PILOT STUDY USING PROPRANOLOL TO PROMOTE PRENYLATION OF THE GTPASE RAP1B IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

Version 2.0

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PROTOCOL SYNOPSIS

Randomized Controlled Pilot Study Using Propranolol to Promote Prenylation of the GTPase Rap1 in Hematopoietic Stem Cell Transplant Recipients

Principal Investigator:	Jennifer M. Knight, MD
Study Design:	This is a an ancillary study designed to explore whether an additional cell signaling pathway (prenylation of Rap1) that was recently identified as being under beta-adrenergic control may be affected by beta-blocker use
Primary Objective:	The primary objective of this ancillary study is to explore whether beta-blocker administration will promote prenylation and membrane localization of Rap1 in peripheral blood mononuclear cells (PBMCs).
Eligibility:	Patients who are enrolled in the parent study are eligible for this ancillary study.
Treatment Description:	N/A – Patients are treated on the parent protocol
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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
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

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1.0 BACKGROUND AND RATIONALE:

1.1 Rap1 in the tumor microenvironment

Rap1, a small GTPase, suppresses metastasis by localizing at the plasma membrane of tumor cells through the process of prenylation, the post-translational attachment of a lipid tail. When Rap1 is localized at the plasma membrane of tumor cells, Rap1 promotes adhesion of the tumor cells and inhibits their metastatic spread. In contrast, when Rap1 prenylation is inhibited, Rap1 cannot anchor at tumor cell membranes, resulting in loss of cell-cell adhesion and increased metastasis of tumor cells.

Recently, it was discovered that prenylation of Rap1 is inhibited by activating adenosine receptors on tumor cells.[1, 2] This was the first demonstration that Rap1 prenylation can be regulated by receptor activation. [3, 4] Researchers subsequently discovered that prenylation of Rap1 is inhibited by activating beta-adrenergic receptors (unpublished data). This discovery supports the novel model that beta-blockers will promote prenylation and membrane localization of Rap1, decreasing tumor aggressiveness. The demonstration that beta-blockers increase the prenylation of Rap1 will support future studies testing the effects of beta-blockers on the prenylation of Rap1 in patients' tumors. The aim of this study is to assess whether beta-blocker administration promotes Rap1 prenylation and membrane localization.

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.[5-9, 11-18] Further, propranolol, a non-selective beta-adrenergic receptor blocker, has apoptotic and anti-proliferative effects on multiple myeloma (MM) cells.[19] Propranolol is the most studied nonselective beta-blocker;[20] this, along with its safe side effect profile, cost-effectiveness, and efficacy in vitro in preventing tumor progression as compared to selective beta-antagonists[16, 21, 22] 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.[23, 24] Psychosocial stress gradually improves over time with a return to pre-transplant psychosocial functioning by about one year post-transplant.[25, 26] 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.[17] Tumor metastasis in mouse models is increased 30-fold as compared to the control group after 20 days of exposure to chronic stress.[27]

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.[27] 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).[28] 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.[29] Given this as well as the variable pharmacokinetic profile of propranolol,[30-32] it is also feasible that 20mg bid may affect prenylation of Rap1.

1.4 Rationale

This study will explore whether prenylation of Rap1 - recently identified as being under beta-adrenergic control - may be affected by beta-blocker use. Further, the proposed study addresses a major goal of the MCW Cancer Center; it promotes collaborative interactions between basic science researchers and clinicians at the Medical College. The proposed investigation of Rap1 prenylation will enhance the translational significance of Dr. Carol Williams's studies of Rap1 and may lead to future clinical trials of beta-blockers as novel therapeutic regulators of Rap1 in cancer.

2.0 STUDY DESIGN

This is a proof of concept randomized controlled pilot study assessing whether prenylation and membrane localization of Rap1 in PBMCs 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 ancillary study is to explore whether beta-blocker administration will promote prenylation and membrane localization of Rap1 in peripheral blood mononuclear cells (PBMCs).

2.2 Patient Eligibility

Patients who are eligible for enrollment in the parent study.

2.3 Treatment Plan

Patients will be treated per the parent protocol.

3.0 STUDY ENDPOINTS

Western blotting on the cytosolic and membrane fractions of isolated PBMCs will determine the distribution of Rap1 in the different fractions as well as the status of Rap1 prenylation in the cells exposed vs. not exposed to beta-blocker therapy.

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 the parent study and this ancillary 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. 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 in the parent study 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 both studies. 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 Western blot analysis, this pilot study will not be blinded. Patients will be enrolled by 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

Same as the parent study

4.3 Data and Safety Monitoring Plan

Same as parent study.

4.4 Specimen Collection

The Cancer Center Lab will draw one tube of blood to be stored in 8 mL BD Vacutainer CPT 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. These 8 mL BD Vacutainer CPT tubes will be transferred to Dr. Williams' lab at MCW for Western blot analysis. Before being transferred 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 Clinical Trials Office under the PI's name.

4.5 Rap1 prenylation

Peripheral blood mononuclear cells (PBMCs) will be isolated from the whole blood samples collected from patients at the three designated time points (Baseline, Day -2, and Day +28). Members of Dr. Carol Williams' laboratory (Professor, Pharmacology and Toxicology, MCW) will conduct western blotting on the cytosolic and membrane fractions of isolated PBMCs to determine the distribution of Rap1 in the different fractions, and the status of Rap1 prenylation in the cells.

4.6 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	Day -7 ± 2 days
Pre-Transplant	Day -2 ± 1 day[1]
4 weeks	28 ± 2 days

[1]Assessment must occur prior to any conditioning regimen

TABLE 4.7b. PATIENT CLINICAL ASSESSMENTS

Study Assessments/ Testing	**Baseline**								
		-2	**7**	**14**	**21**	**28**	**35**	**42**	**100**
Blood draw for Western blot analysis	X	X				X			

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 prenylation data. A targeted final sample of 15 participants in the intervention group will be sufficient for Western blot analysis. This sample size is established for the purpose of the parent study and should be sufficient to describe prenylation.

5.3 Analysis of Primary Endpoints

5.3.1 Rap1 prenylation by beta-blocker exposure

Western blotting on the cytosolic and membrane fractions of isolated PBMCs will determine the distribution of Rap1 in the different fractions as well as the status of Rap1 prenylation in the cells exposed vs. not exposed to beta-blocker therapy. The statistical difference between cells from patients exposed to beta-blocker therapy (intervention arm) vs. those not exposed to beta-blocker therapy (control arm) will be determined by repeated-measures of analysis of variance (ANOVA) individually at the two blood assessment time points on beta-blocker therapy (Day -2, and Day +30). Baseline Rap1 prenylation will also be assessed by the first blood draw and may be compared to later time points. Patients will be included in final analyses if and only if they have been adherent to the study drug during the designated time period as described in the parent protocol.

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