Care Guidelines

There are no recommended anti-emetics or other pre-medications or IV hydration recommended with administration of natalizumab. Routine use of growth factor (G-CSF) support in the absence of documented neutropenia is not recommended. Transfusional support will be given per institutional practice. Anti-infective prophylaxis directed towards: CMV, gram positive (encapsulated bacteria), pneumocystis jirovicii, and fungal infections will be given per standard institutional practice.

5.5 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. Patients will receive a maximum of two doses of natalizumab (see section 5.2.1 on dosing and dosing schedule for eligibility requirements for a second dose of natalizumab).

5.6 Duration of Follow Up

Participants will be followed for 12 months after completion of scheduled treatment even after removal from study or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Removal from Study

Participants will be removed from study when the participant completes the follow up phase, or if lost to follow-up, death or participant withdrawal occurs (whichever comes first). The reason for study removal and the date the participant was removed must be documented in the study specific case report form (CRF).

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator (Dr. Corey Cutler telephone # 617-632-3470).

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 Adverse Event Lists for Natalizumab

Hypersensitivity Reactions
Hypersensitivity reactions are not expected to occur in this setting, but they have been observed in patients receiving natalizumab, including serious systemic reactions (ex. anaphylaxis) which occurred at an incidence of <1%. These reactions usually occur within two hours of the start of the infusion. Symptoms associated with these reactions can include urticaria, dizziness, fever, rash, rigors, pruritus, nausea, flushing, hypotension, dyspnea, and chest pain. Generally, these reactions are associated with antibodies to natalizumab. If a hypersensitivity reaction occurs, discontinue administration of natalizumab and initiate appropriate therapy. Patients who experience a serious hypersensitivity reaction should not be re-treated with natalizumab.

Immunosuppression / Infection

The immune system effects of natalizumab may increase the risk for infections. In studies of natalizumab for the treatment of MS, certain types of infections, including pneumonias and urinary tract infections (including serious cases), gastroenteritis, vaginal infections, tooth infections, tonsillitis, and herpes infections, occurred more often in natalizumab treated patients than in placebo-treated patients. One opportunistic infection, cryptosporidial gastroenteritis with a prolonged course, was observed in a patient who received natalizumab. In Crohn's disease studies, 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. Patients being treated in study will receive infection prophylaxis as per standard institutional guidelines.

Hepatotoxicity
Clinically significant liver injury has been reported in patients treated with natalizumab in the postmarketing setting. Signs of liver injury, including markedly elevated serum hepatic enzymes and elevated total bilirubin, occurred as early as six days after the first dose; signs of liver injury have also been reported for the first time after multiple doses. In some patients, liver injury recurred upon rechallenge, providing evidence that natalizumab caused the injury. If significant liver injury is observed after the first dose of natalizumab, a second dose will not be administered.

Progressive Multifocal Leukoencephalopathy (PML)
As previously outlined, the most concerning toxicity observed with natalizumab remains PML, an opportunistic brain infection caused by JC virus. In a large retrospective analysis, 212 cases of PML were identified amongst 99,571 patients treated with natalizumab (2.1 cases per 1000 patients) (*Bloomgren 2012*). The lowest dose of natalizumab that has been associated with PML is 8 doses, indicating that long-term administration is the major risk attributable to developing PML. Although PML is not expected to occur in this study, if a case is reported, the study will be immediately halted.

6.2 Dose Modifications/Delays

As most patients in this study will receive a single dose of natalizumab, dose reduction or delay are not applicable. If a patient experiences any grade 3 or higher toxicity to the first dose of natalizumab or has a positive JC viral load, he/she will not be considered eligible for a second dose.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Natalizumab

7.1.1 Description

Natalizumab is a recombinant humanized IgG4k monoclonal antibody produced in murine myeloma cells. Natalizumab contains human framework regions and the

complementarity-determining regions of a murine antibody that binds to a4-integrin. The molecular weight of natalizumab is 149 kilodaltons.

In patients with MS, following the repeat intravenous administration of a 300 mg dose of natalizumab, the mean ± SD maximum observed serum concentration was 110 ± 52 mcg/mL. Mean average steady-state trough concentrations ranged from 23 mcg/mL to 29 mcg/mL. The observed time to steady-state was approximately 24 weeks after every four weeks of dosing. The mean ± SD half-life, volume of distribution, and clearance of natalizumab were 11 ± 4 days, 5.7 ± 1.9 L, and 16 ± 5 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.

7.1.2 **Form**

Natalizumab is supplied as a sterile, colorless, and clear to slightly opalescent concentrate for intravenous (IV) infusion. Each 15 mL dose contains 300 mg natalizumab; 123 mg sodium chloride, USP; 17.0 mg sodium phosphate, monobasic, monohydrate, USP; 7.24 mg sodium phosphate, dibasic, heptahydrate, USP; 3.0 mg polysorbate 80, USP/NF, in water for injection, USP at pH 6.1.

7.1.3 **Storage and Stability**

Natalizumab single-use vials must be refrigerated between 2-8°C (36°-46°F) or stored according to instructions in the Package Inset. Do not use beyond the expiration date stamped on the carton and vial label. DO NOT SHAKE OR FREEZE. Protect from light. If not used immediately, store the Natalizumab solution for infusion at 2-8°C (36°-46°F). Natalizumab solution for infusion must be administered within 8 hours of preparation.

7.1.4 **Compatibility**

Other medications should not be injected into infusion set side ports or mixed with natalizumab. Natalizumab concentrate is supplied as 300 mg natalizumab in a sterile, single-use vial free of preservatives. Each package contains a single-use vial.

7.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the study agent in a self-contained and protective environment.

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.

7.1.6 **Availability**

Natalizumab will be commercially supplied free-of-charge from Biogen.

7.1.7 **Preparation**

Natalizumab concentrate is supplied as 300 mg natalizumab in a sterile, single-use vial free of preservatives. Each package contains a single-use vial.

7.1.8 **Administration**

Natalizumab at a dose of 300 mg should be infused in 100 mL 0.9% Sodium Chloride Injection, USP over approximately one hour. After the infusion is complete, flush with 0.9% Sodium Chloride Injection, USP.

7.1.9 **Ordering**

Natalizumab will be supplied by Biogen Idec Inc. and should be obtained by the site research pharmacy using the study-specific drug order form. Natalizumab will be supplied free of charge and will be ordered from Biogen Idec Inc.

7.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

7.1.11 **Destruction and Return**

At the end of the study, unused supplies of natalizumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

7.2 Steroids

Commercial supplies of Methylprednisolone (or equivalent steroid) will be utilized. The formulation, preparation and route of administration will be as per package insert.

7.2.1 Administration

The suggested initial choice and dosing of steroid is methylprednisolone at 2 mg/kg daily. If methylprednisolone is not used, any steroid, in oral or parenteral formulation, can be administered in equivalent dosing to methylprednisolone. For oral doses of steroids, the dose can be rounded to the nearest available tablet strength. Dosing and dose reductions when response is achieved will be at the discretion of the investigator. A suggested taper is 10-25% every 1-2 weeks.

8. CORRELATIVE/SPECIAL STUDIES

Patients who are treated on this trial will have baseline and serial post-treatment peripheral blood samples collected and banked. Peripheral blood samples will be collected on the day of natalizumab infusion, but prior to starting the infusion (day of infusion will be day 0). Further peripheral blood samples will be collected at day 7, day 14, day 21, day 28, day 56, day 100, day 180 and one year after administration of natalizumab. The day 0 peripheral blood sample should be collected within 24 hours prior to the start of the Natalizumab infusion. The correlative data that will be analyzed in these samples will be α4 receptor saturation and pharmacokinetic studies at one week intervals after treatment. Levels of α4 receptor saturation will be correlated with aGVHD and clinical treatment responses. Aliquots of such samples will also be banked for future correlative studies.

All peripheral blood samples collected for correlative studies will be collected in EDTA tubes, with a blood volume of approximately 10mL. At the time of collection, cells will be cryopreserved in FBS + 10% DMSO at –180°C and will be stored in the Pasquarello tissue bank. Mononuclear cells will be isolated by density gradient centrifugation. Peripheral blood mononuclear cells will be washed before analysis by flow cytometry. Flow cytometry will include a panel of T cell markers and α4β7 integrin. Pharmacokinetic studies will be performed using ELISA with an anti-idiotypic monoclonal antibody specific for natalizumab as the capture antibody.

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 1-week prior to start of protocol therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. All assessments must be performed prior to administration of any study medication. All study assessments and medications should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted. Steroids can be administered up to 7 days before administration of natalizumab. Day 0 is the day of infusion of the first dose of natalizumab.

Subjects who develop a positive serum viral load for JC virus will NOT receive a second infusion of natalizumab. For patients who have a less than complete response after the first dose of natalizumab, a second dose of 300mg will be administered in the same fashion as the first dose. If however a patient has a complete response, no response, or progression of aGVHD, experiences a serious hypersensitivity reaction, or has a grade 3 or higher toxicity, then a second dose of natalizumab will not be administered.

	Pre-Study	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 100	Day 180	Day 365
Natalizumab		X				(X)							
Steroid dose	X	X	X----------										----X
Informed consent	X												
History	X					X				X	X	X	X
Concurrent meds	X		X----------										----X
Physical exam (Ht, Wt, BSA, VS)	X	X				X				X	X	X	X
Performance Status	X	X				X				X	X	X	X
CBC w/diff, plts	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[a]	X	X	X	X	X	X	X	X	X	X	X	X	X
Chimerism[b]			X			X				X	X	X	X
JC viral load[c]	X					(X)				X			
Correlative Studies		X	X	X	X	X				X	X	X	X
Adverse event evaluation			X----------										----X

a: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b: includes total and T cell chimerism studies
c: JC viral load will be checked again at day 28 if a second dose of Natalizumab is to be given

10. MEASUREMENT OF EFFECT

Participants will be assessed for response to therapy with natalizumab at day +28, day +56, day +100, day +180 and day +365 after administration of the first dose of Natalizumab. Staging of each organ system involved in aGVHD will be done by standard NIH criteria (see 17.2 – Appendix B).

10.1 Response Criteria for Overall aGVHD

10.1.1 **GVHD-free survival** – days alive with complete resolution of all signs and symptoms of acute GVHD

10.1.2 **Complete Response (CR)** – resolution of all signs and symptoms of acute GVHD

10.1.3 **Very Good Partial Response (VGPR)**

- Skin – no rash or residual erythematous rash involving < 25% of the body surface, without bullae (residual faint erythema and hyperpigmentation do not count) AND
- Liver – total serum bilirubin concentration < 2 mg/dL or <25% of baseline at enrollment AND
- Gut – tolerating food or enteral feeding, predominantly formed stools, no overt gastrointestinal bleeding or abdominal cramping, no more than occasional nausea/vomiting

10.1.4 **Overall Response Rate** = (CR + VGPR)

10.1.5 **Partial Response (PR)** - an improvement of one stage in one or more organs without progression in any other organ

10.1.6 **Mixed Response (MR) –** improvement in one or more organs with deterioration in another organ manifesting symptoms of GVHD or development of symptoms of GVHD in a new organ

10.1.7 **Non-response (NR) to Therapy -** no reduction in any GVHD organ staging on day +15 after the first dose of Natalizumab is administered.

10.1.8 **Progression of Acute GVHD on Therapy**

- New organ involvement (skin, liver, or intestine) on day +8 after the first dose of Natalizumab is administered or thereafter
- Increased organ specific symptoms sufficient to increase the organ stage by one or more on day +8 after the first dose of Natalizumab is administered or thereafter
- Flare of GVHD while tapering steroids +/- other agents will be defined as the need to increase systemic corticosteroid dose >50% of the starting dose on day +1 after the first dose of Natalizumab is administered or thereafter, OR the need to add another systemic agent to treat acute GVHD

10.2 Response Criteria for GI GVHD

10.2.1 **Complete Response (CR)** – resolution of all signs and symptoms of acute GI GVHD

10.2.2 **Very Good Partial Response (VGPR) -** tolerating food or enteral feeding, predominantly formed stools, no overt gastrointestinal bleeding or abdominal cramping, no more than occasional nausea/vomiting

10.2.3 **Overall Response Rate** = (CR + VGPR)

10.2.4 **Partial Response (PR)** - an improvement by one or more stages of GI GVHD, not meeting criteria for VGPR or CR

10.2.5 **Non-response (NR) to Therapy -** no reduction in GI GVHD staging on day +15 after the first dose of Natalizumab is administered

10.2.6 **Progression:**

- Increased GI specific symptoms sufficient to increase the organ stage by one or more on day +8 after the first dose of Natalizumab is administered or thereafter

- Flare of GI GVHD while tapering steroids +/- other agents will be defined as the need to increase systemic corticosteroid dose >50% of the starting dose on day +1 after the first dose of Natalizumab is administered or thereafter, OR the need to add another systemic agent to treat acute GVHD

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of natalizumab. All grade 2 related and unexpected and all grade 3 and higher adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Grade 4 skin rash, diarrhea and increased bilirubin are expected adverse events as a result of aGVHD and do not require reporting. All positive JC viral loads need to be reported regardless of grade.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

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

Events **not** considered to be serious adverse events are hospitalizations for:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- Elective or pre-planned treatment for a pre-existing condition that did not worsen
- Emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- Respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current

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

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

11.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk. Although not explicitly stated as adverse events from the study agent, grade 4 rash, diarrhea, and increased bilirubin are clinical manifestations of acute GVHD and will not be considered unexpected adverse events in this study.

11.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- 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.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised 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 downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

Note: Grade 2 or Grade 3 lab abnormalities that are considered by the investigator to be clinically insignificant and do not require therapy, or adjustment in prior therapy, do not need to be reported.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting. Grade 4 evens previously mentioned that do not require reporting include rash, diarrhea and increase bilirubin.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

- All positive JC viral loads need to be reported regardless of PCR level reported.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Corey Cutler, MD, MPH
617-632-3470 (Phone)
617-632-5168 (Fax)
Email: cscutler@partners.org

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

11.6 Reporting to the Food and Drug Administration (FDA)

The DF/HCC Overall Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. Dr. Corey Cutler, the principal investigator, currently holds the IND for natalizumab (#121195). The DF/HCC Overall Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-800-FDA-0178) using Form FDA 3500A (Mandatory Reporting Form for investigational agents). A formal written submission will be sent in as well. Forms are available at http://www.fda.gov/medwatch/getforms.htm.

11.7 Reporting to the NIH Office of Biotechnology Activities (OBA)

Not applicable

11.8 Reporting to the Institutional Biosafety Committee (IBC)

Not applicable

11.9 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

Dana Farber Cancer Institute will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to Coordinating Center is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives

designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

- Title 21 Part 50 – Protection of Human Subjects
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
- Title 21 Part 54 – Financial Disclosure by Clinical Investigators
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
- Title 21 Part 56 – Institutional Review Boards
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
- Title 21 Part 312 – Investigational New Drug Application
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws
- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Multi-center Guidelines

Not applicable

13.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

Not applicable

14. STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

This is a phase II trial to determine the GVHD free survival rate at day 56 after the first dose of Natalizumab (Tysabri®) is administered plus steroids for initial therapy of GI acute GVHD. The initial treatment will be one dose of 300mg IV. Patients can be treated with a second dose of natalizumab at 28 days if they are deemed eligible (see section 5.2.1 for eligibility requirements for a second dose of natalizumab). The primary endpoint is GVHD free survival rate at day 56 after the first dose of Natalizumab is administered. GVHD free survival is defined as achieving complete response without death or relapse or requiring secondary immunosuppressive therapy. GVHD response criteria are defined in section 10.1 and 10.2. Patients who receive a second dose of Natalizumab will be evaluated at day 56 after the administration of the first treatment dose. Secondary endpoints are outlined in section 1.3 (under secondary objectives).

For the assessment of the primary endpoint, we employ a two-stage design with early stopping for futility after first stage of accrual. The mainstay of acute GVHD treatment remains corticosteroid therapy. The response rate to single-agent steroid therapy when analyzed in large retrospective reviews was approximately 50% (*MacMillan 2002, Martin 1990*). The response rate to GI aGVHD was similar to the overall response rate. Unfortunately, even with an initial response to steroids, the durability of this response remains low at 24% and 40% for matched unrelated and related donor transplantation, respectively (*Roy 1992, Weisdorf 1990*). Based on this information and extrapolating our internal data, we consider Natalizumab efficacious if the GVHD free survival rate at day 56 after the first dose of Natalizumab is administered is 50% or higher and inefficacious if the rate is 30% or lower.

The target accrual goal is 30 evaluable patients. In the first stage, 15 patients will be accrued. If there are 4 or fewer patients with GVHD free survival at day 56 after the first dose of Natalizumab is administered, then this agent will be regarded as futile and the study will be terminated early. If there are 5 or more patients with GVHD free survival in the first 15 patients, additional 15 patients will be accrued in the second stage. After the second stage, if the total number of patients with GVHD free survival at day 56 after the first dose of Natalizumab is administered is 13 or more, then this agent will be regarded as efficacious for the treatment of GI acute GVHD. Conversely, if the total number of GVHD free survival is 12 or fewer, then this agent will be regarded as futile.

With this design, if the true but unknown GVHD free survival rate is 30%, the probability of stopping early for futility is 0.52, but 0.06 if the true but unknown rate is 50%. The overall power is 0.80 with a type I error rate 0.08. Table 1 below presents the operating characteristics of this design.

Table 1. Operating Characteristics

	True but unknown GVHD-free rate at day 56 of treatment				
	0.3	0.35	0.4	0.45	0.5
Futility after Stage 1	0.52	0.35	0.22	0.12	0.06

Overall Power	0.08	0.21	0.41	0.62	0.80
Expected sample size	22.3	24.7	26.7	28.2	29.1

14.2 Sample Size/Accrual Rate

Based on our data in the past 3 years, approximately 6% of patients developed grade 2-4 gastrointestinal acute GVHD after allogeneic stem cell transplantation. Considering about 250 patients are transplanted per year at this institute and assuming about 50% capture rate, we project that the accrual rate will be approximately 7.5 patients per year and thus it will take approximately 4 years to complete the target accrual goal of 30 patients if the study is not terminated after the first stage of accrual. If non-DFCI centers enroll patients, the accrual will complete sooner.

14.3 Stratification Factors

None

14.4 Analysis of Secondary Endpoints

Secondary endpoints are outlined in section 1.3 (under secondary objectives). Proportions will be reported descriptively. Overall survival (OS) and GVHD-free survival will be assessed using the Kaplan-Meier method. Relapse and non-relapse mortality will be assessed in the competing risks framework. If there is enough number of events, we will also explore regression models for OS and GVHD-free survival and relapse although the number of covariates included in the model will be limited. As to the analysis of correlative studies, peripheral blood samples will be collected at day 0, day 7, day 14, day 21, day 28, day 56, day 100, day 180 and one year after the first dose of Natalizumab is administered. Descriptive statistics and graphical assessment will first be employed to characterize pre to post treatment difference in levels of α4 receptor saturation and pre-to-post treatment difference will be tested using either paired t test or Wilcoxon-signed-rank test. If appropriate, change over time will be examined in repeated measures analysis. Due to the exploratory nature, multiple comparisons will not be considered. In particular, we will confirm whether the expression of α4ß7 integrin is up-regulated in patients with GI aGVHD prior to the development of GI aGVHDand assess whether natalizumab reduces the expression level of α4ß7 integrin and whether the level is associated with GVHD-free survival.

14.5 Reporting and Exclusions

Adverse events will be monitored closely. The monitoring guidelines will serve as a trigger for consultation with the Dana-Farber/Harvard Cancer Center Data and Safety Monitoring Committee (DF/HCC DSMC) for additional review, and are not regarded as formal stopping rules that would mandate automatic closure of study enrollment. In the previous studies reported in the literature, natalizumab is generally safe and causing only minimal adverse events (see Section 2.1.2). Based on this information, natalizumab-related grade 4 or higher toxicities is projected to be <10%. If in the first 10 patients, we observe 2 or more patients with non-hematologic CTCAE grade 4 or

higher that are possibly/probably/definitely attributable to natalizumab, further accrual and administration of natalizumab will be halted pending review by the DSMC. Depending on the findings of its review, the DSMC may recommend the permanent closure of enrollment of that arm or continuation of enrollment. In addition, if a case of PML is reported, the study (both accrual and drug administration) will be immediately halted. Patients who developed a positive JC viral load or who have any toxicity to the first dose of natalizumab will not be eligible for a second dose of natalizumab.

With this design, the probability of halting enrollment is 0.09 if the true but unknown rate of grade 4 or higher non-hematologic toxicity that are attributable to natalizumab is 5%, 0.26 if the rate is 10%, and 0.85 if the true rate is 30%.

15. PUBLICATION PLAN

The results of this trial will be made public within 24 months of the end of data collection. The initial release of data will be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes, to be published in a peer-reviewed journal, will be made public no later than three (3) years after the end of data collection.

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17. APPENDICES

17.1 Appendix A: Performance Status Criteria

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

17.2 Appendix B: Acute GVHD Scoring (adapted from Glucksberg, H et al. *Transplantation* 1974;18:295)

Organ	Stage	Description
Skin	1	Maculopapular rash over <25 percent of body area
	2	Maculopapular rash over 25 to 50 percent of body area
	3	Generalized erythroderma
	4	Generalized erythroderma with bullous formation and often with desquamation
Liver	1	Bilirubin 2.0 to 3.0 mg/dL; SGOT 150 to 750 international units
	2	Bilirubin 3.1 to 6.0 mg/dL
	3	Bilirubin 6.1 to 15.0 mg/dL
	4	Bilirubin >15.0 mg/dL
Gut	1	Diarrhea >30 mL/kg or >500 mL/day
	2	Diarrhea >60 mL/kg or >1000 mL/day
	3	Diarrhea >90 mL/kg or >1500 mL/day
	4	Diarrhea >90 mL/kg or >2000 mL/day; or severe abdominal pain with or without ileus
Glucksberg grade		
I – Stage 1 or 2 skin involvement; no liver or gut involvement; ECOG PS 0		
II – Stage 1 to 3 skin involvement; Grade 1 liver or gut involvement; ECOG PS 1		
III – Stage 2 or 3 skin, liver, or gut involvement; ECOG PS 2		
IV – Stage 1 to 4 skin involvement; Stage 2 to 4 liver or gut involvement; ECOG PS 3		

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**14-140**
Protocol Name:	**Phase II Trial of Natalizumab (Tysarbri®) plus Prednisone for Initial Therapy of Acute Graft versus Host Disease (aGVHD) of the Gastrointestinal Tract**
DFCI Site PI:	Corey Cutler, MD, MPH
DFCI Research Nurse:	Susan Stephenson, RN

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
***** Remember to check the ALERT PAGE*****

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	Natalizumab (Tysabri) is a recombinant humanized IgG4 monoclonal antibody. In this study, the initiation of Natalizumab at the onset of symptoms, in addition to steroids, will provide the best chance of preventing crypt necrosis by shutting down the homing of T cells to the gut as early as possible. The absent of α4β7 integrin on hematologic malignancies provides Natalizumab with the unique ability to abrogate gut GVHD without effecting the graft versus tumor effect. Thus, it is hoped that given its specific targeting of integrin, Natalizumab can be successful in treating acute GVHD, yet be less globally immunosuppressive, the main concern with current available treatments of acute GVHD. α4β7 is increased in disease sites of inflammatory bowel disease, providing another potential target for Natalizumab.
Dose Calcul-	• **Natalizumab:** dosed as a flat mg IV, Sections 7.1.8.
Study Drug Administration	*Treatment Plan administration* Guidelines are found in Sections 5 and 7. • Treatment of acute GVHD is an acute process that requires short courses of treatment. • Initial standard dose IV will be used at confirmation of acute gut GVHD. • If at 4 weeks there has been less than a complete response, participants can be treated with a second dose of Natalizumab. • Pre-treatment criteria: ANC ≥ 500/μL (G-CSF) support is allowed); Platelets: ≥ 10,000/μL (platelet transfusions are allowed on the same day). • **Observations**: will be observed for 2 hours following completion of infusion of Natalizumab **for the first infusion** (monitor for possible infusion related reactions). **See Section 5.2.1. for details.** • **Vital Signs:** (temp, Heart rate, blood pressure and respiratory rate) should be monitored approximately every 10 minutes during the infusion of Natalizumab.
Dose Modification	*Expected Toxicities and Dosing Delays / Dose Modifications* are outlined in Section 6.
Concomitant Meds	*General Concomitant Medication and Supportive Care Guidelines* are in Section 5.4. • Routine use of growth factor (G-CSF) support in the absence of documented neutropenia is not recommended. • Prednisone requirements: See Section 7.2 to 7.2.1
Required	Study Calendar data is outlined in Section 9. • Correlative/Special Studies- refer to Section 8.
Charting Tips	**All study drugs require documentation of exact administration time.** Please be sure to DOCUMENT study medication **actual** UP/DOWN times in medical record (e.g. LMR, eMAR, nursing notes). **Edit eMAR as needed to match the exact time given**. • If there is a discrepancy in the infusion time, delay in administration, or the infusion takes longer than is permitted by the guidelines of the protocol, please **document the reason for the discrepancy in the medical record.** Please be sure to also DOCUMENT any required observation periods, any additional vital signs, routes of administration, or injection sites