RANDOMIZED CONTROLLED PILOT STUDY USING PROPRANOLOL TO DECREASE GENE EXPRESSION OF STRESS-MEDIATED BETA-ADRENERGIC PATHWAYS IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

Version 3.0

Principal Investigator:
Jennifer M. Knight, MD
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI 53226
Telephone: 414-955-8908
Fax: 414-955-6285
Email: jmknight@mcw.edu

Co-Investigator:
J. Douglas Rizzo, MD, MS
CIBMTR
Froedtert and the Medical College of Wisconsin
Clinical Cancer Center
9200 W. Wisconsin Avenue Suite C5500
Milwaukee, WI 53226
Telephone: 414-805-0700
Fax: 414-805-0714
Email: drizzo@mcw.edu

Co-Investigator:
Parameswaran Hari, MD, MS
Froedtert and the Medical College of Wisconsin
Clinical Cancer Center
9200 W. Wisconsin Avenue Suite C5500
Milwaukee, WI 53226
Telephone: 414-805-4600
Fax: 414-805-4606
Email: phari@mcw.edu

Consultant and Statistician:
Steve W. Cole, PhD
UCLA-David Geffen School of Medicine
10833 LeConte Ave
11-934 Factor Bldg
Los Angeles, CA 90095-1678
Telephone: 310-267-4243
Email: coles@ucla.edu

Sponsor: Medical College of Wisconsin

Funding Sponsor: This project has an offer of sponsorship from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E.

PROTOCOL SYNOPSIS

Randomized Controlled Pilot Study Using Propranolol to Decrease Gene Expression of Stress-Mediated Beta-Adrenergic Pathways in Hematopoietic Stem Cell Transplant Recipients

Principal Investigator:	Jennifer M. Knight, MD
Study Design:	This is a randomized controlled pilot study designed to evaluate whether the beta-adrenergic antagonist propranolol is effective in decreasing gene expression of stress-mediated beta-adrenergic pathways among a cohort of individuals receiving an autologous hematopoietic stem cell transplant (HCT) for multiple myeloma.
Primary Objective:	The primary objective of this study is to assess whether beta-blocker administration to individuals undergoing HCT alters genome-wide transcriptional pathways involved in beta-adrenergic signaling.
Secondary Objectives:	Explore the impact of socioeconomic status, depression, and anxiety on gene expression as well as on the relationship between beta-blocker and gene expression; assess clinical feasibility and efficacy.
Eligibility:	Eligible patients are between 18 and 75 years undergoing autologous HCT for treatment of multiple myeloma who are ≤ 1 year since initiation of systemic anti-myeloma therapy and who have had no prior progression or relapse of myeloma prior to HCT. They must have an ECOG performance status of 0 or 1, and have a stem cell graft with >2.0 x 10^6 CD34+ cells/kg available for transplant. Patients are excluded if they have had a prior autologous HCT, are on a beta-blocker at the time of study entry, have a previous intolerance to beta-blockers, have any medical contraindications to beta-blockers therapy, or have active depression.
Treatment Description:	Patients enrolled in this study will be randomized to either receive propranolol or not starting 7 (± 2) days prior to transplant and continuing through 28 days post-transplant. Propranolol will start at 20mg

	twice daily and will be titrated to 40mg twice daily as tolerated.
.	
Accrual Objective:	40 patients will be enrolled to the clinical trial.
Accrual Period:	The estimated accrual period is 9 months.
Study Duration:	It is estimated that the entire duration of the study will not exceed one year as follow-up is 30-45 days.

Glossary of Study Abbreviations

AE:	adverse events
ANC:	absolute neutrophil count
CC:	Cancer Center
CR:	complete response
CRC:	clinical research coordinator
CTCAE:	Common Terminology Criteria for Adverse Events
CTRA:	conserved transcriptional response to adversity
DSM:	Data and Safety Monitoring
DSMC:	Data and Safety Monitoring Committee
ECOG:	Eastern Cooperative Oncology Group
FCBP:	Female of Child Bearing Potential
G-CSF:	granulocyte-colony stimulating factor
HADS:	Hospital Anxiety and Depression Scale
HCT:	hematopoietic stem cell transplantation
HED:	human equivalent dose
ICSR:	Individual Case Safety Reports
IMWG:	International Myeloma Working Group
IRB:	Institutional Review Board
MCW:	Medical College of Wisconsin
MM:	multiple myeloma
nCR:	near complete response
OS:	overall survival
PFS:	progression-free survival
PI:	Principal Investigator
SES:	socioeconomic status
sCR:	stringent complete response
SNS:	sympathetic nervous system
TRM:	treatment-related mortality
UCLA:	University of California Los Angeles
VGPR:	very good partial response

Table of Contents

1.0 BACKGROUND AND RATIONALE:

1.1 Beta-adrenergic signaling in the tumor microenvironment

Research suggests beta-adrenergic signaling regulates multiple cellular processes that contribute to the initiation and progression of cancer, including inflammation, angiogenesis, apoptosis/anoikis, cell motility and trafficking, activation of tumor-associated viruses, DNA damage repair, cellular immune response, and epithelial–mesenchymal transition.[1] Within the tumor microenvironment, beta-adrenergic receptors on tumor and stromal cells are activated by catecholamines from local sympathetic nerve fibers (norepinephrine) and circulating blood (epinephrine). Studies of beta-adrenergic influence on tumor biology were motivated by epidemiologic observations in humans associating stressful life circumstances with accelerated progression of incident cancers.[2, 3] In several experimental cancer models, activation of the sympathetic nervous system (SNS) has been shown to promote the metastasis of solid epithelial tumors and the dissemination of hematopoietic malignancies via beta-adrenoreceptor–mediated pathways.[4] SNS regulation of cancer cell biology and the tumor microenvironment is helping to clarify the molecular basis for long-suspected relationships between stress and cancer progression, and now suggests a highly leveraged target for therapeutic intervention.[1]

1.2 Beta-blockade and cancer

Retrospective epidemiologic studies have linked the use of beta-blockers to reduced rates of progression for several solid tumors.[5-9] Recently, a retrospective case-control study of MM patients identified that concurrent use of any beta-blocker is associated with more favorable 5-year overall and disease-specific survival.[10] Preclinical pharmacologic and biomarker studies in animals are now laying the groundwork for translation of beta-blockade as a novel adjuvant to existing therapeutic strategies in clinical oncology, linking the use of beta-adrenergic antagonists (beta-blockers) with reduced disease progression in humans.[4-9, 11-17] Further, propranolol, a non-selective beta-adrenergic receptor blocker, has apoptotic and anti-proliferative effects on multiple myeloma (MM) cells.[18] Propranolol is the most studied nonselective beta-blocker;[19] this, along with its safe side effect profile, cost-effectiveness, and efficacy in vitro in preventing tumor progression as compared to selective beta-antagonists[1, 16, 20] make it our preferred beta-blocker for use in the current proposal. Propranolol does not have any significant drug interactions with the more common antineoplastic (melphalan) and infection-related drugs utilized to treat MM.

The first 30 days following autologous hematopoietic stem cell transplantation (HCT) generally constitute the time period around transplantation of greatest psychological and physiological stress and inflammatory processes.[21, 22] Psychosocial stress gradually improves over time with a return to pre-transplant psychosocial functioning by about one year post-transplant.[23, 24] Therefore, it follows that the first 30 days may also be the time period of highest beta-adrenergic signaling due to increased stress response. Further, data

from mouse models have demonstrated that beta-blockade with propranolol 8 days prior to exogenous stress exposure is effective in blocking beta-adrenergic signaling at the tumor level.[4] Tumor metastasis in mouse models is increased 30-fold as compared to the control group after 20 days of exposure to chronic stress.[25]

1.3 Propranolol dosing for cancer control

Murine models have demonstrated propranolol serum concentrations of 20 ng/ml (range 0.16-0.26 ng/ml) (Sloan, personal communication) with sustained-release propranolol 0.5mg over 21 days to be effective in beta-blockade mediated mitigation of stress-induced tumor progression/metastases.[25] Animal to human dosing may be converted using the dose translation formula based on body surface area where human equivalent dose (HED) (mg/kg) = animal dose (mg/kg) multiplied by Animal K*m*/Human K*m* (where animal K*m* = 3, human K*m* = 37).[26] The HED is 122 mg/day or 142 mg/day for a 60 kg and 70 kg human, respectively. However, the 0.5 mg dose is not a single-dose; therefore, with the sustained-release formulation the HED could be as low as 6 mg/day or 7 mg/day, respectively. The actual HED is likely near the midpoint of the low and high values calculated, which would be 60 to 80 mg per day. Therefore, a goal dose of 40mg orally twice daily in humans should achieve similar serum concentrations as that demonstrated in mice to affect cancer progression. Both 20mg and 40mg of propranolol (one administration of a twice daily administration regimen) are effective for anxiolysis.[27] Given this as well as the variable pharmacokinetic profile of propranolol,[28-30] it is also feasible that 20mg bid may affect change at the level of gene expression.

1.4 Socioenvironmental impact on gene expression

Stress-induced beta-adrenergic signaling within the tumor microenvironment also results in alterations in SNS-mediated gene expression; this was identified in an ovarian cancer cohort of 10 individuals with elevated biobehavioral risk factors compared to relative grade- and stage-matched individuals without these risk factors.[31] This type of socioenvironmental impact on gene expression has been consistently demonstrated, with previous studies showing that circulating immune cells show a systematic shift in basal gene expression profiles during extended periods of stress, threat, or uncertainty, consistent with the physiology of stress-associated illness.[32-36] Recently, we identified increased expression of genes mediated by beta-adrenergic signaling in a cohort of HCT recipients with acute myelogenous leukemia in first complete remission (N=78) under conditions of increased stress as measured by low socioeconomic status (SES) (unpublished data). These changes associated with low SES are reciprocally associated with increased relapse and leukemia-free survival (unpublished data). It is unknown, however, whether a nervous system-targeted intervention such as beta-blocker therapy would affect change at the level of gene expression.

Evaluating a molecular outcome with the magnitude of data that is available as with gene expression studies allows us to assess biobehaviorally-mediated biological changes between two differing groups utilizing a small cohort, as has been done previously.[31, 35] This design provides the advantage of conducting a small, short-term, and inexpensive

pilot study to first assess the feasibility and tolerability of administering a beta-blocker to a cohort of HCT recipients while also obtaining novel information on potential mechanisms.

1.5 Rationale

There has never been a prospective human trial prophylactically administering beta-blocker therapy with conventional cancer treatments to affect change at the level of the tumor microenvironment. We aim to implement the first human pilot study using a beta-blocker to pharmacologically intervene in biobehaviorally mediated gene expression in cancer patients to expand our previous findings. Specifically, we aim to evaluate this effect among patients receiving HCT for MM, the number one indication for autologous transplantation at MCW as well as in the US and worldwide. Using an autologous rather than allogeneic HCT population will minimize the number of potentially confounding variables for purposes of this study. The long-term goal is to utilize findings from this study is to alter tumor progression.

2.0 STUDY DESIGN

This is a proof of concept randomized controlled pilot study assessing whether gene expression of beta-adrenergic signaling pathways can be altered in individuals undergoing autologous HCT for MM by administering a daily beta-blocker (propranolol) to 20 participants. Outcomes of patients on this clinical trial will be compared to 20 participants in a control arm. Study assessment time points are detailed in Table 4.7a.

2.1 Primary Objective

The primary objective of this study is to assess whether beta-blocker administration to individuals undergoing HCT alters genome-wide transcriptional pathways involved in beta-adrenergic signaling.

2.2 Secondary Objectives

The secondary objectives of this study are:

1) To explore additional gene expression endpoints including:
    a) the impact of SES, depression, and anxiety on gene expression as well as on the relationship between beta-blocker administration and gene expression profiles

2) To assess clinical feasibility and efficacy by
    a) testing the feasibility (adherence, tolerance) of using beta-blockers with an intensive conventional antineoplastic treatment regimen (autologous HCT) in a cancer population
    b) describing meaningful clinical endpoints between the intervention vs. control arms including depression and anxiety, proportion of

patients developing engraftment syndrome, time to neutrophil and platelet engraftment, incidence of infection, myeloma response, treatment-related mortality, progression-free survival, and overall survival

2.3 Patient Eligibility

2.3.1 Inclusion Criteria
Patients with multiple myeloma receiving an autologous HCT are eligible when the following criteria are met:

1. 18-75 years of age
2. ≤ 1 year since initiation of systemic anti-myeloma therapy
3. Patient is scheduled for autologous hematopoietic stem cell transplant as the upfront therapy for their multiple myeloma
4. Karnofsky Performance Status of ≥80 %; patients eligible for HCT are eligible for the study
5. All men and women must agree to practice effective contraception during the study period if not otherwise documented to be infertile.

2.3.2 Exclusion Criteria

1. Prior autologous HCT
2. Non secretory multiple myeloma
3. Concurrent beta-blocker therapy at or within 3 weeks of study entry. Refer to section 2.4.4 for complete list.
4. Previous intolerance to beta-blocker therapy
5. Any medical contraindications to beta-blocker therapy including, but not limited to, symptomatic hypotension; drug hypersensitivity; sinus bradycardia, sick sinus syndrome, or 2nd or 3rd degree atrioventricular block without a pacemaker; uncompensated heart failure; or uncontrolled asthma
6. Active, untreated depression screened for by the HCT physician (Patients who screen positive will be offered a referral to the MCW Psycho-Oncology program for further evaluation and treatment)
7. Concurrent use of medications in section 2.4.4 throughout the study or within one week of study entry.
8. Pregnant or lactating women

2.4 Treatment Plan

The study drug will be dispensed. Compliance will be monitored by the clinical research coordinator (CRC). Beta-blocker tolerability will be assessed clinically by the treating physician on a weekly basis and for 1 week following cessation of beta-blocker therapy; subjects will be questioned about specific side effects as well as assessed for blood pressure and heart rate during weekly appointments or by phone.

2.4.1 Beta-blocker dosing schedule

We will take a conservative approach by **initiating propranolol 20 mg orally twice daily** for a week at the time of study commencement (Day -7 ± 2 days with respect to day of transplant being Day 0; nomenclature throughout protocol continues as such; see Figure 1) in an effort to minimize cardiac effects in this medically ill population. The study PI and treating HCT physician will assess drug tolerability and increase the dose to **40 mg orally twice daily on Day 0** if there are no adverse medical or psychiatric side effects as described in Section 2.6. In particular, if study participants have a heart rate of < 50, a blood pressure of < 90/55, significant fatigue impairing their function, or signs or symptoms of congestive heart failure, they will be taken off propranolol. If they have any noticeable side effects but are less severe than described above and the patient is able to tolerate staying on propranolol, their dose will be held at 20mg twice daily. If participants are tolerating propranolol without any side effects, they will be increased to 40mg twice daily. **The maximum dose will be 40 mg twice daily.** Study participants who remain at 20mg orally twice daily will continue on as study subjects. This informs the feasibility aim of the study as well as contributes to the drug-exposed population for gene expression analyses. As demonstrated in the above studies, there is a wide range of serum concentrations that can have clinical effectiveness. The target dose of 40 mg twice daily should possess the desired antitumor effects while minimizing hemodynamic side effects in most patients. This should coordinate well with Sloan's murine model that demonstrated anti-tumor effects at a serum level of 20 ng/ml and should maximize the likelihood of effecting change at the level of gene expression.

Patients taking antihypertensive medications will be monitored in the same manner as the other study participants with regular blood pressure and heart rate assessments. If patients already taking antihypertensive medication(s) develop low blood pressure on propranolol, their antihypertensive medication will be lowered and the propranolol dose will be continued as tolerated. Similarly, as the study drug is weaned, their antihypertensive medication(s) will be titrated as necessary.

2.4.2 Study interruption

Participants needing to stop beta-blocker therapy secondary to intolerance or new onset of a contraindication will not be considered for resumption of therapy. Outcome and medical data will continue to be collected and assessed for intervention arm participants despite an inability to remain on beta-blocker therapy for the study duration.

2.4.3 Beta-blocker weaning

Beta-blocker will be weaned for one of three reasons: 1) completion of drug intervention (28 ± 2 days post-transplant), 2) intolerance secondary to side effects, or 3) onset of new medical symptoms rendering beta-blocker therapy as contraindicated (see section 2.3.2). For patients that are at 40 mg twice daily at the time of weaning, the dose will be reduced to 20 mg twice daily for one week before being discontinued entirely. For patients who are on 20 mg twice daily at the time of weaning, their dose will be stopped altogether.

2.4.4 Prohibited concomitant medications and treatments

- *Beta-Blocker therapy* including but not limited to: Atenolol, Carvedilol, Labetalol, Metoprolol, Nadolol, Propranolol, Sotalol, Timolol
- *Pgp substrates*: Bosutinib, PAZOPanib, Silodosin, Topotecan, VinCRIStine (liposomal)
- Ceritinib, Floctafenine, Methacholine
- *Herbal medications,* including but not limited to: St. John's Wort, ginko biloba, saw palmetto, and ginseng

2.4.5 Concomitant medications to be used with caution: Require an investigational drug pharmacist consult to evaluate drug interaction

- Full list can be found in Appendix A
- Antihypertensives and bradycardia causing agents:
    - *Alpha1 blockers,* including but not limited to: Doxazosin, Prazosin,Tamsulosin; Terazosin
    - *Alpha2 agonist,* including but not limited to: CloNIDine,GuanFACINE,TiZANidine
    - *Alpha/Beta-Agonist:* Dopamine, DOPamine; EPINEPHrine; Isometheptene; Levonordefrin; Norepinephrine
- ***Scheduled and As Needed*** *Beta2-Agonist therapy:* Albuterol; Arformoterol; Bambuterol; Fenoterol; Formoterol; Indacaterol; Levalbuterol; Metaproterenol; Olodaterol; Pirbuterol; Salmeterol; Terbutaline; Vilanterol
- *Pgp substrate:* Afatinib, Colchicine, DOXOrubicin, Dabigatran, Edoxaban, Everolimus, Rivaroxaban, vincristine
- *Strong Cyp1A2 inducers,* including but not limited to: CarBAMazepine, PHENobarbital, Rifampin
- *Strong Cyp1A2 inhibitors,* including but not limited to: Cipro, FluvoxaMINE, Primaquine
- *Cyp1A2 Substrates,* including but not limited to: Vemurafenib
- *Strong Cyp2D6 inhibitors,* including but not limited to: BuPROPion,Cinacalcet, FLUoxetine, Lopinavir, PARoxetine, Ritonavir, Terbinafine
- *Cyp2D6 Substrates,* including but not limited to: Abiraterone

2.5 Study Drug Information

Propranolol is highly protein bound (89%) and undergoes extensive first-pass metabolism (hepatic extraction ratio 0.7-0.9) yielding nonrestrictive elimination where the hepatic extraction ratio is greater than the amount of unbound drug. This results in significant intra-patient variability. At higher doses used to achieve serum concentrations >30 ng/ml, propranolol manifests linear kinetics.[30, 37] Owing to this unique pharmacokinetic profile it is difficult to calculate a predicted serum concentration. However, given that a mouse serum concentration target of 20 ng/ml has been established and that this theoretically correlates with target human serum concentration,[25, 38] we can apply human dose-finding studies to determine HED need to achieve said concentration. At a dose of 120 mg/day mean serum trough concentrations have been reported as 54 ± 34 ng/ml and 41.6 ±37.6

ng/ml while peak serum concentrations are >100 ng/ml.[39, 40] The optimal cardiac dose has been established at 144 mg/day, yielding a serum trough concentration of 30 ± 7 ng/ml; however, some have suggested concentrations > 20 ng/ml manifest cardiac effects.[40] Similarly, other studies associate propranolol 80 mg/day with serum concentration in the 10 to 30 ng/ml range.[28, 29, 41] Therefore, a goal dose of 40 mg orally twice daily in humans should achieve similar serum concentrations as that demonstrated in mice to affect cancer progression, with the possibility that the anxiolytic dose of 20 mg twice daily may have similar effects. Drug supply and storage will be coordinated with the F/MCW Clinical Trials Office.

2.6 Participant Risks

Rare

☐ Dizziness or passing out
☐ Hard stools (constipation)
☐ Loose stools (diarrhea)
☐ Upset stomach or throwing up
☐ Feeling sleepy
☐ Feeling tired or weak
☐ Not able to sleep

Rare but serious adverse events that have been reported

☐ Chest pain that is new or worse
☐ Change in thinking clearly and with logic
☐ Hallucinations
☐ Memory problems or loss
☐ Mood changes
☐ A burning, numbness, or tingling feeling that is not normal
☐ Change in eyesight
☐ Shortness of breath, a big weight gain, selling in the arms or legs
☐ Any bruising or bleeding
☐ Slow heartbeat
☐ A heartbeat that does not feel normal
☐ Feeling cold
☐ Low blood sugar, signs may include dizziness, headache, feeling sleepy, feeling weak, shaking, a fast heartbeat, confusion, hunger, or sweating.
☐ A very bad skin reaction (Stevens-Johnson syndrome/toxic epidermal necrolysis) may happen. It can cause very bad health problems that may not go away, and sometimes death. Get medical help right away if you have signs like red, swollen, blistered or peeling skin (with or without fever), red or irritated eyes, or sores in your mouth, throat, nose, or eyes.
☐ In some cases if you stop taking propranolol all of a sudden you may develop chest pain that is worse and in some cases a heart attack may occur. This risk may be greater if you have certain types of heart disease. To avoid side effects, do not stop taking propranolol all of a sudden, you want to slowly stop this drug as ordered by your doctor. Call your doctor right away if you have new or worse chest pain or if other heart problems occur.

Allergic Reactions
☐ Allergic reaction, such as rash; hives; itching; red, swollen, blistered or peeling skin with or without fever; wheezing; tightness in the chest or throat; trouble breathing or talking; unusual hoarseness; or swelling of the mouth, face, lips, tongue or throat

In some cases if you stop taking propranolol all of a sudden you may develop chest pain that is worse and in some cases a heart attack may occur. This risk may be greater if you have certain types of heart disease. To avoid side effects, do not stop taking propranolol all of a sudden, you want to slowly stop this drug as ordered by your doctor. Call your doctor right away if you have new or worse chest pain or if other heart problems occur.

The FDA black box warning:
In some cases if the patient stops taking propranolol all of a sudden the patient may develop chest pain that is worse and in some cases a heart attack may occur. This risk may be greater if the patient has certain types of heart disease. To avoid side effects, do not stop taking propranolol all of a sudden, if the patient wants to slowly stop this drug as ordered by their doctor. The patient should call the doctor right away if the patient has new or worse chest pain or if other heart problems occur.

3.0 STUDY ENDPOINTS

3.1 Primary Endpoint

Expression levels of beta-adrenergic mediated gene expression will be compared between individuals randomized to propranolol vs. control just prior to HCT as well as 28 days following autologous HCT for MM. Quantification of whole genome RNA production will be performed with specific identification of expression of beta-adrenergic signaling pathways.

3.2 Secondary Endpoints

3.2.1 Gene expression

3.2.1.1 Gene expression based on psychosocial factors

Expression levels of beta-adrenergic mediated gene expression will be compared between individuals with high vs. low levels of depression and anxiety, defined as a score of 8 or above on the Hospital Anxiety and Depression Scale (HADS) anxiety (HADS-A, 7 items) and depression subscales (HADS-D, 7 items)[42] at all three blood draw timepoints. Expression differences will be assessed both dependently and independently of treatment group assignment.

3.2.2 Clinical

3.2.2.1 Feasibility

The feasibility portion of the study is the test of whether patients undergoing autologous HCT for MM can be placed and maintained on a beta-blocker during the immediate peri-transplant period. Feasibility to use propranolol in autologous MM HCT recipients for the adjunct cancer control purposes will be determined by a 55-70% enrollment rate and 65-70% retention rate as described below in sections 3.2.2.1a-b. The study intervention will be deemed feasible if both of the below parameters for enrollment and retention are met.

3.2.2.1a Enrollment rate

Based on similar biobehavioral cancer research efforts at other institutions, the target enrollment rate is 55-70%. Of all eligible patients that are approached, the goal is that 55-70% will provide informed consent and enroll in the study. This target rate also takes into account the possibility that enrollment may be slightly lower due to the present study being a drug intervention trial with potential patient reticence to enroll.

3.2.2.1b Retention rate

Based on similar research and the nature of drug intervention trials (as described in 3.2.2.1a), the target retention rate is 65-70%. Retention rate will be assessed individually for two time periods - prior to transplant (between Time 1 and Time 2) and from transplant through Day +30 (Time 2 to Time 3). This allows for assessing the impact on gene expression in both a run-in period preceding transplantation as well as during the transplant process, whereby there will be a greater number of confounding variables that may potentially impact gene expression and adherence. Participants will be considered to be retained in the study for the given time period assessment if they are 80% adherent to their prescribed dose of medication during the specified time period.

Drug adherence will be measured as a percentage of the prescribed number of pills that were actually taken (either 20 mg or 40 mg twice daily). This will be determined outpatient via pill count on Day -2 and Day +28 and inpatient through nursing documentation. Patients will have one additional pill count upon return of the study supply of propranolol. The return will occur at the next earliest clinic appointment following discontinuation of the propranolol. There is no consensual standard for what constitutes adequate adherence. Clinical trials report average adherence rates of 43 to 78 percent among patients taking drugs for chronic conditions; some clinical trials define rates above 80% as acceptable.[43] A target adherence rate of 80% will be utilized in this study to constitute having adequately retained the patient in the study. Adherence will be recorded as a continuous (0-100%) variable. Adherence will be assessed separately during two time periods – prior to transplant (between Time 1 and Time 2) and from transplant through Day +28 (Time 2 to Time 3). Patients will be included in final analyses if and only if they have been adherent (at least 80% of the prescribed dose) to the study drug during the designated time period.

As the retention rate (participants' ability or inability to be maintained on the study drug) will necessarily be affected by side effects, we will collect side effect data to better

inform the feasibility aim of this study. Common side effects that will be screened for clinically at follow up time points include fatigue, dizziness, constipation, bradycardia, hypotension, depression, insomnia, weakness, disorientation, nausea, diarrhea, hypersensitivity reaction, purpura, alopecia, and impotence. Any side effects deemed secondary to beta-blocker usage will be documented and appropriately treated or referred for treatment collaboratively by the study PI and treating HCT physician, and beta-blocker therapy will be weaned by 20 mg at each dosing time. Patients will remain at the weaned dose for 1 week prior to discontinuing if this dose decrease does not eliminate the drug entirely. Heart rate and blood pressure will be monitored and documented at baseline, once after initiating beta-blocker therapy but before transplant, just prior to transplant, and weekly thereafter; these are two of the most common and dangerous side effects. Given that patients aren't as acutely medically ill pre-transplant and that standard of care following beta-blocker initiation would involve follow-up weeks later, this schedule should allow for adequate study drug monitoring while minimizing patient study visit burden. Blood pressure less than 90/55 or heart rate less than 50 will prompt clinical review by the study PI and treating HCT physician for appropriateness to continue beta-blocker therapy. Calendar collection of adverse events (AEs) will be obtained through toxicity forms (Common Terminology Criteria for Adverse Events [CTCAE]) completed weekly until neutrophil engraftment occurs (absolute neutrophil count > 500/mm^3 for ≥3 consecutive days) and bi-weekly thereafter until the study endpoint at Week 6 (See Tables 2a and 2b). Further details about AE monitoring are included in Section 4.3.

3.2.2.2 Outcomes

- Depression and anxiety: as assessed by the HADS, with scores of 8 or above on HADS-A (7 items) and HADS-D (7 items) defining significant anxiety or depression, respectively.[42] Depression and anxiety will be described both overall and between treatment vs. control groups.
- Engraftment syndrome: presence of fever, diarrhea, or rash within 48 hours before or after neutrophil recovery that requires steroids for treatment. This will be determined at the discretion of the treating physician and will be categorized as yes/no.
- Neutrophil engraftment: time to absolute neutrophil count (ANC) > 0.5 x 10^9/L sustained for three consecutive assessments at least one day apart.
- Platelet engraftment: time to achieve a platelet count of (a) >20 x 10^9/L independent of platelet transfusions for 3 consecutive assessments at least one day apart.
- Infection: number of documented culture positive infections or neutropenic fever episodes defined as fever >100.4 degrees F.
- Response to treatment: The trial will assess the rates of very good partial response (VGPR) or better (near complete response (nCR), CR, and stringent CR (sCR)) according to the International Uniform Response Criteria at day 100 post-HCT.
- Treatment-Related Mortality (TRM): TRM is defined as death occurring in a patient from causes other than disease progression. TRM will be assessed at D100

for both the intervention and control arms. Patients alive and progression-free at 100 days will be censored.

- Progression-Free Survival (PFS): Progression (for patients not in CR) is defined as increase of serum paraprotein by 0.5 g/dL compared to pre-transplant levels, increase in 24 hour urine protein electrophoresis ≥ 200 mg compared to pre-transplant levels, absolute increase in the difference between involved and uninvolved FLC levels of >10 mg/dl (only in patients without measurable paraprotein in the serum and urine), >25% increase in plasma cells in a bone marrow aspirate or on trephine biopsy (must also be an absolute increase of at least 10%), increase in the size of existing bone lesions or soft tissue plasmacytomas, development of new bone lesions or soft tissue plasmacytomas, development of hypercalcemia (corrected serum Ca >11.5 mg/dL or >2.8 mmol/L) not attributable to any other cause. Development of a compression fracture does not exclude continued response and may not indicate progression. PFS will be compared between the two arms as a time to event endpoint censored after 100 days of follow-up on each patient. Patients are considered a failure of the primary endpoint if they die or suffer from disease progression. The time to this event is the time from randomization to progression, death, loss to follow up or Day 100 - whichever comes first.
- Overall Survival (OS): The event is death from any cause. The time to this event is the time from randomization to death, loss to follow-up or the end of 100 days, whichever comes first. Patients alive at the time of last observation are considered censored. Estimates of OS will be described for each group at 100 days post-transplant.

4.0 ENROLLMENT PROCEDURES

4.1 Patient Enrollment

Patients will be approached for this study during the pre-transplant evaluation phase. Transplant physicians will evaluate the patient eligibility onto this study. Eligible patients willing to participate in the trial will sign an MCW IRB approved informed consent form. A Clinical Trials Office CRC will record the documentation of patient consent and proceed with registration procedures. All source documents that support eligibility including a signed informed consent/HIPAA and signed eligibility checklist, will be available, reviewed and eligibility verified. At the point of registration, a member of the study team will register the patient in the electronic database, including demographics, consent and on-study information. The patient will be assigned a unique sequence number for the study. The principal investigator (PI) of the study, Dr. Knight, will be notified prior to enrollment. Pharmacy will be notified upon patient registration. If at any point in time a patient wishes to withdraw from the study, they may notify Dr. Knight verbally or in writing. There will be no further data collection for participants who elect to withdraw from the study.

Target enrollment is 40 (20 per group) and includes patients undergoing autologous HCT for MM at MCW. Half of the participants will be randomized via permuted block assignment with random block sizes to receive propranolol upon study entry. Informed consent will be obtained to access participants' full medical records to ascertain relevant demographic and medical data as outlined in Section 4.2. The patient must have signed informed consent prior to registration on the study. Due to the pilot and feasibility nature of this study, the need to monitor response to prophylactic beta-blocker usage in a medically ill population, as well as the objective nature of gene expression profiling, this pilot study will not be blinded. Patients will be enrolled before study commencement at Day -7. Total study duration will be up to 7 weeks for participants assigned to the intervention group (1 week pre-transplant through 6 weeks post-transplant) and 5 weeks for those in the control arm (1 week pre-transplant through 4 weeks post-transplant). Additional clinical information will be collected for 100 days post-transplant as identified in section 3.2.2.2.

4.2 Baseline Demographic-, Disease-, and Treatment-Related Variables

Patient-related:

- Age at transplant
- Gender
- Race: Caucasian vs. African American vs. Asian/Pacific Islander vs. Other
- Body Mass Index: <18.5 vs. 18.5-24.9 vs. 25-29.9 vs. >=30
- Smoking status in 3 months prior to HCT (Y/N)
-- Household income prior to disease diagnosis: <$23,000, $23,000-$34,999, $35,000-$44,999, $45,000-$54,999, $55,000-$64,999, $65,000-$74,999, >$75,000
- Number of individuals in patient's household
- Education level: < high school vs. high school graduate vs. some post high school education vs. college degree vs. professional degree

Disease-related:

- International Stage
- Disease status prior to transplant: complete response, very good partial response, partial response, or stable disease
- Cytogenetics: high risk vs. standard risk by International Myeloma Working Group (IMWG) criteria

Treatment-related:

- Number of CD34+ cells infused/kg of recipient body weight
- Transplant setting: inpatient vs. outpatient transplant (defined as patients who plan to stay outpatient during entire transplant period)

4.3 Data and Safety Monitoring Plan

4.3.1 Guideline for serious adverse event reporting

Please refer to Appendix B for more details on adverse event reporting.

4.3.2 Monitoring the progress of the trial and safety of participants

This randomized controlled pilot study will be monitored by the PI, Dr. Knight. The PI will review the outcome of the data for each individual patient on an ongoing basis. The PI will have primary responsibility for ensuring that the protocol is conducted as approved by the IRB. The PI will ensure that the monitoring plan is followed and that all data required for oversight of monitoring are accurately reported, that all AEs are reported according to the protocol guidelines, and that any AEs reflecting patient safety concerns are appropriately reported.

4.3.3 Adverse event reporting

The capture of toxicities and AEs in this protocol will follow the same approach and will be done in two levels. First, grades 3-5 AEs will be collected at different time points during the study period. Any AE that fulfill this criterion will be captured and the frequency of each organ toxicity tabulated. The Data and Safety Monitoring Committee (DSMC) and IRB will receive a summary of all the AEs captured in calendar forms in a quarterly to biennial basis depending the schedule of protocol review meetings. Additionally the PIs will review the frequency of toxicities in a quarterly basis.

Second, any AE that is unexpected and of grades 3 to 5 will require expedited report to the oversight committees (MCW IRB, MCW DSMC and FDA – if applicable for studies under IND). The collection grades 3-5 unexpected AEs is event driven and requires a more comprehensive description of the event. The report for these AEs are termed Individual Case Safety Reports (ICSR) and include the following components: 1) summary cover sheet of the event, 2) narrative of the event, 3) associated conditions, medications and diagnostic information, source documents with further explanation of the event, and an evaluation of the event by the PI. The PI will review all these events and will determine if they require expedited reporting. If they are considered grades 3-5 and unexpected the report will be sent to the oversight committees within three days from acknowledgement of the event. If the event is not considered to fulfill criteria of grades 3-5 unexpected AE, it will be included in the DSMC and IRB reports for scheduled protocol reviews and will not require expedited reporting.

Reporting timelines:

1. **Fatal (grade 5) or Life Threatening** events must be reported within 24 hours but not later than three calendar days of the investigator's observation or awareness of the event.
2. **All other events grades 3-4 unexpected** events (non-fatal/non life-threatening) must be reported within 3 to 5 calendar days of the investigator's observation or awareness of the event.

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee (MCW CC DSMC). A summary of the MCW CC DSMC activities are as follows:

- Review the clinical trials for data integrity and safety
- Review all adverse events requiring expedited reporting as defined per protocol
- Review all Data and Safety Monitoring (DSM) reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCW CC DSM Plan and membership roster will be maintained in the DSMC file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary. Any available DSMC letters will be submitted to the IRB of record as required.

4.4 Specimen Collection

The Cancer Center Lab draw blood to be stored in PAXGene RNA tubes at three study time points as described in Table 4.7b. These time points include baseline (Day -7), Day -2 (immediately prior to transplant, central line placement, or administration of any conditioning regimen), and Day +28. Blood will be drawn at one of two locations: in the hospital (if participants are immediately post-transplant and/or still or re-hospitalized) or in the transplant clinic during their regular visits. The PAXGene RNA tubes do not require any additional processing of blood samples before being frozen and sent to UCLA. These PAXGene RNA tubes will be stored at -80C in the Neuroscience Research Center, which is a locked facility, until they are batched and shipped to University of California Los Angeles (UCLA) for gene expression analysis. Before being stored they will be assigned a dummy ID that will be the sole identifier of the samples. The linked identifiers will be stored on a secure computer network through the CIBMTR under the PI's name. Samples will be packaged with dry ice and shipped overnight to Dr. Steve Cole's lab at UCLA at the time of study conclusion. This will be done on a day such that the UCLA lab can receive and keep the samples frozen at -80C. Our groups have collaborated successfully on transfer of samples in the past. All samples will be sent together.

4.5 Gene Expression Profiling

The UCLA Social Genomics Core (Directed by Dr. Steve Cole, Consultant) will conduct all gene expression profiling on obtained blood samples by using Illumina HT-12 human gene expression bead arrays.[44] Total RNA will be extracted from the whole blood samples stored at MCW, subjected to quality assurance assays to test suitable mass (by spectroscopy) and integrity (by Agilent Bioanalyzer RNA Integrity Score) for analysis, and subjected to microarray target synthesis and hybridization in collaboration with the UCLA Neuroscience Genomics Core Laboratory using standard Illumina assay equipment and protocols. The output of these analyses is quantification of whole genome

RNA production, which will be analyzed as per the methods below (section 5.4.1). Patient-level genetic data from Dr. Cole's lab will not be conveyed to the patient or the clinician and no clinical decision will be made on the basis of genetic findings.

4.6 Psychosocial Assessments

4.6.1 Socioeconomic status

SES will be assessed by household income prior to diagnosis with MM. This information will be collected from participants at baseline/time of study enrollment. The 2012 median family income in Wisconsin was $51,059 (US median was $51,371) according to the US American Community Survey (http://www.census.gov/prod/2013pubs/acsbr12-02.pdf) with the poverty threshold of $23,050 for a four-person family. Thus, we categorized family income as <$23,000, $23,000-$34,999, $35,000-$44,999, $45,000-$54,999, $55,000-$64,999, $65,000-$74,999, >$75,000. Education level (< high school vs. high school graduate vs. some post high school education vs. college degree vs. professional degree) will also be collected but not used as the primary indicator of SES in analyses.

4.6.2 Anxiety and depression

Participants will complete a HADS for primary analysis with gene expression at three study time points as described in Table 4.7b. These time points include baseline (Day -21), Day -2 (immediately prior to conditioning regimen), and Day +28. Further, HADS will be administered/assessed weekly in coordination with weekly beta-blocker assessment to ascertain further whether the beta-blocker might be affecting depression or anxiety.

HADS is a 14-item scale that generates ordinal data and is specifically designed to avoid reliance on somatic symptoms that are concomitant with medical illness. Participants can score between 0 and 21 for either anxiety or depression. Each item is scored on a scale of 0-3. A cut-off point of 8 or above on HADS-A (7 items) and HADS-D (7 items) has been established to connote significant anxiety or depression, respectively.[42] Anxiety and depression will be evaluated both continuously as well as categorically with a cutoff of 8 to signify an abnormal level of anxiety or depression. Participants scoring at this level will be offered a referral to the MCW Psycho-Oncology program for further evaluation and treatment.

4.7 Study Monitoring

The follow-up schedule for scheduled study visits is outlined in Table 4.7a with study assessment time points described in Table 4.7b.

TABLE 4.7a. FOLLOW-UP SCHEDULE

Study Assessment Time Point	**Target Day**
Baseline	Up to Day -7
Pre-Transplant	Day -2 ± 1 day[1]
Day 0	Date of transplant
1 week	7 ± 2 days
2 weeks	14 ± 2 days
3 weeks	21 ± 3 days
4 weeks[2]	28 ± 3 days
5 weeks[3]	35 ± 3 days
6 weeks[3]	42 ± 3 days
Next clinic appointment	~8 weeks (post-transplant)
14 weeks	100 ± 2 weeks

[1]Assessment must occur prior to any conditioning regimen
[2] Research samples only can be collected ± 7 days from target day
[3]Assessment time points for intervention arm only

TABLE 4.7b. PATIENT CLINICAL ASSESSMENTS

Study Assessments/ Testing	**Baseline**	**Day**									
		-2	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**Next clinic appt.**	**100**
Demographics (patient-, disease-, and treatment-related)	X										
Additional descriptive outcomes				X	X	X	X				X
Socioeconomic status	X										
Hospital Anxiety and Depression Scale (HADS)	X		X	X	X	X	X				
Blood draw for gene expression analysis	X	X					X				
Pregnancy test for FCBP	X										
Toxicity			X	X	X	X	X	X[1]	X[1]		
Assessment of adherence[2]		X					X			X	
Heart rate and blood pressure	X		X	X	X	X	X			X	
Myeloma response assessment											X

Consent has to occur within 30 days prior to starting study drug
Numbers indicate days relative to day of transplant, with day of transplant being 0
Patients will be enrolled and randomized prior to commencement of study drug/control arm

[1] For Propranolol group only. Patients will have toxicity assessed weekly and once more 7 days ± 3 days after discontinuing drug.
[2]Adherence will be assessed weekly or monthly based on number of pills prescribed.

5.0 STATISTICAL ANALYSIS

5.1 Study Design

5.1.1 Accrual

It is estimated that 9 months – 1 year will be necessary to enroll the targeted sample size.

5.1.2 Primary Endpoint

The primary endpoint is Day +28 (4 weeks) following HCT for MM, with intervention arm patients followed for an additional 1-2 weeks until they are weaned from beta-blocker therapy.

5.2 Sample Size and Power Calculation

We will enroll 40 participants undergoing autologous HCT for MM at MCW. Accounting for study attrition and beta-blocker intolerance, we aim to have 30 patients with a complete set of gene data. A sample size of 40 will inform our feasibility aim, while a targeted final sample of 15 participants in the intervention group will be sufficient for gene expression analysis. The gene expression sample size is based on previous studies with similar or smaller sample sizes that have evaluated gene expression as a function of psychosocial factors and yielded hundreds of differentially expressed genes that generate statistically significant results in higher-order bioinformatics.[35, 45, 46] More specific sample size estimates are difficult to project with any greater accuracy, as there is not data on the effect size for gene expression differences as a function of propranolol in humans. To this end, the current proposal will inform future sample size estimations. This study is intended mainly to generate effect size estimates and 95% confidence intervals to aid with planning future research projects. We will generate both simple estimates of effect size as well as adjusted estimates, attempting to control for any accidental confounding that may occur despite randomization.

5.3 Analysis of Primary Endpoints

5.3.1 Gene Expression Profiling

The final outcome for the gene expression analysis will be expression levels of the group of genes comprising the conserved transcriptional response to adversity (CTRA) profile. The CTRA profile represents a systemic shift in basal gene expression profiles among circulating immune cells during extended periods of stress, threat, or uncertainty consistent with the physiology of stress-associated illness.[32-36] This profile is characterized by 53-genes and includes up-regulated expression of pro-inflammatory genes (*IL1A, IL1B, IL6, IL8, TNF, PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA,* and *RELB*) and down-regulated expression of genes involved in type I interferon (IFN) responses (*GBP1, IFI16, IFI27, IFI27L1-2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1-3, IFIT5, IFIT1L, IFITM1-3, IFITM4P,*

IFITM5, IFNB1, IRF2, IRF7-8, MX1-2, OAS1-3, and *OASL*) and antibody synthesis (*IGJ, IGLL1,* and *IGLL3*).[33, 34, 47, 48] This CTRA profile is significantly regulated through beta-adrenergic mediated signaling. The CTRA profile will include a composite of standardized scores on the 53 a priori defined genes and measured levels of each transcript treated as 53 repeated measurements analyzed by a mixed effect linear model with a residual covariance structure estimated to account for potential correlation among the 53 genes.

Primary analyses will be performed at the time of all 3 blood draws (Baseline, Day -2, and Day +28) and will use general linear model analyses to quantify the association between expression of each of the 53 CTRA contrast genes and beta-blocker usage. To ensure analysis of patients with sufficient beta-blocker exposure, only gene expression data from study participants with an adherence rate of 80% (at either dose) will be used as comparators against the control group. Descriptive tables of patient-, disease-, and transplant-related factors will be prepared with separate columns by propranolol vs. no propranolol groups. Any demographic or psychosocial variables (other than those of primary interest) that are unevenly distributed between groups after randomization will be adjusted for in subsequent analyses as previously described using ANCOVA. Further, differences in gene expression will be compared between groups/variables that might not have been randomly distributed, regardless of beta-blocker treatment assignment, to ascertain whether these differences may be stronger than drug-attributed changes. The UCLA Social Genomics core will provide genetic and statistical analyses for gene expression data.

5.4 Analysis of Secondary Endpoints

5.4.1 Gene expression based on psychosocial factors

Subgroup analyses will describe additional psychosocial factors (SES, depression, anxiety) that may result in altered gene expression or differentially affect the response to beta-blockers in our proposed cohort. As this study is not powered to formally assess this relationship, these will be exploratory analyses to determine whether there is sufficient signal of a response to merit further exploration in future studies.

SES will be assessed by household income prior to diagnosis with MM as described in section 4.6.1. This information will be collected from participants at baseline/time of study enrollment. SES will be assessed by all 7 categorical levels collected as well as by lowest quartile vs. all other quartiles. Anxiety and depression will be evaluated both continuously as well as categorically with a cutoff of 8 or above signifying an abnormal level of anxiety or depression. General linear model analyses will be used to quantify the association between the three psychosocial variables of interest – SES, depression, and anxiety – with expression of the CTRA gene profile, measured continuously. Beta-blocker usage will then be entered into this model to ascertain whether it significantly changes the association between SES, depression, and anxiety and gene expression as measured by the CTRA profile. Significance will be assessed by change in magnitude of the partial regression coefficient relating candidate psychosocial variables (e.g., SES) to

the composite CTRA gene expression z-score in presence vs. absence of control for beta-blocker exposure.

5.4.2 Clinical

5.4.2.1 Feasibility

Feasibility to use propranolol in autologous MM HCT recipients for the adjunct cancer control purposes will be determined by a 55-70% enrollment rate and 65-70% retention rate as described in section 3.2.2.1. The rates of these events will be tabulated.

5.4.2.2 Outcomes

- Depression and anxiety: HADS scores will be tabulated and evaluated comprehensively as well as by HADS-A and HADS-D. The trajectory of these scores over the course of the study will be described. Differences in scores between the treatment vs. control groups over the course of the study will be assessed.
- Engraftment syndrome: Engraftment syndrome occurrences will be tabulated and the incidence described for both treatment and control arms.
- Neutrophil engraftment: Differences in time to neutrophil engraftment will be determined between treatment and control arms.
- Platelet engraftment: Differences in time to platelet engraftment will be determined between treatment and control arms.
- Infection: Infection events will be tabulated and differences in events will be determined for treatment and control arms.
- Response to treatment: The rates of VGPR or better (nCR, CR, and sCR) will be calculated at 100 days post-transplant and compared between intervention and control arms.
- Treatment-Related Mortality (TRM): TRM is defined as death occurring in a patient from causes other than disease progression. Disease progression is a competing event for TRM. The time to this event is the time from randomization to death, disease progression, loss to follow-up or the end of 100 days post-transplant, whichever comes first. Patients alive without disease progression at 100 days are considered censored. The cumulative incidence of TRM at 100 days will be estimated separately for each treatment-group. Overall TRM through 100 days of follow-up will be compared using Gray's test.
- Progression-free survival (PFS): PFS will be compared between the two arms as a time to event endpoint censored after 100 days of follow-up on each patient. Patients are considered a failure of the primary endpoint if they die or suffer from disease progression. The time to this event is the time from randomization to progression, death, loss to follow up or Day 100 - whichever comes first. The Kaplan-Meier estimate of survival will be estimated separately for each treatment group at 100 days post-transplant.
- Overall survival (OS): The event is death from any cause. The time to this event is the time from randomization to death, loss to follow-up or the end of 100 days, whichever comes first. Patients alive at the time of last observation are considered

censored. Estimates of OS will be described for each group at 100 days post-transplant. The Kaplan-Meier estimate of survival will be estimated separately for each treatment group at 100 days post-transplant. Overall survival will be compared using a log-rank test at a two-sided significance level of .05.

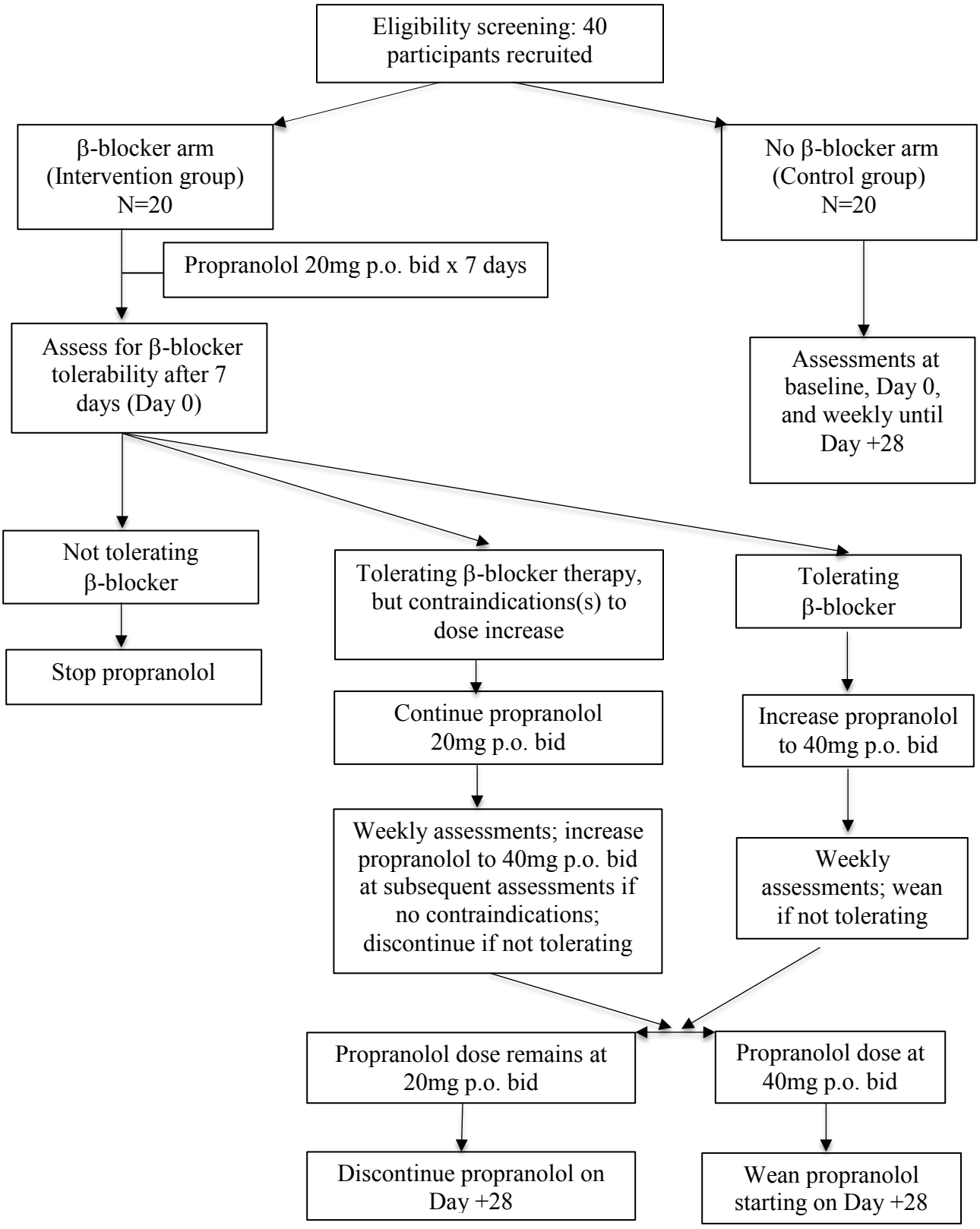

Figure 1. Treatment schema diagram; day values are in reference to transplant as Day 0

APPENDIX A
DRUG INTERACTIONS

Appendix A: Full list of concomitant medications to be used with caution

Abiraterone Acetate	Lopinavir
Afatinib	Methotrimeprazine
Alfuzosin	Methoxsalen (Systemic)
Amifostine	Methyldopa
Aminophylline	Methylergonovine
Brimonidine (Ophthalmic)	Mexiletine
Bromocriptine	Norepinephrine
Bupropion	Norepinephrine
Cabergoline	Obinutuzumab
Carbamazepine	Ofloxacin (Systemic)
Cinacalcet	Paroxetine
Ciprofloxacin (Systemic)	Phenobarbital
Clonidine	Phenoxybenzamine
Cocaine	Phentolamine
Colchicine	Prazosin
Dopamine	Primaquine
Doxorubicin	Primidone
Dabigatran Etexilate	Rituximab
Delavirdine	Rifampin
Dexmedetomidine	Ritonavir
Dihydroergotamine	Rivaroxaban
Doxazosin	Rizatriptan
Dronedarone	Stiripentol
Dyphylline	Silodosin
Epinephrine (Racemic, Systemic, Oral Inhalation, Nasal)	Tamsulosin
Ergoloid Mesylates	Terazosin
Ergonovine	Terbinafine (Systemic)
Ergotamine	Theophylline
Everolimus	Thiabendazole
Fluoxetine	Thioridazine
Fluvoxamine	TiZANidine
Grass Pollen Allergen Extract (5 Grass Extract)	Tipranavir
Guanfacine	QuiNIDine
Isometheptene	Vemurafenib
Levonordefrin	Vincristine

APPENDIX B
ADVERSE EVENT REPORTING

Introduction

Clinical trials that assess interventions in the setting of hematopoietic cell transplantation is challenging as the number of expected AEs is high. The Blood and Marrow Transplant Clinical Trial Network (BMT CTN) has established an AE capture approach specific for transplant trials. This approach has the objective of optimizing the capture of AEs by reducing the noise-signal ratio and identifies AEs that are not expected after transplant or overlaps with manifestations of common complications, such as GVHD or graft failure.

Definitions

Adverse Event (AE) - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An AE can considered therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not related to the medicinal product. AEs are expected in the HCT population.

Expectedness: An adverse event can be Expected or Unexpected

- **Expected adverse events** are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- **Unexpected adverse events** are those that vary in nature, intensity or frequency from information in the current adverse event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator or treating physician, at immediate risk of death from the reaction. Study toxicities are graded using the adapted NCI Common Toxicity Criteria (where appropriate use the criteria for transplant patients.)

• **Serious Adverse Event (SAE)** – Any adverse event occurring that results in any of the following outcomes:
• death – regardless of cause
• life-threatening adverse event (see above)
• persistent or significant disability/incapacity
• congenital anomaly
• requires intervention to prevent permanent impairment or damage

AE Grading: All AEs that occur in this protocol will be graded according to the NCI Common Terminology Criteria for Adverse Event (CTCAE) v 4.0.

AE Reporting: Depending on the type, severity and whether it is expected, each AE will need to be reported in an appropriate timeline to MCW IRB, Data Safety and Monitoring Committee and FDA (if applicable for studies under an Investigational New Drug [IND] protocol).

Adverse Events Reported to the DSMB
All adverse events that are classified as grades 3 to 5 will be reported to the DSMC. The differences are the timing and amount of information associated with each.

The two-tier approach collects adverse events at specific time points during the trial (calendar-driven) and event-driven upon knowledge of an event deemed serious (grades 3 to 5) or unexpected to be seen in a transplant setting.

Grades 3-5 Adverse Events
For the calendar-driven collection of toxicity, the protocol mandates collection of toxicity on a form that captures most of the most important toxicities observed post transplant. The forms would capture all the AEs that occur in a preceding period, for example day 28 toxicity form, collects all the AEs that occurred from enrollment to day 28 and the grade of each AE. This first period includes all non-hematologic toxicity, since it is expected significant hematologic toxicity will occur immediately post transplant as an effect of the transplantation. Hematologic toxicity will be capture when it occurs beyond day 28 post transplant.

Unexpected Grades 3-5
The threshold for collection of adverse events outside the calendar forms is related to the grade (grades 3-5) and expectedness related to the transplant procedure or possibly related to the study drug. These events are required to be reported to the DSMC in a more immediate time frame and also require more information. For any adverse event that meets these criteria, the report would require a narrative of the event, associated laboratory and imaging information, associated medications and any relevant source documents. These reports are uploaded into Oncore and we will notify the DSMC chair and MCW IRB within 24 hours or 3 to 5 calendar days depending on the severity of the event. Both the PI and the CTO BMT Section leader will be responsible to review and determine if an event meet criteria prior to uploading it in Oncore.

Reporting timelines:

1. **Fatal (grade 5) or Life Threatening** events must be reported within 24 hours but not later than 3 calendar day of the investigator's observation or awareness of the event.
2. **All other events grades 3-4 unexpected** events (non-fatal/non life-threatening) must be reported within 3 to 5 calendar days of the investigator's observation or awareness of the event.

The proposed method outlined here follows the same requirements from MCW IRB for prompt reporting of an event or **Unanticipated Problem Involving Risks to Subjects or Others (UPIRSO)**: which is defined as any incident, experience, or outcome that meets all of the following criteria:

1. Unanticipated (in terms of nature, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, instructions for Use/Device Manual and or Investigator's Brochure; and (b) the characteristics of the subject population being studies;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research) or test article; and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

DSMC Reporting

The table below summarizes the reporting practices from the study to the DSMC. For the ongoing review of an opened trial, the DSMC will receive a report that includes in a tabular form all the grades 3 to 5 that occurred in the trial, summarized according to reporting periods: for example 0-28d, 28-56d, 56 to 100d, 100-180d, 180-270d, 270 to 365d and 0-365d. Additionally the report will include all the unexpected grades 3-5 that occurred in trial participants since activation of the trial and lastly the number of patients who met the graft failure stopping guidelines.

For the unexpected grades 3 to 5, upon knowledge of the event, this will be discussed with the study PI and the CTO BMT section leader. If this fulfills criteria for expedited reporting to the DSMC and IRB, a narrative will be uploaded in Oncore and the DSMC will be notified, either through its secretary or directly to the Chair.

Stopping Rules

- Unexpected SAEs attributable to drug in excess of 20% would be unacceptable. If more than 2 out of the first (or subsequent) 10 participants enrolled in the trial experience a SAE resulting in life threatening complications/death, the trial will be stopped.

	All Grades 3-5	**Unexpected Grades 3-5**
Type of collection	Calendar-driven	Event-driven
Reporting format	Tabular or graphical format	Narrative with supporting documents
Timing of reporting	Biennial basis - cumulative	Within 3 to 5 calendar days from knowledge of the event and cumulative in a biennial basis.

References

1. Cole SW and Sood AK. Molecular pathways: Beta-adrenergic signaling in cancer. Clinical Cancer Research. 2012;18(5):1201-1206.
2. Antoni MH, Lutgendorf SK, Cole SW, et al. The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. Nat Rev Cancer. 2006;6(3):240-248.
3. Chida Y, Hamer M, Wardle J, Steptoe A. Do stress-related psychosocial factors contribute to cancer incidence and survival? Nature clinical practice Oncology. 2008;5(8):466-475.
4. Lamkin DM, Sloan EK, Patel AJ, et al. Chronic stress enhances progression of acute lymphoblastic leukemia via β-adrenergic signaling. Brain Behav Immun. 2012.
5. Powe DG, Voss MJ, Zänker KS, et al. Beta-blocker drug therapy reduces secondary cancer formation in breast cancer and improves cancer specific survival. Oncotarget. 2010;1(7):628.
6. Aydiner A, Ciftci R, Karabulut S, Kilic L. Does beta-blocker therapy improve the survival of patients with metastatic non-small cell lung cancer?. Asian Pac J Cancer Prev. 2013;14(10):6109-6114.
7. Barron TI, Connolly RM, Sharp L, Bennett K, Visvanathan K. Beta blockers and breast cancer mortality: a population- based study. J Clin Oncol. 2011;29(19):2635-2644.
8. De Giorgi V, Grazzini M, Gandini S, et al. Treatment with beta-blockers and reduced disease progression in patients with thick melanoma. Arch Intern Med. 2011;171(8):779-781.
9. Wang HM, Liao ZX, Komaki R, et al. Improved survival outcomes with the incidental use of beta-blockers among patients with non-small-cell lung cancer treated with definitive radiation therapy. Ann Oncol. 2013;24(5):1312-1319.
10. Hwa YL, Lacy MQ, Gertz MA, et al. Impact of beta blocker on clinical outcomes of multiple myeloma patients [abstract]. Blood (ASH Annual Meeting Abstracts). 2014;124.
11. Nagaraja AS, Sadaoui NC, Lutgendorf SK, Ramondetta LM, Sood AK. beta-blockers: a new role in cancer chemotherapy?. Expert Opin Investig Drugs. 2013;22(11):1359-1363.
12. Benish M, Bartal I, Goldfarb Y, et al. Perioperative use of beta-blockers and COX-2 inhibitors may improve immune competence and reduce the risk of tumor metastasis. Ann Surg Oncol. 2008;15(7):2042-2052.
13. Melhem-Bertrandt A, Chavez-Macgregor M, Lei X, et al. Beta-blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. J Clin Oncol. 2011;29(19):2645-2652.
14. Lemeshow S, Sorensen HT, Phillips G, et al. beta-Blockers and survival among Danish patients with malignant melanoma: a population-based cohort study. Cancer Epidemiol Biomarkers Prev. 2011;20(10):2273-2279.
15. Guo K, Ma Q, Wang L, et al. Norepinephrine-induced invasion by pancreatic cancer cells is inhibited by propranolol. Oncol Rep. 2009;22(4):825-830.
16. Lin X, Luo K, Lv Z, Huang J. Beta-adrenoceptor action on pancreatic cancer cell proliferation and tumor growth in mice. Hepatogastroenterology. 2012;59(114):584-588.
17. Melamed R, Rosenne E, Shakhar K, Schwartz Y, Abudarham N, Ben-Eliyahu S. Marginating pulmonary-NK activity and resistance to experimental tumor metastasis:

suppression by surgery and the prophylactic use of a β-adrenergic antagonist and a prostaglandin synthesis inhibitor. Brain Behav Immun. 2005;19(2):114-126.
18. Kozanoglu I, Yandim MK, Cincin ZB, Ozdogu H, Cakmakoglu B, Baran Y. New indication for therapeutic potential of an old well-known drug (propranolol) for multiple myeloma. J Cancer Res Clin Oncol. 2013;139(2):327-335.
19. Wong GW and Wright JM. Blood pressure lowering efficacy of nonselective beta-blockers for primary hypertension. status and date: New, published in. 2014(2).
20. Masur K, Niggemann B, Zanker KS, Entschladen F. Norepinephrine-induced migration of SW 480 colon carcinoma cells is inhibited by β-blockers. Cancer Res. 2001;61(7):2866-2869.
21. McQuellon RP, Russell GB, Rambo TD, et al. Quality of life and psychological distress of bone marrow transplant recipients: the 'time trajectory' to recovery over the first year. Bone Marrow Transplant. 1998;21(5):477-486.
22. Wang XS, Shi Q, Shah ND, et al. Inflammatory markers and development of symptom burden in patients with multiple myeloma during autologous stem cell transplantation. Clin Cancer Res. 2014;20(5):1366-1374.
23. Norkin M, Hsu JW, Wingard JR. Quality of life, social challenges, and psychosocial support for long-term survivors after allogeneic hematopoietic stem-cell transplantation. Semin Hematol. 2012;49(1):104-109.
24. Grulke N, Albani C, Bailer H. Quality of life in patients before and after haematopoietic stem cell transplantation measured with the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Core Questionnaire QLQ-C30. Bone Marrow Transplant. 2012;47(4):473-482.
25. Sloan EK, Priceman SJ, Cox BF, et al. The sympathetic nervous system induces a metastatic switch in primary breast cancer. Cancer Res. 2010;70(18):7042-7052.
26. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008;22(3):659-661.
27. Khadke VV, Khadke SV, Khare A. Oral propranolol--efficacy and comparison of two doses for peri-operative anxiolysis. J Indian Med Assoc. 2012;110(7):457-460.
28. Wong L, Nation R, Chiou W, Mehta P. Plasma concentrations of propranolol and 4-hydroxypropranolol during chronic oral propranolol therapy. Br J Clin Pharmacol. 1979;8(2):163-167.
29. Mullane JF, Kaufman J, Dvornik D, Coelho J. Propranolol dosage, plasma concentration, and beta blockade. Clinical Pharmacology & Therapeutics. 1982;32(6):692-700.
30. Inderal® [package insert]. Cranford, NJ: Akrimax Pharmaceuticals. 2010.
31. Lutgendorf SK, DeGeest K, Sung CY, et al. Depression, social support, and beta-adrenergic transcription control in human ovarian cancer. Brain Behav Immun. 2009;23(2):176-183.
32. Powell ND, Sloan EK, Bailey MT, et al. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via beta-adrenergic induction of myelopoiesis. Proc Natl Acad Sci U S A. 2013;110(41):16574-16579.
33. Cole S. Social regulation of gene expression in the immune system. In: Segerstrom S, ed. The Oxford Handbook of Psychoneuroimmunology. New York: Oxford Univ Press; 2012:254-273.

34. Irwin MR and Cole SW. Reciprocal regulation of the neural and innate immune systems. Nature Reviews Immunology. 2011;11(9):625-632.
35. Cole SW, Hawkley LC, Arevalo JM, Sung CY, Rose RM, Cacioppo JT. Social regulation of gene expression in human leukocytes. Genome Biol. 2007;8(9):R189.
36. Cacioppo JT and Hawkley LC. Social isolation and health, with an emphasis on underlying mechanisms. Perspect Biol Med. 2003;46(3 Suppl):S39-52.
37. Shargel L Y, AB. Applied Biopharmaceutics and Pharmacokinetics. McGraw-Hill/Appleton & Lange; 1999.
38. Kubota T, Inoue S, Furukawa T, et al. Similarity of serum–Tumor pharmacokinetics of antitumor agents in man and nude mice. Anticancer Res. 1993;13:1481-1484.
39. Vervloet E, Pluym BF, Cilissen J, Kohlen K, Merkus FW. Propranolol serum levels during twenty-four hours. Clin Pharmacol Ther. 1977;22(6):853-857.
40. Walle T, Byington RP, Furberg CD, McIntyre KM, Vokonas PS. Biologic determinants of propranolol disposition: Results from 1308 patients in the beta-blocker heart attack trial*. Clinical Pharmacology & Therapeutics. 1985;38(5):509-518.
41. Gengo FM, Fagan SC, Kinkel WR, McHugh WB. Serum concentrations of propranolol and migraine prophylaxis. Arch Neurol. 1984;41(12):1306-1307.
42. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale: an updated literature review. J Psychosom Res. 2002;52(2):69-77.
43. Osterberg L and Blaschke T. Adherence to medication. N Engl J Med. 2005;353(5):487-497.
44. Cole SW, Hawkley LC, Arevalo JMG, Cacioppo JT. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. Proceedings of the National Academy of Sciences. 2011;108(7):3080.
45. Miller GE, Chen E, Sze J, et al. A functional genomic fingerprint of chronic stress in humans: Blunted glucocorticoid and increased NF-[kappa] B signaling. Biol Psychiatry. 2008;64(4):266-272.
46. Creswell JD, Irwin MR, Burklund LJ, et al. Mindfulness-based stress reduction training reduces loneliness and pro-inflammatory gene expression in older adults: a small randomized controlled trial. Brain Behav Immun. 2012;26(7):1095-1101.
47. Antoni M, Lutgendorf S, Blomberg B, et al. Transcriptional modulation of human leukocytes by cognitive-behavioral stress management in women undergoing treatment for breast cancer. Biol Psychiatry. 2012;71(4):366-372.
48. Fredrickson BL, Grewen KM, Coffey KA, et al. A functional genomic perspective on human well-being. Proc Natl Acad Sci U S A. 2013;110(33):13684-13689.