Masonic Cancer Center, University of Minnesota
Blood and Marrow Transplantation Program

Phase I/II Study of Human Chorionic Gonadotropin and Epidermal Growth Factor Supplementation (Pregnyl®) to Support Tolerance and Repair As Adjunct Therapy in High-Risk or Refractory Acute Graft-Versus-Host Disease

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Revision History

Revision #	Version Date	Summary Of Changes	Consent Changes
	04/08/2015	original to CPRC	
	05/11/2015	response to CPRC initial review	n/a
	07/07/2015	clarify maintenance in schema and section 7 minor edits/clarification in section 10 prior to IRB/FDA submission	n/a
	08/05/2015	a complete response to the FDA's deficiency request: • Synopsis, section 5.2, appendix I – exclude patients unwilling or unable to stop supplemental sex hormone therapy (estrogen, progesterone, and/or testosterone preparations) • Schema, sections 4 and 8.1 – Add "Each lot of Pregnyl® will be tested for EGF content prior to administration so that the precise EGF dose administered is known." • Schema, sections 4, 7.1, 7.2, 10.1, 10.2, 11.2, 13.1- revise DLT assessment period to "occurring by day 21 after the 1st dose of Pregnyl®", previously was day 7 for males and day 14 for females • Sections 7.1, 7.2 and 7.4 – remove individual dose level reductions due to DLTs • Section 10.3 – delete reference to patients receiving supplement as now such patients are excluded • Section 13.1 – expand CRM section for clarity • Section 13.4 – recalculated early study stopping rule by lowering the expected toxicity rate • Section 15 – update reference 22 from abstract to full publication	Parent
	08/14/2015	A complete response to the FDA's deficiency request (dated 08/13/2015): • Synopsis, Sections 5.1, 10.1 and 10.2, appendix I – add baseline eGRF and LVEF requirements for eligibility • Synopsis, Section 5.2 and appendix I - expand exclusion criteria to exclude patients with a hormone responsive malignancy, an active or recent thrombus (irrespective of anticoagulation status) • Need to correct reference	yes
	09/12/2016	Section 5.1Clarification of inclusion/exclusion criteria, expansion to include patients with steroid-dependent aGVHD, late-onset aGVHD, and overlap syndrome	
	11/30/2016	Section 5, Clarification of eligibility criteria, evaluability, patient replacement, and Appendix 1	No
	03/22/2017	Corrected typo in Section 6.4 in response to CPRC stipulations on version 11/30/16	No
	06/21/2017	Section 5, Updated eligibility to include patients from 0-12 Section 7.1 and Section 7.2, Separated Arm 2 into Arm 2A (steroid-dependent) and 2B (steroid-refractory), and expanded dosing cohorts for Arm 2B given preliminary laboratory analyses.	Yes
	10/28/2017	Section 13, Edited the statistical section due to CPRC stipulations on 6/21/17 amendment	
	05/17/2018	Fixed typographical error in study schema dose levels; Section 5, updated eligibility based upon biomarkers	no

	01/22/2019	Title page, Key personnel, Section 6.1, Section 11.3, Section 12.7: Added 2nd site (Rush University) – throughout document, protocol updated with information for affiliate institutions. Section 8.1, Added bioequivalent form of hCG/EGF from Fresenius Kabi should Merck product not be available. Section 11.2, Added relapse of primary malignancy to reportable events.	Yes
	07/01/2019	Minor administrative clarification – synopsis, sections 7.3 and Section 8.1 - for Arm 2B, dose level 5,to avoid using vials from more than one Lot of study product, the maximum dose is either (1) the contents of a single 10,000 IU vial, or (2) a maximum dose of 10,000 IU from multiple vials. All vials used should be from the same Lot.	No
	05/27/2020	Section 5.1: Arm 1, updated definition of high-risk aGVHD to include Minnesota standard with aGVHD with no response to 7 days of sirolimus plus a calcineurin inhibitor Deleted eligibility checklist (Appendix I) as per SOP – this document is stored in Oncore	

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Protocol Synopsis

Study Design:

This multi-center center phase I/II study will use Continual Reassessment Method (CRM) to establish a maximum tolerated dose (MTD) of human chorionic gonadotropin and epidermal growth factor supplementation (hCG) as an adjunct to facilitate mucosal healing and immune tolerance in conjunction with accepted, standard immunosuppressive therapy in the setting of high-risk (Arm 1) and steroid-dependent (Arm 2A) and steroid-refractory (Arm 2B) acute graft-versus-host disease (aGVHD). Enrollment and dose escalation of Pregnyl® will be independent for each arm.

After completion of the dose finding trial for each arm, the final doses will be carried forward into a two-stage phase II extension trial to confirm safety and make a preliminary determination of the activity level for Arm 1, Arm 2A and Arm 2B. If the phase I trial enrolls fewer than 13 patients at the MTD, we will employ Simon's Minmax two-stage design with the possibility to discontinue after the 1st stage if the response rate is low.

- Stage 1: Enroll a total of 12 patients (including all treated at the MTD in phase I). If 5 or more of these respond, the trial will continue to stage 2.
- Stage 2: Enroll an additional 14 patients. If 14 or more out of 26 patients respond, hCG will be considered promising for further investigation.

If phase I trial enrolls more than 12 patients at the MTD, we will adjust the futility rule above for the two stage design with the giving number of patients enrolled at the MTD.

Primary Objective:

Phase I: To determine the maximum tolerated dose (MTD) of Pregnyl® when given in combination with standard immunosuppressive therapy in pediatric and adult patients with high-risk (Arm 1) or steroid-refractory/dependent (Arm 2) aGVHD

Phase II: To determine the proportions of complete, partial, mixed, and no response among surviving patients at days 28 after initiation of protocol therapy in pediatric and adult patients with high-risk (Arm 1) or steroid-refractory/dependent (Arm 2) aGVHD

Secondary Objectives:

- To determine the safety and feasibility of hCG supplementation with Pregnyl® in combination with standard immunosuppressive therapy in pediatric and adult patients with high-risk or refractory aGVHD
- To determine the incidence of acute GVHD flare after CR/PR requiring increase of steroids or other systemic treatment at days 28 and 56
- To compare the rate of treatment failure for acute GVHD at days 28 and 56 after initiation of protocol therapy to historical controls

Transplant Related Objectives:

- Disease-free survival, overall survival, and non-relapse mortality at day 28, 56, 180 and 1 year of protocol treatment
- Incidence of chronic GVHD at day 180 and 1 year

Correlative Objectives:

- Pharmacokinetics (clearance, half-life, volume of distribution) of the both the hCG and EGF components of urinary hCG when administered to patients with GVHD
- Monitoring for hormonal effects via determination of serum concentrations of hCG, estradiol, progesterone, and testosterone on days 7, 14, 28, and 56, compared to baseline
- Collection of blood samples for measurement of immune and tissue damage/repair biomarkers to determine change from pre-treatment levels to levels obtained on days 7, 14, 28, and 56 post-treatment
- Analysis of previously collected skin and/or gastrointestinal biopsies for biomarkers of refractory aGVHD and response to therapy

Patient Population: HCT recipients 0 to 76 years of age within the first 7 days of initial treatment of high-risk (Arm 1) or steroid-refractory (Arm 2) aGVHD at any time in their treatment course

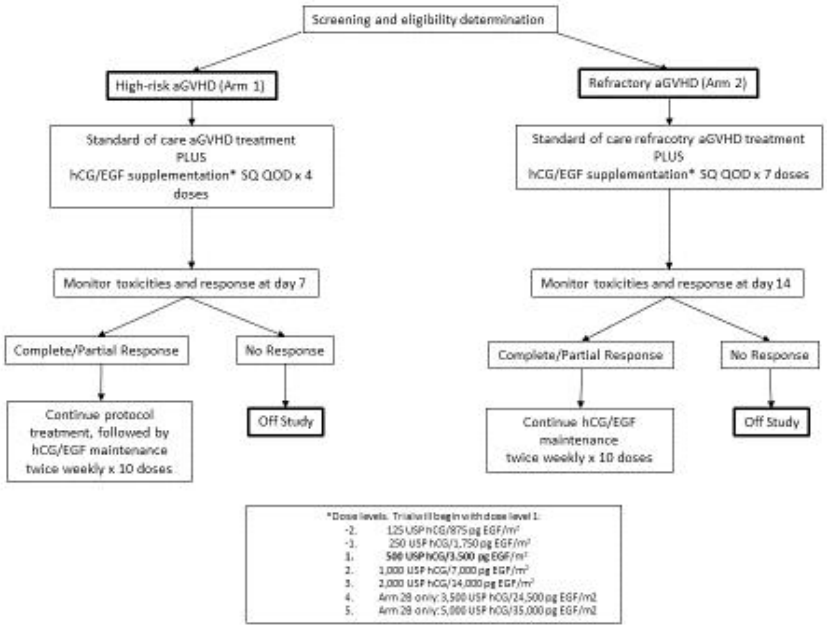
Inclusion Criteria: Renal: Serum creatinine ≤2.5x upper limit of normal (ULN)
Cardiac: Left ventricular ejection fraction (LVEF) ≥ 35%

Exclusion Criteria:

- Progressive malignancy
- Uncontrolled infection at initiation of protocol treatment
- Unwilling or unable to stop supplemental sex hormone therapy (estrogen, progesterone, and/or testosterone preparations)
- Diagnosis of a hormone responsive malignancy
- Current thromboembolic disease requiring full-dose anticoagulation - patients receiving pharmacologic prophylaxis for thromboembolic disease will be eligible
- Active or recent (within prior 3 months) thrombus, irrespective of anticoagulation status
- Pregnancy
- Women or men of childbearing potential unwilling to take adequate precautions to avoid unintended pregnancy from the start of protocol treatment through 28 days after the last treatment

Enrollment Plan: Phase I: Up to 20 patients in each arm with an estimated 2 years to accrue patients
Phase II component (for each arm):
Stage 1: Enroll a total of 12 patients (including all treated at the MTD in phase I). If 5 or more of these respond, the trial will continue to stage 2.

Stage 2: Enroll an additional 14 patients. If 14 or more out of 26 patients respond, hCG will be considered promising for further investigation.



***NOTE: Arm 2B, dose level 5: to avoid using vials from more than one Lot of study product, the maximum dose is either (1) the contents of a single 10,000 IU vial, or (2) a maximum dose of 10,000 IU from multiple vials. All vials used should be from the same Lot.**

Arm 1, Arm 2A and Arm 2B will be treated as separate, independent cohorts. Each lot of Pregnyl® will be tested for EGF content prior to administration so that the precise EGF dose administered is known.

Phase I:
For each arm, dose levels of Pregnyl® will be tested starting at dose level 1. Dose levels -1 and -2 will be used only if dose level 1 proves too toxic.

The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until all patients in the current cohort have reached day 21. Each new cohort of 2 patients will be assigned by the study statistician to the most appropriate dose level based on toxicity probabilities set for this study. The MTD for each arm (high-risk and steroid-refractory aGVHD) will be identified at or before the total sample size of 20 patients per arm is reached.

The dose limiting toxicity (DLT) is defined as any of the following occurring by day 21 after the 1st dose of Pregnyl®:

- In males and females: Grade 2-4 thromboembolic event (CTCAE v4) i.e., any thrombosis more severe than a superficial venous thrombosis where treatment is required
- In males and females: Grade 3-4 ascites (CTCAE v4)
- Specific to females: Ovarian hyperstimulation syndrome, defined as the rapid development of enlarged, cystic, painful ovaries

Phase II Extension:

- **Stage 1:** Enroll a total of 12 patients (including all treated at the MTD in phase I). *If 5 or more of these respond, the trial will continue to stage 2.*
- **Stage 2:** Enroll an additional 14 patients. *If 14 or more out of 26 patients respond, hCG will be considered promising for further investigation.*

1 Study Objectives

1.1 Primary Objective

Phase I: To determine the maximum tolerated dose (MTD) of Pregnyl® when given with standard immunosuppressive therapy in pediatric and adult patients with high-risk or refractory acute graft-versus-host disease (aGVHD).

Phase II: To determine the proportions of complete, partial, mixed, and no response among surviving patients at days 28 after initiation of protocol therapy in pediatric and adult patients with high-risk (Arm 1) or refractory (Arm 2) aGVHD.

1.2 Secondary Objectives

- To determine the safety and feasibility of hCG supplementation with Pregnyl® in combination with standard immunosuppressive therapy in pediatric and adult patients with high-risk or refractory aGVHD
- To determine the incidence of acute GVHD flare after CR/PR requiring increase of steroids or other systemic treatment at days 28 and 56
- To compare the rate of treatment failure for acute GVHD at days 28 and 56 after initiation of protocol therapy to historical controls

1.3 Correlative Objectives

- Pharmacokinetics (clearance, half-life, volume of distribution) of the both the hCG and EGF components of urinary hCG when administered to patients with GVHD
- Monitoring for hormonal effects via determination of serum concentrations of hCG, estradiol, progesterone, and testosterone on days 7, 14, 28, and 56, compared to baseline
- Collection of blood samples for measurement of immune and tissue damage/repair biomarkers to determine change from pre-treatment levels to levels obtained on days 7, 14, 28, and 56 post-treatment
- Analysis of previously collected skin and/or gastrointestinal biopsies for biomarkers of refractory aGVHD and response to therapy

1.4 Transplant Related Objectives

- To determine the disease-free survival, overall survival, and non-relapse mortality at day 28, 56, 180 and 1 year of protocol treatment
- To determine the incidence of chronic GVHD at day 180 and 1 year

2 Background

Patients with high-risk acute graft-versus host disease (HR-aGVHD) have poor responses to initial therapy and an increased risk of death.

In the US, nearly 7,000 patients undergo allogeneic hematopoietic cell transplantation (HCT) annually in an effort to cure hematologic malignancies and other bone marrow disorders. The majority of allogeneic HCT recipients will experience acute graft-versus-host disease (aGVHD), a complication in which cells from the immunocompetent donor graft attack the recipient's organs and tissues [1]. Patients with severe skin or visceral involvement, or multi-organ aGVHD, have high-risk disease, characterized by a less than 50% complete or partial response to first-line, high-dose corticosteroids and a 2-3 fold risk of treatment-related mortality [2]. Before the recent ability to discriminate high-risk versus standard-risk aGVHD based upon organ staging at onset, it was noted that one-half of patients aGVHD did not sustain response to first-line therapy with corticosteroids (i.e., are *steroid-refractory*) [3]. Damage to the gastrointestinal tract is the major cause of morbidity and mortality in patients with severe, refractory aGVHD.

Figure 1 shows typical colonoscopic findings of a University of Minnesota patient with aGVHD with mucosal edema, erythema, loss of normal vascular patterns, and extensive ulcerations. Clinically, such patients have anorexia, abdominal cramping and pain, malabsorption, and large volume (often >2 liters) of diarrhea daily. They become severely malnourished, require intravenous medications and nutrition due to poor oral absorption, and they consequently endure very prolonged hospital stays. 80-100% of patients die within two years of the development of steroid-refractory [4] (and unpublished quality improvement data).

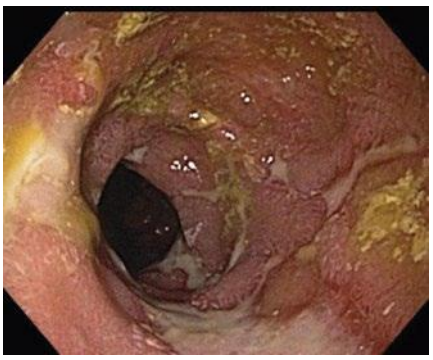


Figure 1. Appearance of transverse colon in a patient suffering from steroid-refractory aGVHD.

Current standard treatment of aGVHD depletes circulating and tissue immune effector cells. The reason for poor outcomes in high-risk and steroid-refractory aGVHD is likely *not solely* due to poor efficacy of immunosuppressant medications. High-dose steroids, calcineurin inhibitors, anti-thymocyte globulin, and anti-cytokine therapies effectively deplete the circulating pool of cytokine-producing cells responsible for GVHD effects. **Figure 2** shows an example of the effects of steroid-refractory aGVHD and its treatment on peripheral blood leukocytes (PBL) in humans (Holtan, unpublished data). The scatterplot on the left depicts the distribution of normal PBL, including TNFα-producing monocytes, neutrophils, and lymphocytes in a healthy donor. The scatterplot on the right depicts PBL of a patient treated for steroid-refractory aGVHD with high-dose steroids, cyclosporine, and TNFα inhibition. It is apparent that TNFα-producing monocytes and lymphocytes, the cell subsets responsible for aGVHD effects, are substantially depleted from the peripheral blood of the steroid-refractory aGVHD patient.

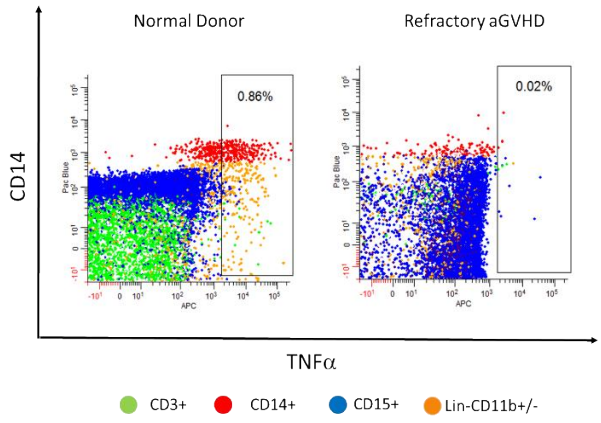


Figure 2. Spectrum of TNFα-producing and non-TNFα-producing peripheral blood leukocytes after stimulation with lipopolysaccharide in a normal donor (left) versus a patient treated for steroid-refractory aGVHD (right).

These inflammatory cytokine-producing cells are also depleted from colonic tissue in patients with refractory aGVHD. **Figure 3** shows that the lamina propria in a patient with refractory aGVHD (right) is relatively devoid of hematopoietic cells (CD45 staining in brown) compared to that of a normal donor (left, Holtan, unpublished data). Based upon these preliminary findings as well as >20 years of intensified immunosuppression as the sole paradigm for high-risk or refractory aGVHD, it is very unlikely that investigational drugs that exert their effects by intensification of immune suppression will be able to facilitate healing or improve organ function. New paradigms aimed at supporting immunologic tolerance as well as restitution of damaged tissues such as the methods proposed in this trial are necessary.

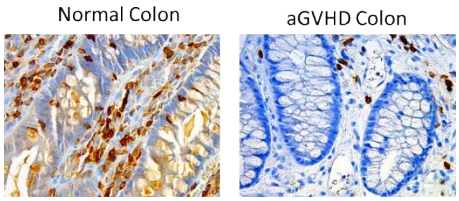


Figure 3. Fewer CD45+ (brown) hematopoietic cells in colon tissue (lamina propria) normal donor (left) versus a patient treated for steroid-refractory aGVHD (right).

Mucosal healing capacity may be reduced in high-risk or refractory GVHD due to deficiency of trophic growth factors.

Markers of endothelial damage (angiopoietin-2) and angiogenesis (vascular endothelial growth factor, VEGF) have been shown to be prognostic in steroid-refractory acute GVHD [5]. A recent broader study of angiogenic factors in allogeneic HCT has been completed. In that pilot study, epidermal growth factor (EGF) levels were over 10-fold lower in 17 patients experiencing severe (grade III or IV) aGVHD compared to 17 allogeneic HCT recipients at 3-months post-HCT who did not experience aGVHD (median 11.5 vs 141.4 pg/ml, p=0.004). The finding of low EGF levels at the onset of aGVHD was subsequently validated in two independent cohorts of patients enrolled on multicenter treatment trials, Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0302 and 0802 compared to a separate cohort of patients without aGVHD [[6], and Holtan et al, manuscript accepted]. Furthermore, in BMT CTN 0802, where plasma (as opposed to serum, which can have EGF levels increase due to release from activated platelets) was analyzed, EGF levels significantly *decreased* at day 28 after initial aGVHD therapy (p=0.01, Wilcoxon signed rank) in patients who had no response. This was particularly apparent in patients who had high-risk aGVHD at onset (**Figure 4, top**), where day 28 median EGF was 6-fold lower (3.8 versus 23.2 pg/mL) in patients with no response to initial therapy. In contrast, patients with complete responses to initial therapy demonstrated *increases* in plasma EGF by day 28 of treatment.

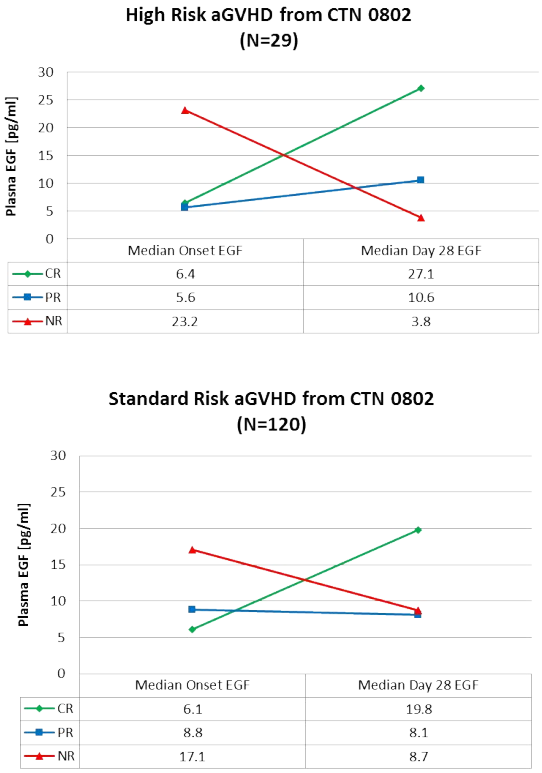


Figure 4. Plasma EGF levels at aGVHD onset and day 28 of initial therapy in patients enrolled in BMT CTN 0802, stratified by high-risk aGVHD and standard risk aGVHD. Patients with high-risk (top) and standard risk aGVHD (bottom) and no response (NR) to therapy show a decline in EGF levels at day 28 of therapy, with the decrease even more dramatic in high-risk aGVHD.

Decreased circulating EGF levels have been identified in inflammatory bowel disease [7], which shares many manifestations with GVHD. EGF has been shown to enhance gut epithelial restitution after radiation injury [8] and protect against the development of colitis in a rat model [9]. Furthermore, EGF treatment can improve ion transport capabilities, especially sodium reabsorption, in inflamed colonic mucosa [10]. In

addition to providing mitogenic signals for intestinal epithelial stem cells, EGF also has been shown to regulate inflammation, intestinal epithelial apoptosis, and autophagy in the setting of necrotizing enterocolitis [11]. Chronic administration of intraluminal EGF enhanced colonic mucosal growth in both a rodent model [12] and in humans [13]. However, intraluminal administration is not practical or likely to be efficacious in steroid-refractory aGVHD due to the typical widespread gastrointestinal involvement. Feasibility of *systemic* treatment with EGF has recently been demonstrated. In a randomized, placebo-controlled trial of intravenous recombinant EGF in premature infants with necrotizing enterocolitis, 6-day continuous IV administration of EGF at 100 ng/kg/hour improved gut mucosal thickness by 54% over baseline as early as day 4 of therapy [14]. No significant infusional or other systemic side effects from EGF administration were noted in the trial. Whether similar responses could be elicited in steroid-refractory aGVHD has not been tested. Unfortunately, pharmaceutical grade recombinant EGF is not currently available for clinical testing in humans, making alternate sources of EGF necessary.

Circulating EGF levels are high and excreted in the urine during pregnancy.

In recent studies of the immune and growth factor milieu of early pregnancy, EGF levels have been identified as being approximately 20-fold elevated in the first trimester of pregnancy, while several inflammatory biomarkers were significantly decreased (Table 1) relative to non-pregnant controls (Holtan et al, manuscript accepted). EGF is critical for the initiation and maintenance of pregnancy by stimulating hCG production in the early developing placenta [15]. As pregnancy progresses, deficiency of EGF leads to intrauterine growth retardation [16] and can predispose to necrotizing enterocolitis [17]. Considering its importance in placentation and fetal growth, it is not a surprise that EGF is a biologically functional component of commercial urinary-derived hCG preparations. With testing by the Cytokine Reference Laboratory at the University of Minnesota, the concentration of EGF in Pregnyl® was determined to be 7,037 pg/mL (Panoskaltsis-Mortari, unpublished data).

Analyte	Non-pregnant Median (N=11)	Min	Max	Pregnant Median (N=16)	Min	Max	p
EGF	**24.8**	**8.6**	**178.2**	**489.4**	**14.6**	**1506**	**0.003**
sCD40L	424.7	132.7	1423	29.2	0	1583	0.004
L-17	14.8	2.2	161.6	5.5	<LLD	46.9	0.03
IL-6	7.9	<LLD	82	<LLD	<LLD	34.3	0.03

Table 1. Differences in serum growth factors and markers of inflammation between pregnant (first trimester) and non-pregnant individuals. LLD=lower limit of detection.

Tolerance induction during pregnancy: central role of hCG.

HCG is widely studied for its immune tolerance-inducing effects. Recently described immunomodulatory properties of hCG include stimulating an increase in regulatory T cells with a concomitant reduction of inflammatory cytokines [18], maintenance of dendritic cells in a tolerogenic state [19], and modulation of innate immunity [20]. The molecular mechanisms of hCG-induced immune modulation are not completely known but involve inhibition of nuclear transcription factor-κB and activator protein-1 gene transcription driven by TNFα [21]. Recently, chronic administration of low-dose hCG was piloted as a therapy for refractory chronic GVHD and found to be tolerable with suggestion of clinical efficacy [22]. In this study, an increase in indoleamine 2,3-dioxygenase (IDO) and IL-10 blood levels were observed, both beneficial for runaway GVHD immune responses. It is possible that some of the results, including the gut epithelial restitution depicted in this report, were also due to supplementation of EGF.

Potential complementary roles of EGF and hCG in high-risk and steroid-refractory aGVHD.

It is possible that both the hCG and EGF components of Pregnyl® will contribute to improvement of clinical outcomes in high-risk and refractory aGVHD. HCG is well known for its ability to induce immunologic tolerance, and EGF is well known for its role in repair of damaged tissues. Considering the gut trophic properties of EGF and the immune modulatory properties of hCG, our **central hypothesis** is that administration of an EGF-containing urinary preparation of hCG to patients with steroid-refractory aGVHD may help restore organ function, support the development of immunologic tolerance, and improve quality of life, and without adding significant toxicity to standard immunosuppressive therapy. Before large scale application of this approach, the safety, feasibility, pharmacokinetic, immunologic and other pharmacodynamic correlates of its administration in a limited number of patients must first be determined.

Subcutaneously-delivered urinary hCG is has safely been given to both men and women for endocrinologic indications, with data regarding safety and efficacy spanning over 4 decades [23, 24, 25, 26, 27]. Severe reactions including arterial thromboembolism and ovarian hyperstimulation syndrome occur in <1% of patients. The doses administered on this protocol are within the range of established standard of care dosing for endocrinologic indications today. Similar low doses of urinary hCG given to a cohort of patients with steroid-refractory chronic GVHD was well tolerated. Therefore, we believe the safety risks of this study are very minimal. However, the

doses required to significantly increase plasma and tissue EGF levels and exert immunologic effects are not known, making the studies proposed herein necessary for further repurposing of the drug.

3 Summary and Rationale

High-risk and steroid-refractory acute graft-versus-host disease (aGVHD) are frequent and often fatal complications of allogeneic hematopoietic cell transplantation (HCT). The current treatment paradigm, intensification of immunosuppression, does not yield durable improvement of organ (especially gastrointestinal) function or long-term survival in most patients. Many patients die of consequences of severe mucosal damage of the gastrointestinal tract and complications of profound immunosuppression. This study takes an entirely different approach to the treatment of otherwise fatal aGVHD by hastening mucosal healing and inducing immune tolerance with the hormonal supplement, Pregnyl®. We recently identified that circulating epidermal growth factor (EGF) levels are very low in patients with severe aGVHD compared to allogeneic HCT recipients without aGVHD. EGF is an important protein involved in the proliferation of epidermal and epithelial cells, and it is involved in healing of damaged mucosal tissues. Extensive preclinical and clinical data has demonstrated that administration of EGF induces remission of other life-threatening forms of intestinal inflammation, such as necrotizing enterocolitis and inflammatory bowel disease, but this approach has never been tested in aGVHD. A readily available and inexpensive source of EGF is urinary-derived human chorionic gonadotropin (hCG) (e.g., Pregnyl® and other brands) which is commonly used for reproductive endocrinology indications. Pregnyl® is isolated from the urine of pregnant women which contains both hCG and EGF. Although the EGF component contained in Pregnyl® is not essential for reproductive endocrinology indications, it may be highly effective in providing EGF for repair of tissues damaged by aGVHD.

HCG is a hormone critical for the development of maternal immune tolerance towards a fetus during pregnancy, arguably the most commonly encountered and effective example of induced immune tolerance in nature. HCG induces immune tolerance in pregnancy by mechanisms including induction of indoleamine 2,3 dioxygenase (IDO) and expansion of endogenous regulatory T cells (Tregs), both of which are protective against GVHD. Therefore, hCG may be effective in promoting immune tolerance in aGVHD. Importantly, a urinary-derived preparation of hCG has already shown early promise in inducing tolerance and reversing organ damage without untoward toxicity in 12 of 20 (60%) of patients with heavily pre-treated patients with steroid-refractory or intolerant chronic GVHD [22].

The objective of this study is to test for the first time the use of supplemental EGF and hCG (Pregnyl®) as an adjunct to facilitate improved mucosal healing and immune tolerance in addition to accepted, standard immunosuppressive therapy in the setting of high-risk (Arm 1) and steroid-refractory (Arm 2) aGVHD. Our central hypothesis is that administration of urinary hCG and EGF will provide (a) EGF for facilitation of gastrointestinal healing and (b) hCG for induction of immune tolerance, while not worsening the rates of infectious or other complications, in patients with severe aGVHD. However, the optimal supplemental doses of EGF necessary to achieve an epithelial restitution effect are not known. Therefore, this study will use the Continuous Reassessment Method (CRM) to test up to five dose levels of Pregnyl® in order to identify the maximum tolerated dose (MTD) by each patient cohort (high-risk (arm 1), steroid dependent (arm 2A) and steroid-refractory (arm 2B)). We will determine pharmacokinetics of both the EGF and hCG components of urinary HCG and identify correlative biomarkers of aGVHD response. The pharmacokinetic and correlative studies would not be possible without intercollegiate collaborations. The knowledge gained from these aims will provide critical feasibility, preliminary safety, and correlative data for efficacious dose selection and identification of leading biomarkers for the phase II expansion of this trial. These data will be necessary to successfully compete for National Institutes of Health or similar funding for a larger scale, multicenter trial, if the approach proves to be feasible, tolerable, and efficacious in this initial study.

Urinary HCG is an inexpensive, commercially-available hormone with a long track record of safety and tolerability. Therefore, if effective, we anticipate this could quickly be repurposed into a standard adjunctive treatment for high-risk or refractory aGVHD.

The first goal of this study is to find the dose of Pregnyl® needed to at least double patients' plasma EGF levels from baseline levels while not exceeding the maximum tolerated dose. Our preliminary data indicate that baseline plasma EGF levels are in the range of 10-30 pg/ml, whereas the median plasma EGF level of a normal allogeneic HCT recipient without aGVHD is over 10-fold higher at 140 pg/mL.

Dose justification: The doses selected for testing in this trial are within the range of doses of Pregnyl® used for endocrinology purposes today and do not represent "high" doses. The standard dose for ovulation trigger is 10,000 USP of the hCG component, which is more than double the amount that patients on the highest dose level on this protocol will receive. The starting doses for this protocol are estimated to be the minimum sufficient to determine an increase in plasma EGF with therapy. The 1-week cumulative EGF dose obtained from Pregnyl® at dose level 1 is expected to be equivalent to approximately 50% of the circulating EGF in a typical patient with severe aGVHD. The 1-week cumulative EGF dose at dose level 2 is expected to be

approximately 100% of (effectively doubling) circulating EGF in patients with severe aGVHD. Each lot of Pregnyl® will be tested for EGF content prior to administration so that the precise EGF dose administered is known.

4 Study Design

This multi-center phase I/II study will use Continual Reassessment Method (CRM) to establish a maximum tolerated dose (MTD) of human chorionic gonadotropin and epidermal growth factor supplementation (hCG) as an adjunct to facilitate improved mucosal healing and immune tolerance in conjunction with accepted, standard immunosuppressive therapy in the setting of high-risk (Arm 1), steroid dependent (Arm 2A), and steroid-refractory (Arm 2B) acute graft-versus-host disease (aGVHD).

Enrollment and dose escalation of Pregnyl® will be independent for each arm.

Arm 1, Phase I: For each arm up to 5 dose levels of Pregnyl® will be tested; however dose levels -1 and -2 will be used only if dose level 1 proves too toxic. In additional to Pregnyl®, patients will continue on their current standard of care aGVHD treatment.

The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until all patients in the current cohort have reached day 21. Each new cohort of 2 patients will be assigned by the study statistician to the most appropriate dose level based on toxicity probabilities set for this study. The MTD for each arm (high-risk and steroid-refractory aGVHD) will be identified when the total sample size of 20 patients within an arm is reached. Enrollment on phase I is expected to take 2 years based on an enrollment of approximately 20 patients per year for each arm.

Phase II Extension: After completion of the dose finding trial for each arm, the final doses will be carried forward into a two-stage phase II extension trial to confirm safety and make a preliminary determination of the activity level for Arm 1 and Arm 2. If the phase I trial enrolls fewer than 13 patients at the MTD, we will employ Simon's Minmax two-stage design with the possibility to discontinue after the 1st stage if the response rate is low.

- **Stage 1:** Enroll a total of 12 patients (including all treated at the MTD in phase I). *If 5 or more of these respond, the trial will continue to stage 2.*
- **Stage 2:** Enroll an additional 14 patients. *If 14 or more out of 26 patients respond, hCG will be considered promising for further investigation.*

If phase I trial enrolls more than 12 patients at the MTD, we will adjust the futility rule above for the two stage design with the giving number of patients enrolled at the MTD.

Arm 2A (steroid-dependent), Phase I: For each arm up to 5 dose levels of Pregnyl® will be tested; however dose levels -1 and -2 will be used only if dose level 1 proves too toxic. In additional to Pregnyl®, patients will continue on their current standard of care aGVHD treatment.

The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until all patients in the current cohort have reached day 21. Each new cohort of 2 patients will be assigned by the study statistician to the most appropriate dose level based on toxicity probabilities set for this study. The MTD for each arm (high-risk and steroid-refractory aGVHD) will be identified when the total sample size of 20 patients within an arm is reached. Enrollment on phase I is expected to take 2 years based on an enrollment of approximately 20 patients per year for each arm.

Phase II Extension: After completion of the dose finding trial for each arm, the final doses will be carried forward into a two-stage phase II extension trial to confirm safety and make a preliminary determination of the activity level for Arm 1 and Arm 2. If the phase I trial enrolls fewer than 13 patients at the MTD, we will employ Simon's Minmax two-stage design with the possibility to discontinue after the 1st stage if the response rate is low.

- **Stage 1:** Enroll a total of 12 patients (including all treated at the MTD in phase I). *If 5 or more of these respond, the trial will continue to stage 2.*
- **Stage 2:** Enroll an additional 14 patients. *If 14 or more out of 26 patients respond, hCG will be considered promising for further investigation.*

  If phase I trial enrolls more than 12 patients at the MTD, we will adjust the futility rule above for the two stage design with the giving number of patients enrolled at the MTD.

Arm 2B (steroid-refractory), Phase I: For each arm up to 7 dose levels of Pregnyl® will be tested; however dose levels -1 and -2 will be used only if dose level 1 proves too toxic. In additional to Pregnyl®, patients will continue on their current standard of care aGVHD treatment.

The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until all patients in the current cohort have reached day 21. Each new cohort of 2 patients will be assigned by the study statistician to the most appropriate dose level based on toxicity probabilities set for this study. The MTD for each arm (high-risk and steroid-refractory aGVHD) will be identified when the total sample size of 20 patients within an arm is reached. Enrollment on phase I is expected to take 2 years based on an enrollment of approximately 20 patients per year for each arm.

Phase II Extension: After completion of the dose finding trial for each arm, the final doses will be carried forward into a two-stage phase II extension trial to confirm safety and make a preliminary determination of the activity level for Arm 1 and Arm 2. If the phase I trial enrolls fewer than 13 patients at the MTD, we will employ Simon's Minmax two-stage design with the possibility to discontinue after the 1st stage if the response rate is low.

- **Stage 1:** Enroll a total of 12 patients (including all treated at the MTD in phase I). *If 5 or more of these respond, the trial will continue to stage 2.*
- **Stage 2:** Enroll an additional 14 patients. *If 14 or more out of 26 patients respond, hCG will be considered promising for further investigation.*

If phase I trial enrolls more than 12 patients at the MTD, we will adjust the futility rule above for the two stage design with the giving number of patients enrolled at the MTD.

5 Patient Selection

Study entry is open to persons 0 to 76 years of age regardless of gender or ethnic background. While there will be every effort to seek out and include women and minority patients, the patient population is dependent upon the transplant population at the University of Minnesota.

5.1 Inclusion Criteria

- Acute graft versus host disease (GVHD) fitting one of the following categories:

High-Risk aGVHD (ARM 1)

Pediatric or adult (ages 0-76 years) HCT recipients with:

- high-risk acute GVHD, as determined either by:
  - the refined MN acute GVHD risk score [28]: http://z.umn.edu/MNAcuteGVHDRiskScore
  - OR high risk on the basis of blood biomarkers (Ann Arbor Score 3 or amphiregulin ≥ 33 pg/ml) [[29, 30]]
- OR Minnesota standard (http://z.umn.edu/MNAcuteGVHDRiskScore) with aGVHD with no response to 7 days of sirolimus plus a calcineurin inhibitor.

Patients in this arm must start treatment within the first 7 days after onset of high-risk aGVHD.

or

Steroid- Dependent aGVHD (ARM 2A)

Pediatric or adult (ages 0-76 years) HCT recipient with grade II-IV steroid-dependent acute GVHD, defined as any one of the following:

- Flare of acute GVHD of at least grade II/IV severity within 8 weeks of tapering down or (off of) immunosuppression for acute GVHD, with the flare occurring on ≤0.5 mg/kg prednisone. This can include late-onset aGVHD and overlap syndrome.

or

Steroid-Refractory aGVHD (ARM 2B)

Pediatric or adult (ages 0-76 years) HCT recipient with grade II-IV steroid refractory acute GVHD, defined as any one of the following:

- No response of acute GVHD after at least 4 days of systemic corticosteroids of at least 2 mg/kg prednisone or equivalent
- Progression of acute GVHD within 3 days of systemic corticosteroids of at least 2 mg/kg prednisone or equivalent
- Failure to improve to at least grade II acute GVHD after 14 days of systemic corticosteroids, with initial doses of at least 2 mg/kg prednisone or equivalent
- Flare of acute GVHD of at least grade II/IV severity while on steroids at a dose >0.5/mg/kg/day. This can include late-onset aGVHD and overlap syndrome.

- Adequate organ function at study enrollment defined as:
  Renal: Serum creatinine ≤2.5x ULN (within 7 days)
  Cardiac: LVEF ≥ 35% (within 42 days)

- Voluntary written consent (adult or parent/guardian with minor assent for 12 through 17 year olds)

5.2 Exclusion Criteria

- Progressive malignancy
- Diagnosis of a hormone responsive malignancy
- Uncontrolled infection at initiation of protocol treatment
- Current thromboembolic disease requiring full-dose anticoagulation - patients receiving pharmacologic prophylaxis for thromboembolic disease will be eligible

- Active or recent (within prior 3 months) thrombus, irrespective of anticoagulation status
- Pregnancy as assessed on baseline blood hCG level
- Unwilling or unable to stop supplemental sex hormone therapy (estrogen, progesterone, and/or testosterone preparations)
- Women or men of childbearing potential unwilling to take adequate precautions to avoid pregnancy from the start of protocol treatment through 28 days after the last treatment

6 Patient Registration

Registration will occur after the patient has signed the subject consent and eligibility is confirmed, but before any treatment has been administered. To be eligible for registration to this study, the patient must meet each criteria listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record. A copy of the eligibility checklist is under attachments within the study in OnCore.

6.1 Registration with the Masonic Cancer Center Clinical Trials Office

Upon completion of the screening evaluation, eligibility confirmation and obtaining written consent, the study coordinator or designee will enroll the patient into OnCore. At the time of registration the patient will be assigned to either Arm 1 (high-risk aGVHD) or Arm 2A (steroid-dependent aGVHD) or Arm 2B (steroid-refractory aGVHD) in OnCore.

Complete registration information is found in the study's Procedures Manual for Affiliate Sites.

Affiliate sites only: At the time of registration, the signed consents will be uploaded into OnCore as an attachment under the appropriate record.

Affiliates are responsible for fulfilling any local registration requirements.

6.2 Pregnyl® Dose Level Assignment

The dose level of Pregnyl® will be assigned based on the currently enrolling dose for the patient specific aGVHD status (Arm 1, Arm 2A or Arm 2B) and Phase.

6.3 Patients Who Are Registered and Do Not Receive Study Treatment

If a patient is registered to the study, and is later found not able to begin study treatment, for whatever reason, the patient will be removed from study and treated at the physician's discretion. The study coordinator or designee will update OnCore of the patient's non-treatment status and notify the

Principal Investigator. The reason for removal from study will be clearly documented in OnCore. Such patients will be replaced within their assigned arm (Arm 1 – high-risk aGVHD or Arm 2A (steroid-dependent aGVHD) or Arm 2B steroid-refractory aGVHD).

6.4 Patients Who Do Not Complete All Study Treatments

A patient must receive at least 3 doses of Pregnyl to be evaluable for safety/tolerability in the phase I portion of the study. If a patient receives fewer doses and discontinues study therapy for a reason unrelated to study drug toxicity, the patient will be replaced within their assigned arm, however these patients may re-consent and enroll on the study again if they are eligible at a later time.

In the phase II portion of the study, a patient must receive at least 3 doses of Pregnyl to be assessed for response in Arm 1. In Arm 2A and 2B, a patient must receive at least 6 doses to be assessed for response. If a patient does not complete the number of doses necessary for response assessment, the patient will be replaced within their assigned arm, however these patients may re-consent and enroll on the study again if they are eligible at a later time.

7 Treatment Plan

Patients may remain on their standard of care current acute GVHD therapy, including calcineurin inhibitors, sirolimus, and systemic corticosteroids at the start of study treatment, unless any of these agents are determined to unsafe due to an unforeseen complication.

7.1 Arm 1 – High-Risk aGVHD

Standard of care immunosuppression, plus Pregnyl® (hCG supplementation) at assigned dose subcutaneously every other day for 4 doses

If a patient has a toxicity that fits the definition of dose limiting as defined in the phase I section below, the patient will discontinue study treatment.

Continue protocol treatment through day 7. Assess response at day 7:

- **If a complete or partial response**, the patient is eligible to receive Pregnyl maintenance at the same dose twice weekly for 10 doses beginning day 9 to 12. Refer to section 9 for response definitions.
- **If no response,** the patient will be taken off study treatment

Pregnyl® dose level assignment will be done by treatment arm, with Arm 1 independent of Arms 2A and 2B.
For phase I, up to 5 dose levels of Pregnyl® will be tested; however dose levels -1 and -2 will be used only if dose level 1 proves too toxic.

Dose Level	Dose
-2	125 USP hCG/875 pg EGF/m^2
-1	250 USP hCG/1,750 pg EGF/m^2
1 (start)	500 USP hCG/3,500 pg EGF/m^2
2	1,000 USP hCG/7,000 pg EGF/m^2
3	2,000 USP hCG/14,000 pg EGF/m^2

The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until all patients in the current cohort have reached day 21 in order to assess for dose limiting toxicity (DLT).

Dose limiting toxicity (DLT) is defined as any of the following occurring by day 21 after the 1st dose of Pregnyl® (phase I component only):

- In males and females: Grade 2-4 thromboembolic event (CTCAE v4) i.e., any thrombosis more severe than a superficial venous thrombosis develops and treatment with anticoagulation is required
- In males and females: Grade 3-4 ascites (CTCAE v4)
- Specific to females: Grade 3-4 ovarian hyperstimulation syndrome, defined as the rapid development of enlarged, cystic, painful ovaries - refer to section 11.2 for CTCAE grading guidance

Each new cohort of 2 patients will be assigned by the study statistician to the most appropriate dose level based on toxicity probabilities set for this study. The MTD will be identified when the total sample size of 20 patients is reached.

For the phase II the dose of Pregnyl will be that identified as the MTD during phase I.

7.2 Arm 2A – Steroid-Dependent aGVHD

Standard of care immunosuppression, plus Pregnyl® (hCG supplementation) at assigned dose subcutaneously every other day for 7 doses.

If a patient has a toxicity that fits the definition of dose limiting as defined in the phase I section below, the patient will discontinue study treatment.

Continue protocol treatment through day 14. Assess response at day 14:

- **If a complete or partial response**, the patient is eligible to receive Pregnyl maintenance at the same dose twice weekly for 10 doses beginning day 15 to 17. Refer to section 9 for response definitions.
- **If no response,** the patient will be taken off study treatment

Pregnyl® dose level assignment will be done by treatment arm, with Arm 2 independent of Arm 1.

For phase I, up to 5 dose levels of Pregnyl® will be tested; however dose levels -1 and -2 will be used only if dose level 1 proves too toxic.

Dose Level	Dose
-2	125 USP hCG/875 pg EGF/m^2
-1	250 USP hCG/1,750 pg EGF/m^2
1 (start)	500 USP hCG/3,500 pg EGF/m^2
2	1,000 USP hCG/7,000 pg EGF/m^2
3	2,000 USP hCG/14,000 pg EGF/m^2

The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until all patients in the current cohort has reached day 21 to assess for dose limiting toxicity (DLT).

DLT is defined as any of the following occurring by day 21 after the 1st dose of Pregnyl® (phase I component only):

- In males and females: Grade 2-4 thromboembolic event (CTCAE v4) i.e., any thrombosis more severe than a superficial venous thrombosis develops and treatment with anticoagulation is required
- In males and females: Grade 3-4 ascites (CTCAE v4)
- Specific to females: Grade 3-4 ovarian hyperstimulation syndrome, defined as the rapid development of enlarged, cystic, painful ovaries– refer to section 11.2 for CTCAE grading guidance

Each new cohort of 2 patients will be assigned by the study statistician to the most appropriate dose level based on toxicity probabilities set for this study. The MTD will be identified when the total sample size of 20 patients is reached.

For the phase II the dose of Pregnyl will be that identified as the MTD during phase I.

7.3 Arm 2B – Steroid-Refractory aGVHD

Standard of care immunosuppression, plus Pregnyl® (hCG supplementation) at assigned dose subcutaneously every other day for 7 doses.

If a patient has a toxicity that fits the definition of dose limiting as defined in the phase I section below, the patient will discontinue study treatment.

Continue protocol treatment through day 14. Assess response at day 14:

- **If a complete or partial response**, the patient is eligible to receive Pregnyl maintenance at the same dose twice weekly for 10 doses beginning day 15 to 17. Refer to section 9 for response definitions.
- **If no response,** the patient will be taken off study treatment

Pregnyl® dose level assignment will be done by treatment arm, with Arm 2 independent of Arm 1.

For phase I, up to 7 dose levels of Pregnyl® will be tested; however dose levels -1 and -2 will be used only if dose level 1 proves too toxic. The reason for this expansion is lack of rise in plasma EGF levels with Pregnyl therapy in patients with the most severe aGVHD in the first 6 patients in phase 1.

Dose Level	Dose
-2	125 USP hCG/875 pg EGF/m^2
-1	250 USP hCG/1,750 pg EGF/m^2
1 (start)	500 USP hCG/3,500 pg EGF/m^2
2	1,000 USP hCG/7,000 pg EGF/m^2
3	2,000 USP hCG/14,000 pg EGF/m^2
4	3,500 USP hCG/24,500 pg EGF/m^2
5*	5,000 USP hCG/35,000 pg EGF/m^2

***NOTE: To avoid using vials from more than one Lot of study product, the maximum dose is either (1) the contents of a single 10,000 IU vial, or (2) a maximum dose of 10,000 IU from multiple vials. All vials used should be from the same Lot.**

The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until all patients in the current cohort has reached day 21 to assess for dose limiting toxicity (DLT).

DLT is defined as any of the following occurring by day 21 after the 1st dose of Pregnyl® (phase I component only):

- In males and females: Grade 2-4 thromboembolic event (CTCAE v4) i.e., any thrombosis more severe than a superficial venous thrombosis develops and treatment with anticoagulation is required
- In males and females: Grade 3-4 ascites (CTCAE v4)
- Specific to females: Grade 3-4 ovarian hyperstimulation syndrome, defined as the rapid development of enlarged, cystic, painful ovaries– refer to section 11.2 for CTCAE grading guidance

Each new cohort of 2 patients will be assigned by the study statistician to the most appropriate dose level based on toxicity probabilities set for this study. The MTD will be identified when the total sample size of 20 patients is reached.

For the phase II the dose of Pregnyl will be that identified as the MTD during phase I.

7.4 Supportive Care

Patients will receive transfusions, infection prophylaxis (including appropriate viral, fungal, bacterial prophylaxis and preemptive viral monitoring per standard of care), growth factor support, and nutritional support according to the current University of Minnesota supportive care guidelines.

7.5 Duration of Study Therapy

Arm 1 (high-risk aGVHD): Study treatment will be for a minimum of 7 days with study therapy continuing for an additional 5 weeks if a complete or partial response is documented at the initial response evaluation. Non-responders at 7 days will be taken off study therapy and treated at the discretion of the treating physician. The treating physician may remove patients from study participation sooner if concerns regarding safety emerge prior to completing 7 days on protocol.

Arm 2A and 2B (steroid-refractory/dependent aGVHD): Study treatment will be for a minimum of 14 days with study therapy continuing for an additional 5 weeks if a complete or partial response is documented at the initial response evaluation. Non-responders at 14 days will be taken off study

therapy and treated at the discretion of the treating physician. The treating physician may remove patients from study participation sooner if concerns regarding safety emerge prior to completing 14 days on protocol.

An exception to the above is if a patient experiences an event that fits the definition of a dose limiting toxicity (Grade 2-4 thromboembolic event, Grade 3-4 ascites or Grade 3-4 ovarian hyperstimulation syndrome). Study treatment will be permanently discontinued in this situation.

7.6 Duration of Study Participation

All participants will be followed for adverse events through day 56, or if a non-responder at initial assessment; for 28 days after the last dose of Pregnyl® to rule-out early thromboembolic events. The remainder of follow up data for study endpoints will coincide with routine transplant related follow-up (chart review database query).

8 Study Drug Formulation, Preparation, Side Effects

8.1 Human Chorionic Gonadotropin (HCG) (Pregnyl® (Merck), or generic urinary-derived hCG from Fresenius Kabi)

Pregnyl® or its generic equivalent (chorionic gonadotropin for injection USP) is a highly purified pyrogen-free preparation obtained from the urine of pregnant females.

Complete prescribing information for urinary HCG can be located at the following internet address:

http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/017692s021lbl.pdf

PROCUREMENT: Pregnyl® or its generic equivalent will be purchased from a commercial source for the purposes of this study.

DOSAGE AND ADMINISTRATION: This drug is currently licensed by the FDA for intramuscular use only. However, in clinical practice, it is commonly given subcutaneously. Because of the risk of bleeding due to IM injections, and because of the otherwise off-label use for this drug, we have sought an IND from the FDA to administer Pregnyl® or its generic equivalent via subcutaneous route during this clinical trial.

PREPARATION: The drug is supplied in two-vial package containing:

1. 1-10 mL lyophilized multiple dose vial containing: 10,000 USP Units chorionic gonadotropin per vial, NDC 0052-0315-10 (Pregnyl) or NDC 63323 (Fresenius Kabi).
2. 1-10 mL vial of solvent containing: water for injection with sodium chloride 0.56% and benzyl alcohol 0.9%, NDC 0052-0325-10 (Pregnyl) or NDC 63323 (Fresenius Kabi).

Each lot of Pregnyl® or its generic equivalent will be tested for EGF content prior to administration so that the precise EGF dose administered is known.

When reconstituted, each 10 mL vial contains:

- Chorionic gonadotropin 10,000 USP Units
- Monobasic sodium phosphate 5 mg
- Dibasic sodium phosphate 4.4 mg
- Sodium chloride 0.56%
- Benzyl alcohol 0.9%

If required pH adjusted with sodium hydroxide and/or phosphoric acid.

NOTE: For Arm 2B, Dose level 5, in order to avoid using vials from more than one Lot of study product, the maximum dose is either (1) the contents of a single 10,000 IU vial, or (2) a maximum dose of 10,000 IU from multiple vials. All vials used should be from the same Lot.

IMPORTANT: RECONSTITUTED SOLUTION IS STABLE FOR 60 DAYS WHEN REFRIGERATED.

PRECAUTIONS

1. General: Since androgens may cause fluid retention, hCG should be used with caution in patients with cardiac or renal disease, epilepsy, migraine, or asthma.
2. Pediatric Use: Induction of androgen secretion by hCG may induce precocious puberty in pediatric patients treated for cryptorchidism. Therapy should be discontinued if signs of precocious puberty occur.
3. Geriatric Use: Clinical studies of PREGNYL® (chorionic gonadotropin for injection, USP) and its generic equivalent did not include subjects aged 65 and over.
4. ADVERSE REACTIONS: Headache, irritability, restlessness, depression, fatigue, edema, precocious puberty, gynecomastia, pain at

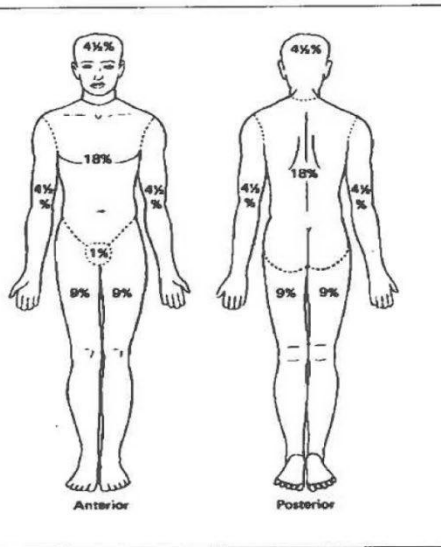
the site of injection. Hypersensitivity reactions, both localized and systemic in nature, have been reported.

9 Disease Reassessment and Response Definitions

Participants will be staged according to the Minnesota grading schema below:

GUIDE FOR GVHD STAGING

ORGAN	CLINICAL STAGE	
SKIN	1 = Rash <25% BSA 2 = Rash 25-50% BSA 3 = Generalized erythroderma 4 = Generalized erythroderma with bullous formation or desquamation	
	ADULT	PEDS
LOWER GI	0 = <500	(<280ml/m^2)
	1 = 500-1000	(281-555ml/m^2)
	2 = 1000-1500	(556-833ml/m^2)
	3 = >1500	(>834ml/m^2)
	4 = Severe abdomen pain with or without ileus, or stool with frank blood	
Liver	1 = Bili 2.1-3.0 2 = Bili 3.1-6.0 3 = Bili 6.1-15.0 4 = Bili >15.1	
UPPER GI	0 = No prolonged nausea or vomiting 1 = Persistent nausea, vomiting or anorexia	



Overall Grade	Organ Stage			
	Skin	Liver	Lower GI	Upper GI
I	1-2	0	0	0
II	3	1	1	1
III	-	2-4	2-3	
IV	4	-	4	

Response will be based on the following definitions:

- **Complete Response (CR):** a Stage of 0 for all organs with no additional intervening therapy for their GVHD.
- **Partial Response (PR):** improvement by at least 1 stage in all involved organs without progression in others with no additional intervening therapy for their GVHD.
- **Mixed Response (MR)**: improvement in one organ with deterioration in another organ manifesting symptoms of GVHD or development of symptoms of GVHD in a new organ.
- **Progression:** deterioration of any organ involved.

- **No Response (NR):** absence of any improvement or progression as defined above.

10 Schedule of Patient Activities

Scheduled evaluations after screening and through day 19 of the last treatment cycle may be performed +/-2 days from the targeted date; assessments performed after day 28 and through day 56 may be done +/-5 days of the targeted date. After day 56, assessments will coincide with the post-transplant follow-up schedule (i.e., additional clinic visits specifically for this study will not be required). In addition, targeted days may be altered as clinically appropriate, and will not constitute a protocol deviation.

Schedule of Treatment, Tests and Procedures – Arm 1

	Baseline	Treatment Days +/- 2 days											Phase I only	Follow Up +/- 5 days***	
Procedures, evaluations, and treatment		1	3	5	7	9	11	13	14	15	17	19	21	28	56
Standard of Care															
Vital signs and weight	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Performance status (Karnofsky or Lansky)	X				X				X			X		X	X
Acute GVHD staging	X				X				X					X	X
Complete blood count	X				X				X			X		X	X
Complete metabolic panel	X				X				X			X		X	X
Serum creatinine	X														
Ejection Fraction by echocardiogram or MUGA	X														
Research															
Pregnyl® dosing*		X	X	X	X	*									
Assess for toxicity		beginning with the 1st dose of Pregnyl® and continuing for 4 weeks after the last dose per section 11.2 (if phase I assess for DLT at day 21)													
Pharmacokinetics**		X			X										
Serum hCG	X^				X				X					X	X
Estradiol, progesterone, and testosterone	X				X				X					X	X
Collection of blood samples for immune and tissue damage/repair biomarkers (refer to section 10.3 for blood volume and tube type)s	X				X				X					X	X

Research related blood samples will go to Translational Therapy Lab (TTL) for processing and storage until study related testing.

*If a patient achieves a complete or partial response, he/she may continue to receive maintenance Pregnyl® doses twice per week, in the dose level he or she was already assigned, for another 10 doses, beginning during protocol day 9-12.
**Blood samples (2 ml plasma) collected 30 (± 10) minutes pre-dose, and at post-dose 1 hour (± 5 min), 3 hours (± 5 min), 6 hours (± 10 min), and 24 hours (± 30 min).
***After day 56 endpoint assessments will coincide with the post-transplant anniversary visits and the information may be abstracted from the medical record and/or the BMT database.
^ testing to rule-out pregnancy will be performed on females for eligibility screening

Schedule of Treatment, Tests and Procedures – Arm 2

	Baseline	Treatment Days +/- 2 days											Phase I only	Follow Up +/- 5 days***	
Procedures, evaluations, and treatment		1	3	5	7	9	11	13	14	15	17	19	21	28	56
Standard of Care															
Vital signs and weight	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Performance status (Karnofsky or Lansky)	X				X				X			X		X	X
Acute GVHD staging	X				X				X			X		X	X
Complete blood count	X				X				X			X		X	X
Complete metabolic panel	X				X				X			X		X	X
Serum creatinine	X														
Ejection Fraction by echocardiogram or MUGA	X														
Research															
Pregnyl® dosing*		X	X	X	X	X	X	X		*					
Assess for toxicity		beginning with the 1st dose of Pregnyl® and continuing for 4 weeks after the last dose per section 11.2 (if phase I assess for DLT at day 21)													
Pharmacokinetics**		X						X							
Serum hCG	X^				X				X					X	X
Estradiol, progesterone, and testosterone	X				X				X					X	X
Collection of blood samples for immune and tissue damage/repair biomarkers (refer to section 10.1 for blood volume and tube type)	X				X				X					X	X

Research related blood samples will go to Translational Therapy Lab (TTL) for processing and storage until study related testing.

*If a patient achieves a complete or partial response, he/she may continue to receive maintenance Pregnyl® doses twice per week, in the dose level he or she was already assigned, for another 10 doses, beginning during protocol day 15-17.
**Blood samples (plasma) collected 30 (± 10) minutes pre-dose, and at post-dose 1 hour (± 5 min), 3 hours (± 5 min), 6 hours (± 10 min), and 24 hours (± 30 min).
***After day 56 endpoint assessments will coincide with the post-transplant anniversary visits and the information may be abstracted from the medical record and/or the BMT database.
^ testing to rule-out pregnancy will be performed on females for eligibility screening

10.1 Correlative Studies and Hypotheses

1. Pharmacokinetics: Clearance, half-life, and volume of distribution of the both the hCG and EGF components of urinary hCG when administered to patients with GVHD. This objective tests the hypothesis that as enteropathy improves, the PK profile of both hCG and EGF will change.

2. Hormonal effects: concentrations of hCG, estradiol and progesterone, and testosterone at baseline, and on days 7, 14, 28, and 56 of protocol treatment. This objective tests the hypothesis that any increase in sex hormones induced by the hCG will be reversible and associated with no adverse events.

3. Biomarkers of response in routine clinical laboratory parameters: This objective tests the hypothesis that those receiving HCG will have (a) higher circulating numbers of total lymphocytes, monocyte, platelets, and albumin levels as measured by routine clinical laboratory testing and that all will correlate positively, with HCG treatment and plasma levels of both EGF and hCG.

The following studies detailed below in number 4-7 will be exploratory laboratory studies performed on blood samples collected for the study protocol. At each time point, the following samples will be collected:

For patients ≥ 40 kg

- 60 ml of heparinized blood (green top tubes) for PBMCs, and plasma stored in 15 x 1 ml aliquots.

For patients < 40 kg

- *Collect up to 2 cc/kg to a maximum of 65 ml total blood volume for placed in green top tubes. Plasma will be stored in 1 ml aliquots.*

4. Angiogenic biomarkers of response: This objective tests the hypothesis that higher plasma levels of EGF and VEGF-A and lower levels of follistatin will be observed in those receiving HCG treatment and will correlate with response to treatment.

5. Suppressive/regulatory markers of response: This objective tests the hypothesis that higher blood levels of suppressive/regulatory cytokines cells (IL-10, IL-22 – measured by multiplex array, and regulatory T cells – measured by flow cytometry) will be observed in those receiving HCG treatment and correlate with response to therapy.

6. Inflammatory markers of response: This objective tests the hypothesis that lower blood levels of inflammatory markers (TNFα, IL-6, IL-17, IL-33,– measured by multiplex array) will be observed in those receiving HCG treatment and correlate with response to therapy.

7. Accepted biomarkers of GVHD response: This objective tests the hypothesis that reduced levels of validated biomarkers of GVHD (e.g., TNFR1, REG3α, ST2, where elevated levels are indicative of poor response – measured by ELISA) will be observed in those receiving HCG treatment and will correlate with response to therapy.

8. Tissue expression of markers of regenerative capacity: This objective tests the hypothesis that tissue expression of EGF, IL-22, IDO, Ki-67 will be higher in patients with complete response to therapy. Analytes will be measured by immunohistochemistry (IHC) performed on slides prepared from gastrointestinal specimens previously collected for clinical purposes. No additional biopsies are required for protocol participation.

Separation of PBMCs and plasma, and enumeration of regulatory T cells by flow cytometry, will be performed by the Translational Therapy Laboratory. ELISAS and multiplex arrays will be performed by the Cytokine Reference Laboratory. IHC will be performed by the Histology core/AHC Bionet.

11 Adverse Event Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page http://evs.nci.nih.gov/ftp1/CTCAE/About.html

11.1 Definitions

Note: throughout this section the generic term “drug” refers to the Pregnyl®, or its generic equivalent, injection.

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.

Serious Adverse Event Or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

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

If either the IND sponsor or the investigator believes the event is life-threatening or serious, the event must be evaluated by the sponsor for expedited reporting (21CRF 312.32(a)).

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. Thus, adverse events that occur as part of the disease process or underlying medical conditions are considered unexpected; however, they will not be reportable per section 11.4.

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research.

Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

Expedited (Rapid) Reporting: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB, FDA) as detailed in section 11.4. For the IRB this is 5 business days from discovery. For studies under an IND, it is 7 or 15 calendar days.

11.2 Adverse Event Documentation

Due to the complex medical condition of the post-transplant patient, monitoring for adverse events will focus on the following events beginning with the 1st dose of Pregnyl® and continuing for 4 weeks after the last dose. Any of these events will be documented on the study specific case report form:

- death
- anaphylaxis
- thromboembolic event
- Grade 3-4 skin injection site reaction
- Grade 3 headache
- Grade 3 gynecomastia
- Grade 3-4 ascites

In addition, the following events occurring by day 21 after the 1st dose of Pregnyl® are considered a dose limiting toxicity (DLT) and should be reported as such for patients enrolled in the Phase I component per section 11.4:

- In males and females: Grade 2-4 thromboembolic event (CTCAE v4)
- In males and females: Grade 3-4 ascites (CTCAE v4)
- Specific to females: Grade 3-4 ovarian hyperstimulation syndrome, defined as the rapid development of enlarged, cystic, painful ovaries, Note: as there is no CTCAE grading for this event, the generic grading will apply as follows:

Grade	Description
1	mild; asymptomatic or mild symptoms; clinical or diagnostic observations
2	moderate; minimal or non-invasive intervention indicated

Grade	Description
3	severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated
4	life-threatening consequences; urgent intervention indicated

For the Phase II expansion cohort, an early study stopping rule was developed for excessive Grade 4/5 non-hematologic, non-infectious treatment probably or definitely attributed to the study drug occurring by day 28 after the last dose of Pregnyl® and is reportable per section 11.4. Refer to section 13.4 for details.

For both cohorts, relapse of the patient's malignancy and/or development of a new one occurring 4 weeks after the last dose is reportable per section 11.4.

11.3 Institutional Event Reporting Table

Individual institutional sites will be responsible for reporting any event meeting local reporting requirements to their institutional IRB and/or other research oversight committees.

Additionally institutional sites will be responsible for reporting events detailed in sections 11.1 and 11.2 within the following time frames to the MCC Affiliate Sites Manager

Event Type	Reporting Timeframe	Form in OnCore to Use	Report to
Any event meeting the definition of a SAE within 4 weeks after last dose of pregnyl	Within 24 hours of knowledge	SAE Report Form	Masonic Cancer Center (MCC) Affiliate Sites Manager affiliates@umn.edu Local institutional IRB or other entities per institutional policies and guidelines.
Event counting toward the safety stopping rule as defined in Section 13.4	Within 24 hours of knowledge	Stopping Rule Event Form	
Major Deviations, as defined in Section 11.1.	Within 5 working days of knowledge	Deviation Report Form	
Minor Deviations, as defined in Section 11.1.	Per Institutional Policy	n/a (record in Deviations Tab) but it is not necessary to report the event to MCC	For UMN MCC only: minor deviations are reported to the UMN IRB by the study's regulatory specialist per IRB reporting requirements. For Affiliate Sites: minor deviations are not reportable to the Masonic Cancer Center. Report to local institutional IRB or other entities per institutional policies and guidelines.

11.4 Required Reporting: FDA, IRB and Masonic Cancer Center's SAE Coordinator

As the study sponsor, the Masonic Cancer Center has the following expedited reporting responsibilities for local events and events reported in Section 11.3.

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers
U of MN IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm refer to http://www.research.umn.edu/irb/guidance/ae.html#.VC7xral0-sh	Within 5 business days of event discovery	IRB's Report Form	irb@umn.edu
FDA	Unexpected and fatal or life threatening suspected adverse reaction	As soon as possible but no later than 7 Calendar-Days	MCC SAE	Submit to CBER as an amendment to IND
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	As soon as possible but no later than 15 Calendar-Days		
	All other events per CRF 312.33	At time of IND annual report	Summary format	Submit as part of the IND annual report
Masonic Cancer Center SAE Coordinator	Events that meet the criteria of dose limiting toxicity (DLT) or an early study stopping rule	At time of reporting	Event Form	SAE Coordinator mcc-saes@umn.edu

In each IND safety report, the sponsor must identify all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of the previous, similar reports.

The SAE Coordinator will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

12 Study Data Collection and Monitoring

12.1 Data Management

This study will report clinical data using The Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database utilizing study specific electronic case report forms. Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore. Patient demographics, patient specific study treatment calendars, targeted adverse events and other information required for IND annual reporting will be placed in OnCore and other databases maintained by the Cancer Center.

12.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRF) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by study's Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRF based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

12.3 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp.

For the purposes of data and safety monitoring, this study is classified as high-risk (phase I). Therefore the following requirements will be fulfilled:

- The PI will complete and submit a quarterly Trial Progress Report to the Masonic Cancer Center Data and Safety Monitoring Council (DSMC) with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in section 11.1 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.
- The PI with the CTO has oversight responsibility for trial monitoring at affiliate sites.

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the Cancer Protocol Review Committee (CPRC).

12.4 IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the sponsor-investigator with assistance from the MCC Clinical Trials Office (CTO) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

12.5 Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the sponsor-investigator and/or sponsor/investigator designee, IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

12.6 Teleconferences – Lead Site and Affiliate Site

Regular teleconferences to facilitate communication between participating sites regarding the study's progress, patient updates, summary of safety reports, case report form completion, and other issues for discussion. The University of Minnesota Affiliate Manager will be responsible for arranging these teleconferences and preparing the agenda. Meetings will occur at least every 3 weeks; however, these may be scheduled more or less frequently at the discretion of the lead institution. Participation of a minimum of one representative from each affiliate site will be required. These teleconferences are in addition to other previously described site interactions including centralized patient registration, institutional and MCC required reporting of safety related issues, case report form completion in the study's central database (OnCore) and affiliate oversight through self-monitoring in compliance with the Masonic Cancer Center's Data and Safety Monitoring plan.

12.7 Affiliate Site Monitoring

The PI (Dr. Holtan) with the CTO has oversight responsibility for trial monitoring at affiliate sites. Affiliate sites must self-monitor following the University of Minnesota Masonic Cancer Center Data and Safety Monitoring

Plan (DSMP - http://z.umn.edu/dmsp) and the CTO Affiliate and Satellite Site Monitoring SOPs.

The investigator will permit study-related monitoring, audits, and inspections by the study's Principal Investigator or any designees, the local IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

12.8 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at 6 years after the study file is closed with the IRB and FDA.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO before destroying any study related records.

13 Statistical Considerations

13.1 Study Design

This is a multi-center phase I/II study of supplemental hCG as an adjunct to facilitate improved mucosal healing and immune tolerance in conjunction with accepted, standard immunosuppressive therapy in the setting of high-risk (Arm 1), steroid-dependent (Arm 2A) aGVHD and steroid-refractory (Arm 2B). Once the MTD is identified, the objective will be to confirm safety and obtain preliminary estimates of the primary trial endpoint defined as complete response (CR) and partial response (PR) at day 28.

Phase I component: Estimating the MTD of hCG for Arm 1 and Arm 2A

The first phase is a dose finding study. The dose escalation will be two independent processes with CRM for Arm 1 (high-risk aGVHD) and Arm 2A (steroid-dependent aGVHD). The target toxicity rate was determined based on clinical input and acceptability. Continual reassessment method (CRM)

is originally proposed by O'Quigley et al [31]. The goal for CRM is to identify the dose level which corresponds to the desired toxicity rate of 25% or less. We are choosing a lower target toxicity than would be chosen under the standard design. A standard "3+3" design will often choose a dose with corresponding to a toxicity rate of 33% or lower. The trial will stop if the posterior probability (that the lowest dose is more toxic than the target dose) is 0.90.

Dose Level	hCG Dose
-2**	125 USP hCG/875 pg EGF/m^2
-1*	250 USP hCG/1,750 pg EGF/m^2
1	500 USP hCG/3,500 pg EGF/m^2
2	1,000 USP hCG/7,000 pg EGF/m^2
3	2,000 USP hCG/14,000 pg EGF/m^2

*Dose level -1 (250 USP hCG/1,750 pg EGF/m^2) will be used only if Dose Level 1 (500 USP hCG/3,500 pg EGF/m^2) proves too toxic.
**Dose level -2 (125 USP hCG/875 pg EGF/m^2) will be used only if Dose Level -1 (250 USP hCG/1,750 pg EGF/m^2) proves too toxic.

Initial estimates of toxicity or the "skeleton" estimates are given by the clinicians. The estimates of toxicity for Arm 1 and Arm2 are listed as 1%, 2%, 5%, 10% and 20% for the 5 dose levels, respectively. The 1st 2 patients will be enrolled in dose level 1.

The next cohort of 2 patients will not begin treatment until the last patient in the current cohort has reached day 21. Each new cohort of 2 patients will be assigned by the study statistician (Qing Cao email: caox075@umn.edu). The CRM assumes the working dose-toxicity model or power model. The probability (toxicity at d_i) = $p_i^{\exp(\alpha)}$ for each dose level i. The parameter α is distributed a priori as a normal random variable with mean 0 and variance 2. After simulation, a variance of 2 was predicted to balance both patient safety and accuracy. Pre-specified constants p_i for each dose are 1st determined by using the skeleton estimates at the beginning of the trial. After initiation of the trial and collection of events, the unknown parameter α is continuously updated. Calculation of posterior means of probability of toxicity from dose level i are calculated from Bayes theorem using the likelihood function based on a binomial distribution for the binary outcome of toxicity. After calculation of these means, each new cohort is assigned to the dose with an estimated toxicity probability closest to the pre-specified target toxicity of 25%.

Eventually the MTD will be identified when the total sample size of 20 patients is exhausted.

The dose limiting toxicity (DLT) is defined as any of the following occurring by day 21 after the 1st dose of hCG.

- In males and females: Grade 2-4 thromboemcolic event (CTCAE v4)
- In males and females: Grade 3-4 ascites (CTCAE v4)
- Specific to females: Ovarian hyperstimulation syndrome, defined as the rapid development of enlarged, cystic, painful ovaries

Software within the BMA-CRMSimulator Version 2.1.0, downloaded from the MD Anderson biostatistics department website, will be used to calculate the posterior means of the toxicity probabilities for all of the doses under consideration:

http://biostatistics.mdanderson.org/SoftwareDownload. Dose escalation of more than one level is not permitted with this design.

Phase I component: Estimating the MTD of hCG for Arm 2B

The phase I design for Arm2B (steroid-refractory aGVHD) is similar to Arm 1 and Arm 2B. The goal for CRM is to identify the dose level which corresponds to the desired toxicity rate of 30% or less. We are choosing a lower target toxicity than would be chosen under the standard design. A standard "3+3" design will often choose a dose with corresponding to a toxicity rate of 33% or lower. The trial will stop if the posterior probability (that the lowest dose is more toxic than the target dose) is 0.90.

Dose Level	hCG Dose
-2**	125 USP hCG/875 pg EGF/m^2
-1*	250 USP hCG/1,750 pg EGF/m^2
1	500 USP hCG/3,500 pg EGF/m^2
2	1,000 USP hCG/7,000 pg EGF/m^2
3	2,000 USP hCG/14,000 pg EGF/m^2
4	3,500 USP hCG/24,500 pg EGF/m^2
5	5,000 USP hCG/35,000 pg EGF/m^2

*Dose level -1 (250 USP hCG/1,750 pg EGF/m^2) will be used only if Dose Level 1 (500 USP hCG/3,500 pg EGF/m^2) proves too toxic.
**Dose level -2 (125 USP hCG/875 pg EGF/m^2) will be used only if Dose Level -1 (250 USP hCG/1,750 pg EGF/m^2) proves too toxic.

Initial estimates of toxicity or the "skeleton" estimates are given by the clinicians. The estimates of toxicity for Arm 1 and Arm2 are listed as 1%, 2%, 5%, 10%, 15%, 20% and 25% for the 7 dose levels, respectively. The 1st 2 patients will be enrolled in dose level 1. The next cohort of 2 patients will not begin treatment until the last patient in the current cohort has reached day 21. Each new cohort of 2 patients will be assigned by the study

statistician (Qing Cao email: caox075@umn.edu). With same calculation described in Arm 1 and Arm 2A, each new cohort is assigned to the dose with an estimated toxicity probability closest to the pre-specified target toxicity of 30%. DLT has the same definition as the other two arms.

Operating Characteristics for Phase I (Arm 1 and Arm 2A)

Operating characteristics are calculated by BMA-CRMSimulator for two different scenarios, one in which the "true" probabilities of DLTs are the same as those specified by the clinicians and one in which the "true" probabilities of DLTs are much higher than expected. Table 2 shows that there is a 88% chance of selecting the maximum dose of 2,000 USP hCG/14,000 pg EGF/^{m2}, which is closest to our target toxicity of 25%. Table 3 shows that there is a 11% chance of stopping the study or a 28% chance of selecting the dose associated with a 25% toxicity rate.

Table 2. Simulations based on "true" probabilities equal to initial skeleton estimates.

Dose	Actual probability in theory of having a DLT at each dose	Probability of Selecting the specified dose given the probabilities of having a DLT listed in the 2nd column	Estimated Number of subjects treated at each dose level during the study
125 USP hCG/875 pg EGF/m^2	0.01	0.00	0.2
250 USP hCG/1,750 pg EGF/m^2	0.02	0.00	0.3
500 USP hCG/3,500 pg EGF/m^2	0.05	0.01	2.9
1,000 USP hCG/7,000 pg EGF/m^2	0.10	0.11	3.9
2,000 USP hCG/14,000 pg EGF/m^2	0.20	0.88	12.7

Table 3. Simulations based on "true" probabilities equal to excessively high toxicity.

Probability of stopping: 11%

Dose	Actual probability in theory of having a DLT at each dose	Probability of Selecting the specified dose given the probabilities of having a DLT listed in the 2nd column	Estimated Number of subjects treated at each dose level during the study
125 USP hCG/875 pg EGF/m^2	0.20	0.29	6.2
250 USP hCG/1,750 pg EGF/m^2	0.25	0.28	3.7
500 USP hCG/3,500 pg EGF/m^2	0.35	0.22	5.2
1,000 USP hCG/7,000 pg EGF/m^2	0.45	0.09	2.3
2,000 USP hCG/14,000 pg EGF/m^2	0.55	0.01	1.0

Operating Characteristics for Phase I (Arm 2B)

Operating characteristics are calculated by BMA-CRMSimulator for two different scenarios, one in which the "true" probabilities of DLTs are the

same as those specified by the clinicians and one in which the "true" probabilities of DLTs are much higher than expected. Table 4 shows that there is a 78% chance of selecting the maximum dose of 5,000 USP hCG/35,000 pg EGF/^{m2}, which is closest to our target toxicity of 30%. Table 5 shows that there is a 11% chance of stopping the study or a 29% chance of selecting the dose associated with a 30% toxicity rate.

Table 4. Simulations based on "true" probabilities equal to initial skeleton estimates.

Dose	Actual probability in theory of having a DLT at each dose	Probability of Selecting the specified dose given the probabilities of having a DLT listed in the 2nd column	Estimated Number of subjects treated at each dose level during the study
125 USP hCG/875 pg EGF/m^2	0.01	0.00	0.2
250 USP hCG/1,750 pg EGF/m^2	0.02	0.00	0.0
500 USP hCG/3,500 pg EGF/m^2	0.05	0.00	2.3
1,000 USP hCG/7,000 pg EGF/m^2	0.10	0.02	2.9
2,000 USP hCG/14,000 pg EGF/m^2	0.15	0.07	3.1
3,500 USP hCG/24,500 pg EGF/m^2	0.20	0.14	2.9
5,000 USP hCG/35,000 pg EGF/m^2	0.25	0.78	8.6

Table 5. Simulations based on "true" probabilities equal to excessively high toxicity.

Probability of stopping: 11%

Dose	Actual probability in theory of having a DLT at each dose	Probability of Selecting the specified dose given the probabilities of having a DLT listed in the 2nd column	Estimated Number of subjects treated at each dose level during the study
125 USP hCG/875 pg EGF/m^2	0.25	0.28	6.7
250 USP hCG/1,750 pg EGF/m^2	0.30	0.29	3.1
500 USP hCG/3,500 pg EGF/m^2	0.40	0.23	5.3
1,000 USP hCG/7,000 pg EGF/m^2	0.50	0.07	2.6
2,000 USP hCG/14,000 pg EGF/m^2	0.60	0.02	0.7
3,500 USP hCG/24,500 pg EGF/m^2	0.70	0.00	0.2
5,000 USP hCG/35,000 pg EGF/m^2	0.75	0.00	0.1

Phase II extension: Studying the efficacy of hCG (Arm 1, Arm 2A and Arm 2B)

After completion of the dose finding trial for each arm, the final doses will be carried forward into a two-stage phase II extension trial to confirm safety and make a preliminary determination of the activity level for Arm 1 and Arm 2. If the phase I trial enrolls fewer than 13 patients at the MTD, we will employ Simon's Minmax two-stage design (Simon, 1989) with the possibility to discontinue after the 1st stage if the response rate is low.

- **Stage 1:** Enroll a total of 12 patients (including all treated at the MTD in phase I). *If 5 or more of these respond, the trial will continue to stage 2.*

- **Stage 2:** Enroll an additional 14 patients. *If 14 or more out of 26 patients respond, hCG will be considered promising for further investigation.*

If phase I trial enrolls more than 12 patients at the MTD, we will adjust the futility rule above for the two stage design with the giving number of patients enrolled at the MTD.

13.2 Sample Size Consideration

Resulting from laboratory studies conducted on the first several patients on this study, the investigative team plans to test higher doses in steroid-refractory patients with aGVHD (separating of Arms into 2A and 2B). This necessitates expanding the number of participants treated on the arms as described below:

Arm 1 Treated Patients	Arm 2A Treated Patients	Arm 2B Treated Patients
Level 1: N = 2, DLT = 0	Level 1: N = 1, DLT = 0	Level 1: N = 1, DLT = 0
Level 2: N = 2, DLT = 0	Level 2: N = 0, DLT = 0	Level 2: N = 2, DLT = 0
Level 3: N = 6, DLT = 0	Level 3: N = 3, DLT = 0	Level 3: N = 4, DLT = 0
Enroll 10 more patients Next dose level 3: N = 2	Enroll 16 more patients Next dose level 3: N = 2	Enroll 13 more patients Next dose level 4: N = 2

- The next dose was calculated with R package "dfcrm".
- The next dose for Arm 2B was dose level 5, but restrict the dose jump, so the next dose will be dose level 4.

We now anticipate recruitment of 20 participants in each of the 3 arms to complete phase I.

In phase I, based on resources, the maximum number of allowable evaluable patients is 20 for each arm (total of 60 for all three arms). The simulations show it should be sufficient and safe to define the MTD for future efficacy trials.

For Arm 1 or 2A, if fewer than 13 patients are enrolled at the MTD, the phase II extension trial using Simon's two-stage Minmax design will require 26 patients to test the null hypothesis that the response rate at day 28 is <40% versus the alternative that the response rate is ≥ 65% after assuming 80% power and a significance level of 0.05 and using a maximum of 26 patients. We will choose the rule that minimizes the expected sample size giving a true response rate equivalent to the null hypothesis. We expect that for each arm, phase I and II will require approximately 33 patients due to use of patients from the phase I trial in the phase II extension (table 2 shows about 13 patients will be treated under MTD). But the sample size for each

arm could be as high as 44 when only two patients were treated at MTD in phase I. Therefore we expect the sample size of the phase I/II study for Arm 1 and Arm 2A will be 66 to 88 patients.

For Arm 2B, same as the other two arms, if fewer than 13 patients are enrolled at the MTD, the phase II extension trial using Simon's two-stage Minmax design will require 26 patients to test the null hypothesis that the response rate at day 28 is <40% versus the alternative that the response rate is ≥ 65% after assuming 80% power and a significance level of 0.05 and using a maximum of 26 patients. We expect that for each arm, phase I and II will require approximately 37 patients due to use of patients from the phase I trial in the phase II extension (table 4 shows about 9 patients will be treated under MTD). But the sample size could be as high as 44 when only two patients were treated at MTD in phase I. We expected the sample size of phase I/II study for Arm 2B will be 37 to 44 patients.

The number of evaluable patients per year will be approximately 20 for each arm. We expect accrual to be completed within 7 years.

13.3 Statistical Analysis

SAS 9.3 (Cary, NC, USA) will be used for the statistical analyses. For binary endpoints such as toxicity, summary statistics including proportions and their 95% confidence intervals will be calculated for each arm. All toxicity and safety assessments will be presented in the data listings and summarized with descriptive statistics. Response at day 28 will be estimate with simple proportions and descriptive plots.

The secondary endpoint, proportions of response among the surviving patients at day 7, 14, 56 and 180, treatment failure proportion for acute GVHD at day 28, 56 and 180 after initiation of protocol therapy, the incidence of acute GVHD flare after CR/PR requiring increase of steroids or other systemic treatment at days 28, 56, and 180 will be estimated. Overall survival and disease free survival probabilities after the initial treatment will be estimated using Kaplan-Meier's method. Treatment-related mortality and the incidence of chronic GVHD after the initial treatment will be estimated by competing risk analysis. For other correlative endpoints, continuous measures will be summarized with mean and SD for each arm and time point (if skewed, we will report median and interquatile range) and categorical measures will be summarized with frequency and percentage.

13.4 Early Stopping Rule

During Phase I CRM, the trial will stop if the posterior probability that the lowest dose is more toxic than the target is greater than 90%. Stopping rules will also be employed to monitor excess toxicity outside of the phase I trial. Stopping rule was developed using Pocock stopping boundaries [32]. The stopping rule will be monitored for Arm 1, 2A and Arm 2B separately.

Early Stopping Rule for Toxicity

Stopping rules were developed for excessive Grade 4/5 non-hematologic, non-infectious toxicity attributed to the study drug occurring by day 28 after the last dose of hCG. The goal is to construct a boundary based on toxicity such that the probability of early stopping is at most 10% if the toxicity rate is equal to 20% and assuming 13 more patients of each arm are enrolled after phase I. Given these parameters, the upper stopping boundary for toxicity is 2 events out of 2 patients, 3 events out of 3 patients, 5 out of 8, or 6 at any time. If the true toxicity rate is as high as 50% then chance of early stopping is 80% and the expected sample size is 7.3.

14 Conduct of the Study

14.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

14.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

14.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of

the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

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