Hackensack Meridian *Health*

Hackensack University Medical Center

Pilot Study of Telmisartan (Micardis) For the Prevention of Acute Graft vs. Host Disease Post Allogeneic Hematopoietic Stem Cell Transplantation

PRINCIPLE INVESTIGATOR

Scott D. Rowley, MD, FACP

SUB-INVESTIGATORS

Michelle Donato, MD
David Vesole, MD, PhD
Alan Skarbnik, MD
Sujatha Iyengar, PhD
Phyllis McKiernan, APN
Michele Boonstra, APN
Melissa Baker, APN
Pam Sutherland, APN
Michele Simone, APN

Pro00005465
Version 9 .0

Version Date **14Jul2017**

1. BACKGROUND MATERIAL

1.1 Graft vs. Host Disease

Acute graft vs. host disease (GVHD) caused by mature donor lymphocyte alloreactivity to host tissue antigens (Ags), following allogeneic blood and marrow transplantation (BMT) for hematologic malignancies, is a major cause of morbidity and mortality. Multiple organs can be involved, including the skin, liver, and lungs, but the main cause of death appears to be damage to the intestinal tract (IT) small and large bowel, resulting in sepsis, diarrhea, and shock [1, 2]. A prominent finding is loss of villous crypt Paneth cells, which produce antimicrobial alpha1 defensin [3]. Acute and chronic GVHD are prevented by purging donor hematopoietic cell transplants (HCTs) of mature lymphocytes prior to transplantation [4-6] but this leads to untenable rates of tumor relapse, due to loss of graft vs. tumor effect (GVTE), also mediated by mature donor lymphocytes (primarily T cells). In fact, an inverse correlation exists between severity of GVHD and incidence of relapse [7-9]. This conundrum has spurred efforts to mitigate GVHD while preserving GVTE.

1.2 Overcoming GVHD While Preserving GVTE

Removal of mature alloreactive donor T cells from allogeneic hematopoietic cell transplant (HCT) grafts prevents graft vs host disease (GVHD), but also eliminates graft vs. tumor effect (GVTE), resulting in unacceptable rates of cancer relapse. A solution to this conundrum appears to be possible. In mice, several different strategies for preventing intestinal tract (IT) reduce or eliminate GVHD mortality while preserving systemic alloreactivity and GVTE.

One strategy is to identify tumor specific Ags (TSAs) and the T cell clones recognizing them, so that these may be selectively expanded, while all other alloreactive clones are removed [10, 11]. The limited number of well-defined TSAs is an obstacle to this approach. So, too, is the removal of alloreactivity, which comprises a much broader, stronger, and less readily evaded response repertoire than that generated against a single TSA. Therefore, our long term objective is to ameliorate IT GVHD, while preserving highly effective systemic alloreactive GVTE.

Four general strategies can be envisioned for protecting the gut against GVHD while preserving general alloreactivity: *1)* *Reduce accumulation* of alloreactive effector lymphocytes at the most vulnerable IT sites via tighter endothelial barriers, decreased diapedesis and motility of alloreactive T cells (Teffs), and/or decreased gut-specific homing. *2)* *Inhibit IT neo-vascularization* by donor derived endothelial cells (ECs) differentiating from precursors (EPCs) under hypoxic conditions—recently revealed to be a major source of IT pathology during GVHD [12-15]. *3)* *Activate and expand allospecific IT regulatory T cells* (Tregs) to suppress the inflammatory and cytotoxic responses of effector T cells (Teffs) in a localized manner. In this scenario, Tregs protect the most vulnerable GVHD sites (gut mucosa), while alloreactive Teffs remain in circulation throughout the rest of the body, available to encounter and eliminate residual host derived tumor cells. *4)* *Reduce intestinal leakage* of bacteria and bacterial products such as endotoxin, which induce local and systemic inflammation at sites of GVHD.

The potential utility of the first approach is supported by dramatically reduced GVHD in mice receiving allogenic cells from donors genetically defective for gut homing integrin a4b7 [16], or retinoic acid receptors which transduce signals leading to a4b7 upregulation [2]. Comparable protection against IT GVHD was seen when allograft donor lymphocytes were depleted of a4b7+ populations *prior* to transplantation [16]. In these three cases where a4b7 mediated gut homing cells were absent, host syngeneic tumors were still strongly rejected. In transplant patients, maraviroc (a CCR5 blocker) prevented IT GVHD and acute (within 100 days) death, but was associated with ~20% greater relapse at 1 year (vs. historical controls) – not statistically significant, but suggestive of immune suppression with respect to GVTE, perhaps due to the widespread distribution of CCR5 on immune cells [17].

The potential utility of the second strategy (inhibition of neovascularization) is supported by analogous mouse studies with anti-vascular endothelial (VE)-cadherin mAb, which reduced IT neovascularization and IT GVHD while leaving anti-tumor alloreactivity intact [15]. Blocking hypoxia-induced neovascularization with other agents capable of decreasing IT hypoxia and neovascularization (e.g. telmisartan) may provide similar benefits.

The third approach also appears promising, based on clinical studies. Crowe's group demonstrated an inverse correlation between T regs bearing IT homing receptors a4b7 and acute GVHD in patients [18, 19]. Addition of donor Tregs (not a4b7 selected) suppressed GVHD without significant early interference with anti-tumor immunity [20]. Selective injection of a4b7 Tregs does not appear to have been attempted, but, presumably, would be beneficial with respect to IT GVHD without suppressing systemic GVTE. There is clinical evidence that telmisartan may increase Treg levels and function, while reducing self-reactive T17 cells [21]. Thus, it is possible that telmisartan will also increase the ratio of Treg : alloreactive Teffectors.

The fourth approach has been supported by numerous pre-clinial and clinical studies showing reduced GVHD in mice and patients pre-treated with gut sterilization, or in some cases, pro-biotics prior to allogeneic transplantation, and by studies showing that inhibition of rho kinase prevents intestinal leak syndrome after irradiation [22], and that telmisartan ameliorates colon inflammation in experimental colitis in rodents [23].

1.3 Telmisartan

1.3.1 Mechanisms of action

Telmisartan (Micardis) was developed and FDA approved and licensed as an angiotensin receptor blocker (ARB). It has been safely used for > 18 years as an anti-hypertensive drug. Importantly, immune suppression has not been reported as an increased risk during the post-marketing period. About 6 years ago, it was discovered that telmisartan has a second mechanism of action that may account for much of its anti-hypertensive activity. It was found to be an agonist for peroxisome proliferator activated receptor gamma (PPARγ). PPARγ agonists (e.g. glitazone) are used as insulin sensitizing drugs to treat type 2 diabetes, and also have anti-hyperlipidemia benefits. More recently, the anti-inflammatory functions of PPARγ agonists have been elucidated. Some of these anti-inflammatory effects appear to be due to reduction in the activity of rho associated coiled coil kinase (rho kinase, or ROCK). In fact, telmisartan was found to be as

potent an inhibitor of ROCK as Y27632, a specific ROCK inhibitor [24]. Thus, the anti-hypertensive effects of telmisartan are now thought to reflect the combination of ARB, PPARγ activation, and ROCK inhibition.

Because recognition of telmisartan as a PPARγ agonist has been slow, and its potency as a ROCK inhibitor is still not widely appreciated, many of the obvious pre-clinical and clinical trials have not been done. Additionally, there may be concerns that any positive effects will not be cleanly attributable to a single mechanism of action. In the case of GVHD, however, this poses no problem. We have demonstrated protection from GVHD by the ROCK inhibitor, fasudil, similar in potency and mechanism of action to Y27632 [25] (and see below). Meanwhile rosiglitazone, a specific PPARγ agonist, has been shown to suppress GVHD inflammation in a similar mouse model although survival curves were not followed [26]. Telmisartan's protective effect in experimental rat colitis has been noted, above [23]. Thus, telmisartan is likely to be an effective drug for the treatment and prophylaxis of GVHD, by PPARγ activation *and* downstream ROCK suppression.

With respect to mechanism(s), there is potential for inhibition of T cell migration, as telmisartan abrogates lymphocyte chemotaxis, in part by abrogation of SDF-1 induced chemotaxis [27]. Additionally, telmisartan may maintain gut endothelial barriers by protecting endothelial cells (ECs) from inflammation mediated destruction [28, 29]. Telmisartan has also been shown to prevent neovascularization in corneal systems [30, 31]. Finally, as noted, telmisartan has been shown to increase the ratio of protective Tregs : autoreactive Th17 cells [21], and a very recent report demonstrates anti-inflammatory effects of telmisartan in the setting of chemically induced acute colitis [23]. Multiple genetic pathways activated by inflammation and oxidative stress, along with inflammatory cell infiltrates and gross pathology of weight loss and diarrhea, were attenuated by pre-treatment with telmisartan. Considering the shared pathways between colitis and acute IT GVHD, this is encouraging.

Additional support for the use of telmisartan comes from a review of the literature, and our own work, on ROCK and its inhibitors, fasudil and Y27632. These have been much more extensively studied than telmisartan, over the past two decades, with respect to the three protective mechanisms envisioned. Fasudil occupies the ATP binding pocket of ROCK's enzymatically functional kinase domain, thereby preventing its phosphorylation of myosin light chain II (MLC) and MLC phosphatase (MLCP) [32-34]. Phosphorylation of MLC activates it for actin filament binding and ratcheting, while phosphorylation of MLCP prevents this enzyme from de-phosphorylating (inactivating) MLC. Thus, ROCK potentiates smooth muscle contraction from two angles, both inhibited by fasudil. This explains fasudil's anti-spasmodic properties on arterial smooth muscle, and its anti-hypertensive effects in pre-clinical and clinical studies [35-42], as well as anti-asthmatic effects in OVA induced asthma models [43]. Fasudil, unavailable for clinical use in Europe or USA, has been safely used in Japan and other Asian countries for almost two decades without evidence of immune suppression.

MLC phosphorylation is also important in the context of cell motility and signal transduction. Over the past 15 years, numerous cell functions were shown to be ROCK dependent, including leukocyte chemotaxis, diapedesis, cytokine secretion and responsiveness [44-52], and tumor cell invasiveness and metastasis [53-65]. Additional targets of ROCK **ser / thre** kinase activity include

cytoskeletal ezrin, radixin, moesin, and focal adhesion kinase [66]. Further, ROCK inhibits endothelial nitric oxide synthase (eNOS) production of NO [67, 68]. Thus, it decreases blood flow in NO regulated vascular beds. ROCK inhibition ***increases*** blood flow in NO regulated vasculature, and also appears to enhance the integrity of endothelial barriers, decreasing capillary leakage and endocyte apoptosis [44-52, 69-71]. Additionally, ROCK inhibition antagonizes vascular endothelial growth factor (VEGF) by more than one pathway [72-78]. Finally, a recent paper indicates that, in the context of experimental autoimmune encephalomyelitis, ROCK inhibition promotes T reg differentiation by shifting macrophages from M1 to the Treg inducing M2 phenotype [79].

Given these functions, net effects of ROCK inhibition by telmisartan in the context of allogeneic BMT plus tumor burden could include: i) decreased inflammatory donor cell migration to, penetration of, and inflammation within key target organs of GVHD; ii) vasodilation, decreased hypoxia, and reduced IT neovascularization; iii) decreased Teff function, possibly due to iv) increased Tregs, and v) anti-tumor effects. Indeed, there is pre-clinical evidence suggesting a potential anti-tumor effect of telmisartan [80].

1.3.2 Clinical experience with Micardis brand of telmisartan

Telmisartan has not been previously used for the specific purpose of preventing or ameliorating GVHD.

Telmisartan is used clinically for hypertension and prophylaxis of heart attacks at doses ranging from 20 mg to 160 mg. Typically, patients whose hypertension remains uncontrolled on 80 mg will be given a combination of telmisartan and hydrochlorothiazide in mg combinations of the two drugs at 80/12.5, 80/25, or 160/25.
The onset of antihypertensive activity occurs within 3 hours after administration of a single oral dose. At doses of 20, 40, and 80 mg, the antihypertensive effect of once daily administration of telmisartan is maintained for the full 24-hour dose interval. With automated ambulatory blood pressure monitoring and conventional blood pressure measurements, the 24-hour trough-to-peak ratio for 40 to 80 mg doses of telmisartan was 70 to 100% for both systolic and diastolic blood pressure.

The incidence of symptomatic orthostasis after the first dose in all controlled trials was low (0.04%). Upon initiation of antihypertensive treatment with telmisartan, blood pressure was reduced after the first dose, with a maximal reduction by about 4 weeks. Most of the BP lowering effect occurs within the first 2 weeks of treatment. With cessation of treatment with micardis tablets, blood pressure gradually returned to baseline values over a period of several days to one week. The antihypertensive effect of telmisartan is not influenced by patient age, gender, weight, or body mass index. Blood pressure response in black patients (usually a low-renin population) is noticeably less than that in Caucasian patients. Limited data are available with regard to overdosage in humans. The most likely manifestation of overdosage with MICARDIS tablets would be hypotension, dizziness and tachycardia; bradycardia could occur from parasympathetic (vagal) stimulation.

1.3.3 Drug interactions and cautions

Telmisartan is contraindicated during pregnancy, and no subjects will be enrolled if there is a chance of pregnancy during the telmisartan treatment phase of the trial. We do not anticipate any such volunteers in an HCT based study, because all female HCT patients who have not been menopausal for at least 1 year, have been serum screened with serum pregnancy tests and are placed on high dose Ovral to prevent pregnancy, which will continue for the 100 days' duration of telmisartan administration, as per Standard Operating Procedures (SOP). Similarly, women are told not to breast feed children during HCT as per SOP. Any women who choose to nurse children will not be eligible for the study, or will be removed from the study if they have started to matriculate.

In patients with an activated renin-angiotensin system, such as volume- or salt-depleted patients (e.g., those being treated with high doses of diuretics), symptomatic hypotension may occur after initiation of therapy with telmisartan. We will take patients off their non-telmisartan anti-hypertensives for two days prior to administration of telmisartan. Blood chemistries, blood pressure, and urination frequency will be monitored to ensure adequate hydration and normokalemia prior to starting telmisartan. For patients already taking telmisartan as part of their normal medical regimen, their daily dose will be replaced by the starting dose of 160 mg and adjusted as needed (see section 5.3.3.). Patients who require additional anti-hypertensive medications will have an appropriate drug added. For example, hydrochlorothiazide and aldosterone have been used in standardized ratios with telmisartan.

Hyperkalemia may occur in patients on telmisartan, particularly in patients with advanced renal impairment, heart failure, on renal replacement therapy, or on potassium supplements, potassium-sparing diuretics, potassium-containing salt substitutes or other drugs that increase potassium levels.

As the majority of telmisartan is eliminated by biliary excretion, patients with biliary obstructive disorders or hepatic insufficiency can be expected to have reduced clearance.

As a consequence of inhibiting the renin-angiotensin-aldosterone system, changes in renal function may occur in susceptible individuals. In patients whose renal function may depend on the activity of the renin-angiotensin-aldosterone system (e.g., patients with severe congestive heart failure or renal dysfunction), treatment with angiotensin receptor antagonists has been associated with oliguria and/or progressive azotemia and (rarely) with acute renal failure and/or death. Patients with such pre-existing conditions would not be candidates for HCT, in any case.

In patients who are elderly, volume-depleted (including those on diuretic therapy), or with compromised renal function, co-administration of NSAIDs, including selective COX-2 inhibitors, with telmisartan, may result in deterioration of renal function, including possible acute renal failure. These effects are usually reversible. The antihypertensive effect of angiotensin II receptor antagonists, including telmisartan may be attenuated by NSAIDs including selective COX-2 inhibitors.

Aliskiren is associated with increased risks of hypotension, hyperkalemia, and changes in renal function (including acute renal failure) compared to monotherapy. Patients cannot be on Aliskiren while on the study. Aliskiren should not be co-administered with Micardis in patients with diabetes.

When Micardis was co-administered with digoxin, median increases in digoxin peak plasma concentration (49%) and in trough concentration (20%) were observed. Therefore, we would monitor digoxin levels when initiating, adjusting, and discontinuing telmisartan for the purpose of keeping the digoxin level within the therapeutic range.

Reversible increases in serum lithium concentrations and toxicity have been reported during concomitant administration of lithium with angiotensin II receptor antagonists including Micardis. Therefore, we will monitor serum lithium levels during concomitant use.

Co-administration of telmisartan 80 mg once daily and ramipril 10 mg once daily to healthy subjects increases steady-state Cmax and AUC of ramipril 2.3- and 2.1-fold, respectively, and Cmax and AUC of ramiprilat 2.4- and 1.5-fold, respectively. In contrast, Cmax and AUC of telmisartan decrease by 31% and 16%, respectively. When co-administering telmisartan and ramipril, the response may be greater because of the possibly additive pharmacodynamic effects of the combined drugs, and also because of the increased exposure to ramipril and ramiprilat in the presence of telmisartan. Concomitant use of Micardis and ACE inhibitors such as ramipril is not permitted on this study.

Drugs ***without*** known telmisartan interactions include: acetaminophen, amlodipine, glyburide, simvastatin, hydrochlorothiazide, warfarin, and ibuprofen. Telmisartan is not metabolized by the cytochrome P450 system and had no effects in vitro on cytochrome P450 enzymes, except for some inhibition of CYP2C19. Telmisartan is not expected to interact with drugs that inhibit cytochrome P450 enzymes; it is also not expected to interact with drugs metabolized by cytochrome P450 enzymes, except for possible inhibition of the metabolism of drugs metabolized by CYP2C19.

1.3.4 Adverse Events

A comprehensive meta-analysis of telmisartan safety and side effects [108] summarized the findings of 30 double blind (~ 8,000 volunteers) and 20 open label studies (~ 8,400 volunteers). Treatments investigated were placebo, telmisartan 10-160 mg, or telmisartan 10-160 mg plus Hydrochlorothiazide (HCT) 6.25-25 mg. The incidence and causality of all adverse events (AEs) and laboratory abnormalities occurring during treatment were recorded.

The incidences of all-cause AEs in the double-blind studies were: 2.73 per patient-year (PY) in placebo), 2.03/PY with telmisartan monotherapy, and 2.09/PY for telmisartan plus HCT. The respective numbers in the open-label studies were: 0.65/PY (telmisartan monotherapy) and 0.70/PY (telmisartan plus HCT). The most frequent suspected adverse reactions were dizziness and headache, which were comparable across groups and studies. The overall incidence of drug-

related laboratory abnormalities was low in all treatment groups. Incidences of discontinuation due to an AE were 4.6%, 4.5% and 4.7%, respectively, for the placebo, telmisartan and telmisartan plus HCT treatment groups.

The most common adverse events (≥ 1%) reported in hypertension trials of Micardis are back pain, sinusitis, and diarrhea (see **Table 1**). When Micardis was used for the reduction of cardiovascular risk, the serious adverse events (≥1%) were intermittent claudication and skin ulcer. The incidence of adverse events ***was not dose-related*** and did not correlate with gender, age, or race of patients.

Table 1. Adverse Events Occurring at an Incidence of ≥ 1% in Patients Treated with MICARDIS and at a Greater Rate Than Patients Treated with Placebo

	Telmisartan *n=1455* (%)	Placebo *n=380* (%)
Upper respiratory tract infection	7	6
Back pain	3	1
Sinusitis	3	2
Diarrhea	3	2
Pharyngitis	1	0

In addition to these adverse events, the following events occurred at a rate of ≥1% but were at least as frequent in the placebo group: influenza-like symptoms, dyspepsia, myalgia, urinary tract infection, abdominal pain, headache, dizziness, pain, fatigue, coughing, hypertension, chest pain, nausea, and peripheral edema. Discontinuation of therapy because of adverse events was required in 2.8% of 1455 patients treated with Micardis tablets and 6.1% of 380 placebo patients in placebo-controlled clinical trials. The incidence of cough occurring with telmisartan in 6 placebo-controlled trials was identical to that noted for placebo-treated patients (1.6%).

In addition to those listed above, adverse events that occurred in more than 0.3%-1% of 3,500 patients treated with MICARDIS in past clinical trials are listed below. We do not know if these events were related to MICARDIS brand telmisartan or not:

Nervous System: impotence, increased sweating, flushing
Whole body: allergy, fever, leg pain, malaise
Cardiovascular: palpitations, leg swelling, chest pain, fast heart rate, abnormal ECG
Central nervous system: insomnia, sleepiness, migraine, vertigo, dizziness, involuntary muscle contractions, decreased sense of touch Gastrointestinal: flatulence, constipation, heartburn, vomiting, dry mouth, hemorrhoids, gastroenteritis, enteritis, acid reflux, toothache
Metabolic: gout, high cholesterol, diabetes Musculoskeletal: arthritis, joint pain, leg cramps
Psychiatric: anxiety, depression, nervousness
Immune system: infection, fungal infection, abscess, ear infection
Respiratory: asthma, bronchitis, nasal inflammation, difficulty breathing, nose bleed
Skin: rash, eczema, itchiness
Urinary: frequency urinating, bladder infection
Vascular: strokes
Special Senses: abnormal vision, conjunctivitis, ringing in the ears, earache.

During initial clinical studies, a single case of angioedema (swelling of the deeper layers of the skin) was reported (among a total of 3781 patients treated).

Post-Marketing: During the years that telmisartan has been sold around the world, the following events have been reported in people taking telmisartan, but there is no evidence that they are directly related to use of the drug. The most frequent reported events include: headache, dizziness, coughing, nausea, fatigue, weakness, swelling of the body, limbs or face, hypersensitivity, sweating increased, redness of skin, chest pain, abnormal heartbeat, congestive heart failure, heart attack, high blood pressure, low blood pressure, high potassium, fainting, indigestion, diarrhea, pain, urinary tract infection, erectile dysfunction, back pain, abdominal pain, muscle cramps (including leg cramps), muscle soreness, slow heart rate, eosinophilia, lowering of platelets, uric acid increased, abnormal liver function, kidney problems includingkidney failure, low hemoglobin, increased CPK, allergic reaction, tendon pain, severe skin rash, low blood sugar (in diabetic patients), and deep skin swelling (with fatal outcome). Rare cases of rhabdomyolysis (muscle damage) have been reported in patients receiving drugs similar to MICARDIS.

1.3.5 Pharmacokinetics

Following oral administration, peak concentrations (Cmax) of telmisartan are reached in 0.5 to 1 hour after dosing. Food slightly reduces the bioavailability of telmisartan, with a reduction in the area under the plasma concentration-time curve (AUC) of about 6% with the 40 mg tablet and about 20% after a 160 mg dose. The absolute bioavailability of telmisartan is dose dependent. At 40 and 160 mg the bioavailability was 42% and 58%, respectively. The pharmacokinetics of orally administered telmisartan are nonlinear over the dose range 20 to 160 mg, with greater than proportional increases of plasma concentrations (C_{max} and AUC) with increasing doses. Telmisartan shows bi-exponential decay kinetics with a terminal elimination half-life of approximately 24 hours. Trough plasma concentrations of telmisartan with once daily dosing are about 10 to 25% of peak plasma concentrations. Telmisartan has an accumulation index in plasma of 1.5 to 2.0 upon repeated once daily dosing.

Telmisartan is highly bound to plasma proteins (>99.5%), mainly albumin and α1 - acid glycoprotein. Plasma protein binding is constant over the concentration range achieved with recommended doses. The volume of distribution for telmisartan is approximately 500 liters indicating additional tissue binding.

Following either intravenous or oral administration of ^{14}C-labeled telmisartan, most of the administered dose (>97%) was eliminated unchanged in feces via biliary excretion; only minute amounts were found in the urine (0.91% and 0.49% of total radioactivity, respectively).

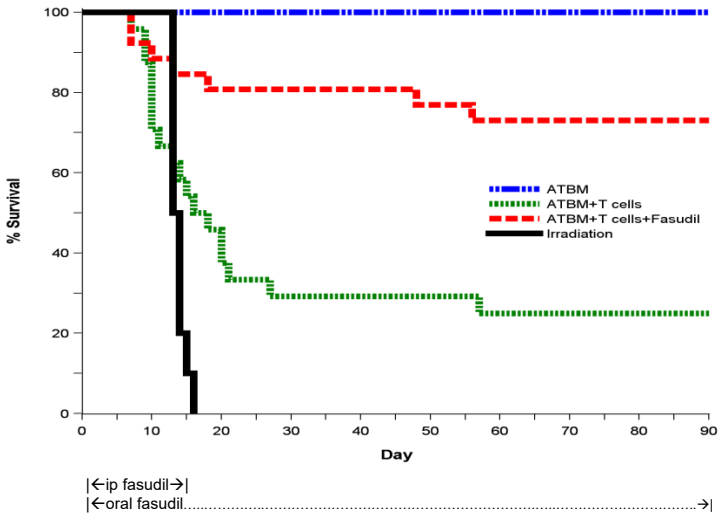
1.4 Relevant preliminary pre-clinical studies

In our own *pre-clinical studies* with the ROCK inhibitor, fasudil, in the C3H → [C57Bl/6 x C3H]F1 (B6C3F1) MHC allogeneic BMT model of acute GVHD, healthy young adult male B6C3F1 mice

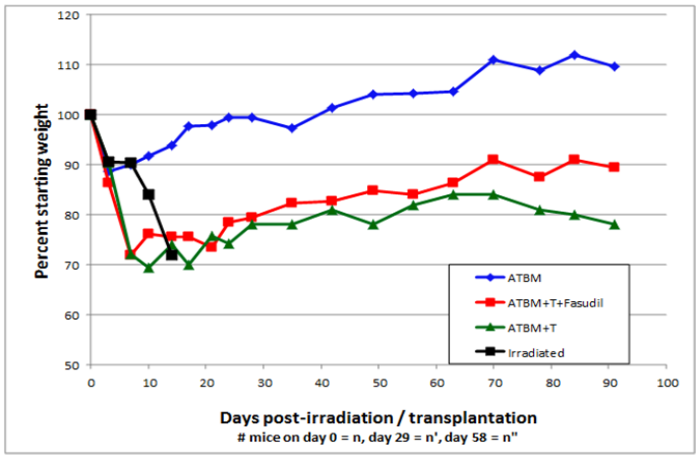
receive a split dose (550 cGy x 2) of marrow ablating radiation and, on the same day, 2 x10^6 anti-thymocyte treated C3H bone marrow (ATBM). Some groups of mice also receive 5 x 10^6 splenic T cells at the same time, or 5 x 10^6 splenic T cells plus fasudil. The fasudil treated mice start drug 24 hrs prior to irradiation and transplant, receiving both i.p. (200 ug twice daily) and oral (1 mg/ml drinking water = ~ 3 mg per day) for 11 days, after which time i.p. injections are discontinued and p.o. drug maintained.

Figure 1A illustrates the cumulative 90 day survival results of 3 experiments. Despite the highly significant ($p < 0.0001$) increase in survival among fasudil treated groups (73%) vs. untreated groups (25%) receiving donor T cells, mice experienced significant weight loss with or without drug within the first two weeks (25-30%) compared with ATBM only recipients (10%). On average, fasudil treated and untreated surviving mice lost the same percentage of weight in the first 1-2 wks, with comparable kinetics of weight stabilization and gradual increase among 1 month survivors. There was continued gradual, but incomplete, weight recovery over the remaining period of observation (Figure 1B). Among these progressively fewer animals in both groups surviving beyond day 28, weight recovery of fasudil treated mice was significantly greater than among untreated mice receiving ATBM + T cells ($p < 0.001$). Mice receiving only ATBM, without T cells, lost only about 10% of their total body weight, which they recovered by 2 weeks, and then continued to grow normally over the next two months, reaching 120% of starting weight by time of sacrifice.

Figure 1. A. Survival of irradiated B6C3F1 mice receiving ATBM + T cells +/- fasudil



B. Weight of surviving F1 hosts over time



ATBM n=n'=n"=18; ATBM+T+fasudil n=26, n'=21, n"=19
ATBM + T n=24, n'=9, n"=8; Irradiated n=10, n'=n"=0

Despite similar weight losses among fasudil treated and untreated T cell recipients in the first two weeks, the two groups had different rates of diarrhea. The majority (> 70%) of untreated mice (vs. 20% of treated) had loose stools for one or more days between the second and third weeks post-transplantation, the period of highest mortality incidence. Diarrhea resolved by the fourth week among survivors in both groups. Irradiated mice not receiving donor cells were not observed to have diarrhea prior to death.

Fasudil also failed to fully prevent skin inflammation, which started in the 4th week and gradually waned over the next two weeks. ATBM controls lost fur, which grew back lighter colored, but developed no skin inflammation. Histopathology on treated mice sacrificed at ~ 90 days showed an absence of inflammation with completely normal histology (**Fig. 2A**) or, at most, in some mice, a moderate mononuclear infiltrate at the base of small bowel crypts (**Fig. 2B**). Interestingly, long term *untreated* survivors also had little or no infiltration.

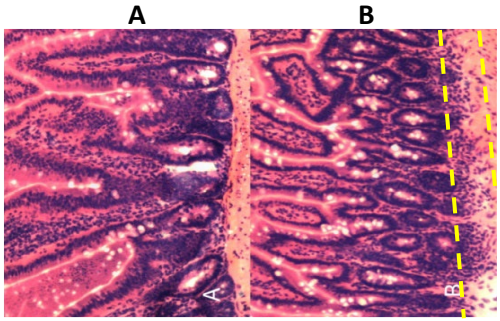


Figure 2. Fasudil treated mice show minimal small intestinal crypt inflammation. Basilar crypts showed (A) no infiltration, or (B) limited inflammation within yelow dashed lines of basal layer and lamina propria, with intact crypts, in 90 day survivor fasudil treated mice. A similar picture of mild or absent inflammation was seen at 90 days in the much smaller number of surviving untreated mice.

Fasudil treatment does not induce systemic donor allotolerance for host. Given the evidence of GVHD in fasudil treated mice, we asked whether their spleen cells were alloreactive to B6C3F1 host cells. Spleens were harvested at day 10, or at day 84 or 98 post-transplant. The day 10 samples represent donor T cells migrating to the spleen, without *de novo* generated T cells emigrating from the host thymus. The day 84-98 spleens contain some newly generated T cells maturing within the host thymus (and, therefore, allotolerant of host B6 parental antigens), as well as mature T cells from C3H donors. Spleens were homogenized and stimulated at a 3:1 ratio with irradiated B6C3F1 or BALB/c spleen cells in one-way mixed lymphocyte reactions (MLRs). After 5 days of stimulation, the very limited numbers of lymphocytes present in day 10 spleens were assayed by IFN-γ ELIspot, a surrogate for CD8+ CTL activity. The more numerous day 84 and 98 lymphocytes were assayed for proliferation by CFSE labeling and analysis of dilution peaks (= cell divisions).

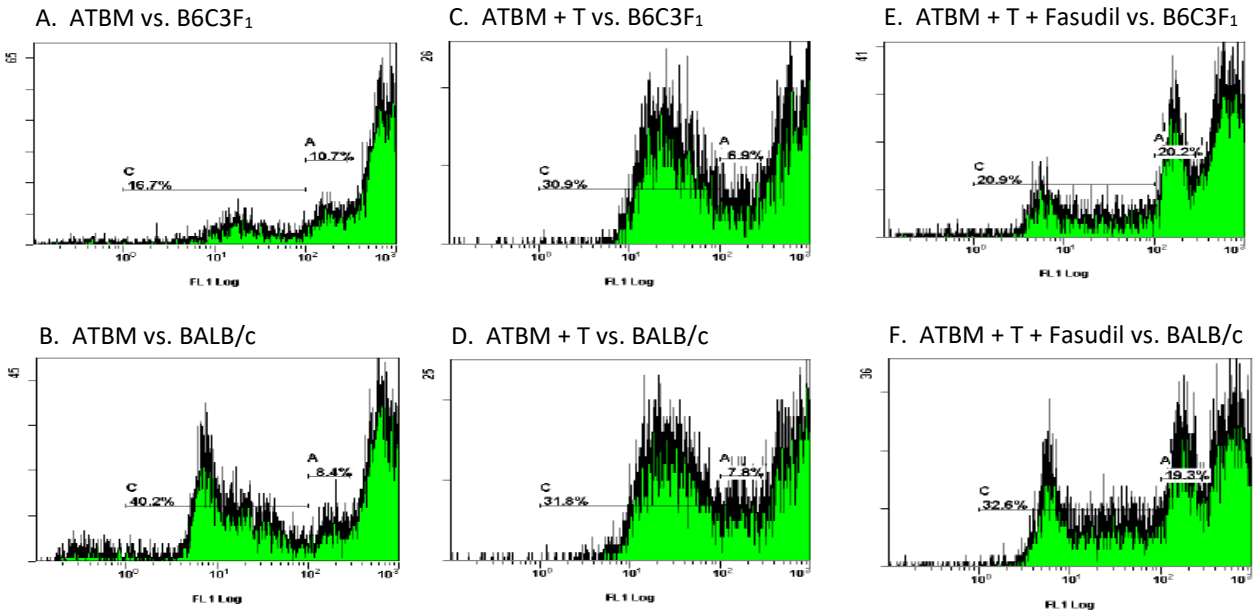
As shown in **Table 2**, there was *no evidence of fasudil induced allotolerance to host B6C3F1*, as measured by IFN-γ ELISpot after one-way donor C3H vs. irradiated B6C3F1 stimulation of splenocytes in vitro. Early time point donor derived T cells responded at least as robustly as splenocytes from untreated mice. Indeed, the greater response to B6 parental Ags than to DBA alloantigens suggests in vivo priming within the B6C3F1 host. As expected, spleens from mice receiving no mature donor T cells (ATBM only) made no responses to any of the stimuli, as no newly formed T cells had yet emerged from the thymus.

Table 2. Day 10 IFN-γ secreting spleen cell frequencies (per 100,000 cells) after 5 day one-way MLRs vs. self (C3H), host B6C3F1, or third party (DBA, H-2^d) stimulators.

Irradiated Stimulators	Responding Splenocytes from Hosts Receiving:		
	ATBM only	ATBM + T cells	ATBM + T cells + Fasudil
C3H	0	4.6	2.3
B6C3F1	0	82.3	115.6
DBA (H-2^d)	0	16	18

Figure 3 shows results from spleens harvested on day 98. Very similar results were obtained with day 84 spleens (not shown). CFSE-labeled splenic lymphocytes from B6C3F1 recipients of ATBM cells alone had a relatively low proportion of cells undergoing multiple rounds of division after 5 days of stimulation with irradiated host cells (A), compared with the proportion of cells undergoing multiple rounds of division in response to third-party allogeneic BALB/c stimulators (B). The limited response to parental B6 MHC antigens is expected from C3H stem cell-derived T cells generated de novo within the F1 host thymic environment. By contrast, splenocytes from recipients of ATBM + donor T cells made a much stronger response to B6C3F1 cell stimulation (C), very similar in magnitude to their response to BALB/c stimulation (D). Fasudil treated recipients of ATBM + T cells also contained splenocytes that made a strong response to B6C3F1 stimulation, with multiple rounds of cell division which yielded weakly fluorescent peaks (E), comparable to their response to BALB/c stimulation (F). When assayed by EIA (not shown), supernatants from 90 day surviving ATBM + T + fasudil treated mice and ATBM + T mice stimulated in one way MLR cultures with irradiated B6C3F1 cells contained significantly more IFN-γ than supernatants from spleens of mice receiving ATBM with no transferred T cells. This is consistent with intact host alloreactivity in fasudil treated survivors. No IL-10 was detected in any MLR cultures. Similar results were obtained with cells from long term survivors sacrificed on day 84.

Figure 3. Proliferation in One-Way MLR Determined by CFSE Dilutional Peaks



To the extent that telmisartan's inhibition of ROCK is comparable to fasudil's, we would expect similarly beneficial results in this mouse model. More importantly, we would expect beneficial results in human trials.

1.5 Biomarkers of GVHD as potential predictors of GVHD

1.5.1 Plasma proteins detected by Enzyme Linked Immunoassay (EIA)

Progress has been made in identifying blood markers of GVHD [83-87]. Levine et al. [83] showed that a panel of 6 plasma markers (elefin, IL-8, TNFR1, HGF, reg3a, IL-2Ra) significantly correlated with non-responsive GVHD and non-relapse mortality (NRM). More recently, the same group, using a more extensive panel, partially overlapping with their previous one, found that essentially ***full predictive power was provided by a single marker, ST2*** (suppression of tumorigenicity 2), a recently described member of the IL-1R family, which binds IL-33, thereby driving T cells toward a Th1 effector phenotype [86, 87]. High ST2 levels in the first two weeks post-HCT were strongly predictive of non-relapse mortality (NRM) in patients with IT GVHD, but not with skin GVHD only. The Paneth cell regeneration marker, ***reg3a, had independent and additive predictive value for IT GVHD*** [88]. This is consistent with very recent findings from models showing that early immune mediated destruction of paneth cells in the intestinal villous crypts eliminates their production of beneficial alpha-defensins, leading, in turn, to altered gut flora, with overgrowth by *E. coli*, *C. difficile,* seeding of organs, and septic complications causing death [88-92]. In a somewhat analogous, but converse manner, Levine at al. found that plasma elafin levels rose at the onset of skin GVHD, but not IT GVHD, and were predictive of NRM [83]. By contrast with elafin or reg3a and ST-2, ceruloplasmin plasma levels between days 7 - 28 post-HCT were highly significant predictors of *both* skin and IT GVHD [93].

1.5.2 Endotoxin as a potential biomarker

Given the importance of the IT microbiome and tissue disruption in intestinal GVHD, there has been great interest throughout many fields of medicine, in monitoring the release of LPS or its toxic Lipid A moiety in the blood. Unfortunately, measurement of LPS or Lipid A in blood, plasma, or serum is fraught with difficulties, due to the presence of various binding proteins and cells, with varying rates of dissociation under different conditions. Recently, however, an Endotoxin Activity Assay (EAA™, Spectral Diagnostics, Inc.) has been developed that measures Lipid A endotoxin in whole blood with sufficient sensitivity, specificity, and reproducibility to be FDA approved for detection of endotoxin in whole blood [94-98]. We will monitor blood LPS with the EAA in ***the first such study to evaluate its predictive and/or correlative value in the context of acute GVHD.***

1.6 GVHD and the Microbiome

Sequencing the diversity of stool and urine microbiota is a new and promising area of investigation for allogeneic transplantation in general. Changes in stool bacteria (increased *Lactobacilli species*, decreased *Clostridia species*) have been correlated with acute GVHD in both humans and mice by Jenq et al. [99]. In allogeneic renal transplants, Fricke et al. also found longitudinal alterations in stool, oral, and urinary microbiota [100]. Surprisingly, urines were positive for a diverse flora in one third of cases, with no symptomatic evidence of UTI. While it is reasonable to expect disruptions in the IT microbiome to correlate strongly with IT GVHD, current attention is also focused on the “gut-skin” axis. Evidence is accumulating that gut

function influences skin health. Arck et al., have reviewed their own data and others' documenting improvement in mouse skin inflammation following the oral administration of probiotics [101].

1.7 Measurement of ROCK activity in vivo

As noted above, ROCK is associated with a variety of inflammatory functions. Thus, ROCK activity in vivo could be a marker for GVHD. ROCK is also a potential target of telmisartan, and could be suppressed by telmisartan independently of, or concomitantly with suppression of GVHD. We will use standard Western Blot methodology to monitor ROCK phosphorylation of a major substrate, myosin binding subunit (MBS) of Myosin Phosphatase within peripheral blood mononuclear cells (PBMCs). We will measure the relative amount of phosphorylated (Threonine 853 and Threonine 696) MBS : total MBS in cell lysates, using anti-phospho-MYPT1 (thr696), and anti-phospho-MYPT1 (thr853) mAbs (Millipore, Billerica, MA, USA). We will also measure total ROCK II in cells using anti-ROCK2 antibodies (AbCam, Cambridge, MA, USA). After blotting for specific proteins, membranes are stripped and blotted with glutaraldehyde 3–phosphate dehydrogenase (GAPDH) antibody for protein normalization. We will obtain samples from baseline, post-engraftment on telmisartan (~ 6, 10, and 14 weeks post-HCT), and at two time points after stopping telmisartan. Assays will be run on batched cryopreserved samples.

2.0 Study Rationale and Overview

In humans, as in mice, IT GVHD is the main cause of death within the first 100 days of HCT. A single clinical trial has tested the concept of selective suppression of IT GVHD, using the CCR5 blocker, maraviroc, which effectively targets a significant proportion of donor T cells homing to the IT [17]. There were no acute deaths among treated patients—a remarkable finding—but there was a 20% increase in cancer relapse at 1 year, when compared with historical controls. While this did not reach statistical significance, it raises questions about the specificity of CCR5+ cell targeting and loss of systemic alloreactivity at the doses used.

We propose to test a different drug, telmisartan, with anti-inflammatory PPARγ agonistic and Rho kinase (ROCK) inhibitory mechanisms of action in conjunction with standard GvHD prophylaxis. Telmisartan has never been used specifically for ROCK inhibition or GVHD. Telmisartan will be used for the initial 100 days post-transplant, which is considered the acute period for GVHD. Reducing acute GVHD will save lives and reduce morbidity of HCT recipients. Cancer recurrence may decline, as less immune suppression will be required. Most cancers would benefit from potent GVTE, and selectively controlling GVHD would greatly broaden the applicability of this therapy to non-hematologic solid tumors. The pilot trial with telmisartan, which we propose here, is modeled loosely on the maraviroc trial [17]. Primary endpoints are drug tolerability, Grade II-IV GVHD, non-relapse mortality (NRM, including Grade V GVHD), primary or secondary graft failure, and cancer progression or relapse at 100 and 180 days.

A secondary (exploratory) goal of this study is to examine the correlation of various blood biomarkers with the onset of GVHD. Knowledge gained about the correlations of bio markers

with GVHD could permit more efficient and sparing use of potent immuno-suppressive steroids or cytotoxic agents. To the extent that telmisartan prevents severe GVHD, we will have fewer events to correlate with our selected biomarkers, but that is an outcome we would welcome, as it would reflect effective treatment. If telmisartan does not suppress GVHD, we anticipate that one or more of our selected markers will exhibit statistical correlation with development of GVHD.

The blood markers to be assessed are ST2 and reg3a (which have been associated with IT GVHD), elafin (associated with skin GVHD) and ceruloplasmin (previously correlated with both gut and skin GVHD). Additionally, we will use the whole blood endotoxin activity assay (EAA, Spectral Diagnostics, Inc.). This assay has been FDA and European Community approved for detecting endotoxin in patients entering emergency rooms with a diagnosis of possible sepsis. We believe it may be sensitive enough to pick up LPS translocation thought to occur during GVHD. This will be the first such trial of this assay in the context of allogeneic transplant and GVHD. Confirmation of one or more biomarkers' association with GVHD would allow more timely use of intensified immune suppressive drugs, ideally for shorter periods, thereby decreasing GVHD and toxicity.

We will look at the ratio of certain Treg and Teff subsets in peripheral blood. While there is no question that such subsets are crucial for the outcome of GVHD, it has been difficult to use them as peripherally circulating markers, since they rapidly leave the blood stream and lodge in bone marrow or tissue sites of potential GVHD. We will look at CD4+FoxP3+CD73+ and CD4+ FoxP3+CD39+ T regulatory cells (Tregs), as they have been controversial as peripheral markers of protection from GVHD, and we will also look at CD4+CD146+CCR5+ cells, which were very recently reported as being positively correlated with GVHD [102]. We will also stain for CD8+FoxP3+CD39+ and CD8+FoxP3+ CD73+ Tregs. Finally, we will look for a4b7+CD4+FoxP3+ Tregs that may reflect cells homing to the IT. The biggest caveat with these measurements is that they can only capture points in time from the peripheral circulation, and may not always reflect the relative proportions of these subsets within the intestinal intraepithelial space, the mesenteric nodes, or the villous epithelium and *lamina propria* layers.

While the ROCK inhibitory activity of telmisartan has been documented in rodent models and in vitro, it has never been demonstrated directly in vivo in humans. This may not be possible using only peripheral blood mononuclear cells, but that is the sample type we have readily available for assaying. ROCK inhibition is expected to correlate with telmisartan in vivo biological activity (e.g., lowering of blood pressure), and may also correlate with suppression of GVHD. Obviously, the feasibility of this assay will depend on the baseline levels of ROCK activity seen in patients. There is a limited literature on this. We will follow the procedures and methods of Cheng et al., who recently showed a statistically significant increase in ROCK activity among stroke patients, with ROCK activity, measured as phosphorylated : total Myosin Binding Subunit, revealed to be an independent predictor of stroke in high risk ischemic patients [103]. In that study, the non-stroke patients had relatively weak ROCK signal, so our results will depend heavily on the baseline and post-treatment levels of Phospho-MBS, which may or may not be high enough to reveal telmisartan mediated suppression. Even if telmisartan suppression of ROCK is not detectable, we do anticipate positive correlation of ROCK activity with IT GVHD, so biomarker utility may be demonstrable.

Finally stool and urine microflora samples will be batch analyzed for diversity, firmaceutes: bacteroidetes: enterocci ratios, and *Clostridia : Lactobacilli* ratios. This will be a *post-hoc* analysis that may contribute to the growing understanding of the role of the gut microbiome in the development of GVHD.

2.1 Rationale for Dose Selection of 160 mg/day.

Telmisartan is sometimes prescribed at 160 mg per day, but this is usually in patients with persistent hypertension, and in those cases telmisartan 160 mg is paired with an additional anti-hypertensive, such as hydrochlorothiazide (12.5 or 25 mg) or amlodipine (10 mg).

The antihypertensive effects of Micardis brand of telmisartan have been demonstrated in six principal placebo-controlled clinical trials, studying a range of 20 to 160 mg; one of these examined the antihypertensive effects of telmisartan and hydrochlorothiazide in combination. The studies involved a total of 1773 patients with mild to moderate hypertension (diastolic blood pressure of 95 to 114 mmHg), 1031 of whom were treated with telmisartan. Following once daily administration of telmisartan, the magnitude of blood pressure reduction from baseline after placebo subtraction was approximately (SBP/DBP) 6-8/6 mmHg for 20 mg, 9-13/6-8 mmHg for 40 mg, and 12-13/7-8 mmHg for 80 mg***. Larger doses (up to 160 mg) did not appear to cause a further decrease in blood pressure.***

One large study showed no further decrease, on average, in blood pressure between 80 and 160 mg of telmisartan alone, without additional anti-hypertensive drugs [81]. Another showed that daily doses of 40, 80 or 160 were all in the same plateau range for anti-hypertensive effects at steady state, despite roughly linear increases in single dose peak circulating blood levels [82]. Thus, subjects are likely to tolerate the highest dose (160 mg daily) without undue drops in blood pressure, allowing us to assess the anti-GVHD properties of telmisartan at this dose without a high frequency of adverse side effects from excessive lowering of blood pressure.

In two studies of normotensive,healthy volunteers, doses of telmisartan at 160 mg per day and 320 mg/d were well tolerated, without any further anti-hypotensive effect compared to 80 mg per day [82a, 82b].

Despite the lack of increased hypotensive effect of increasing telmisartan from 80 to 160 mg per day, we want to use the higher dose because it may have a greater anti-inflammatory effect, even if the impact on hypotension is not enhanced. In particular, since telmisartan is eliminated via the biliary-intestinal tract, higher levels of drug will be present at the intestinal mucosal surface as a consequence of increased drug elimination. The intestinal tract mucosa is the primary target of GvHD that we are trying to protect in this trial, as it accounts for the majority of sever acute GvHD cases.

3.0 STUDY OBJECTIVES

3.1 Primary Objective

To assess the utility of adding telmisartan at 160 mg/day (or the highest tolerated dose of 40, 80, or 160 mg, daily) to standard GvHD prophylaxis for the prevention of grade II or greater acute graft vs. host disease (aGVHD) in patients receiving allogeneic HCT.

3.2 Secondary Objectives

To assess the safety and tolerability of telmisartan at doses of 160 mg per day (adjusted downward to 80 or 40 mg/day as necessary, on an individual basis for each patient), given orally to patients receiving allogeneic HCT.

3.3 Exploratory Objectives

3.3.1 To identify biomarkers that correlate with, or are predictive of, grade III-V GVHD

3.3.2 To identify gut and urine flora diversity (Shannon Diversity Index) and changes in the percentages of firmicutes, bacteroidetes, and enterocci, ratio of Clostridia and Lactobacillus species over time, pre- and post- transplantation, in the presence and absence of telmisartan and/or GVHD. The healthy microbiome contains 40-50% firmicutes, 30-40% bacteroidetes, and < 10% enterocci. Some dysbiosis occurs as a result of conditioning and transplantation, with enterocci increasing to ~ 25%. However, during GVHD, these ratios change dramatically, with enterocci increasing to ~50%, and bacteroidetes dramatically dropping.

3.3.3 To assess the level of ROCK activity in peripheral blood mononuclear cells, on and off telmisartan treatment, in the presence or absence of GVHD.

3.3.4 To measure percentages of lymphocyte subsets, described above, associated with GVHD or suppression of GVHD.

4.0 STUDY POPULATION

The study population will consist of 60 patients undergoing allogeneic HCT for treatment of hematologic malignancies meeting the following criteria. Subject eligibility will be documented by a qualified member of the study team.

4.1 Eligibility Criteria:

4.1.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Meet the eligibility criteria for HCT as defined by the Standard Operating Procedures (SOP) of the blood and marrow transplantation program of Hackensack University Medical Center.

2. Diagnosis of:
   - Acute myeloid or lymphoid leukemia in remission,
   - Myelodysplastic syndrome,
   - Chronic lymphoid leukemia,
   - Non-Hodgkin lymphoma,
   - Hodgkin lymphoma,
   - Chronic myeloid leukemia in chronic or accelerated phase,
   - Myeloproliferative disorder, or
   - Multiple myeloma

3. Undergoing allogeneic HSC transplantation from a related or unrelated donor matched at least at 7 of 8 of the HLA-A, -B, -C, and DR loci ("8/8" or "7/8" match)
4. Planned to receive standard GvHD prophylaxis regimen. Subjects receiving rabbit ATG (Thymoglobulin) as part of their conditioning regimen are eligible to participate.
5. Male or female patient age 18 years or older

6. Karnofsky performance status $\geq$ 70% at time of initiation of pre-transplant conditioning

7. Transplantation-specific co-morbidity score of <5 at time of initiation of pre-transplant conditioning

8. Patients taking antihypertensive medications (including telmisartan) are eligible but the patient must discontinue treatment at least 48 hours prior to first dose of study medication. In cases where the patient's physicians feel it would be inadvisable to stop all anti-hypertensive treatment prior to starting telmisartan, the patient may continue on amlodipine, hydrochlorothiazide, furosemide, or other non-ARB, non-ACE inhibitors, or non-potassium sparing anti-hypertensives. If ARBs, ACE inhibitors, renin inhibitors, spironolactone, or other potassium sparing drugs cannot be discontinued prior to starting telmisartan, the patient will be ineligible for the study.

9. Capable of giving informed consent and having signed the informed consent form

4.1.2 Exclusion criteria:

Subjects who meet any of the following exclusion criteria will not be eligible for enrollment in this study:

1. Inability to provide informed consent

2. Subjects with known heart failure, advanced renal impairment requiring renal replacement therapy, or liver failure although these patients would most likely not be eligible for HCT, as per SOP of the blood and marrow transplantation program of Hackensack University Medical Center.

3. Subjects taking ACE inhibitors, ARBs, renin inhibitors, potassium supplements, or spironolactone (or any other potassium-sparing diuretics) who cannot discontinue use prior to initiation of study treatment OR who require a high-potassium diet.

4. Patient unable to discontinue current ARB, ACE inhibitor, renin inhibitors, spironolactone, or other potassium sparing hypertension medication for medical or other reasons for two days prior to starting telmisartan

5. Chronic symptomatic hypotension, volume depletion.

6. Planned to receive GvHD prophylaxis regimen with an investigational agent.

7. Pregnant or likely to become pregnant during the period of study, or unwilling to stop nursing.

4.2 Recruitment Procedures

4.2.1 Informed consent process. Patients who are already selected to undergo HCT for a hematologic malignancy (as per eligibility criteria) will be recruited of the John Theurer Cancer Center at Hackensack University Medical Center (Hackensack UMC). Once a potential subject's eligibility has been determined via review of medical records, the subject will be approached for participation by the PI, sub-I, or another qualified member of the research team. The patient will be given a copy of the informed consent form to review. No recruitment materials (e.g., flyers, advertisements) will be used to recruit patients into this study. Patients will not be offered financial or other material incentives to participate in this study.

All patients enrolled in this study must have first met all the eligibility criteria for transplantation in accordance with the treatment plan being used. A conference will be held with the patient and family to discuss this research study. All potential risks associated with participation in this study will be discussed as objectively as possible. From their prior discussions with the transplant team, patients will already have been made aware of the risk of GVHD resulting from allogeneic HCT, as per SOP of the blood and marrow transplantation program of Hackensack University Medical Center.

The issue of how patients on this study may be directly benefited by this treatment protocol, as it is designed to reduce the incidence and severity of GVHD, will be discussed. It will be explained that participation in this study is voluntary. Patients can choose not to participate in this study and still undergo their scheduled allogeneic transplant. All patients who meet the eligibility requirements will be offered the option to participate in this transplant protocol.

4.2.2. Contacting primary physicians. Patients will be asked to provide a list of physicians who prescribe medicine for them or manage their health care. These physicians will be contacted by mail and telephone with information regarding the patient's participation in the telmisartan study and a request that no additional medicines be prescribed by these physicians during the time the patient is receiving telmisartan for the study.

Prior to the study drug being discontinued on day 98, or if the drug is discontinued before day 98, these same primary physicians will be contacted to discuss any anti-hypertensive medications the patient may need as part of their ongoing care.

5.0 STUDY DESIGN

5.1 Overall Study Design

This is multi-center, open-label, prospective study of telmisartan in addition to standard GvHD prophylaxis and treatment for the prevention of acute GVHD in approximately 60 subjects undergoing allogeneic HCT for treatment of a hematologic malignancy. The first 5 patients enrolled will receive telmisartan in an escalating dose according to the following schedule, with day 0 as the day of HCT: day -5 (40 mg), day -4 (80 mg), day -3 (80 mg), day -2 (160 mg), day -1 (160 mg). For these first five enrollees, telmisartan dose will be adjusted for grade 3 or 4 hypotension, as defined by CTCAEv4, as follows: the next day's dose will be one half the previously given dose (e.g. 20 vs. 40, or 40 vs. 80, or 80 vs. 160). If grade 3 or greater hypotension occurs at 20 mg, drug will be discontinued and the patient will be removed from the study. If the patient tolerates 40, or 80 mg, without hypotension, the dose will be increased to 80 or 160 mg, respectively, for the following day. If that (higher) next day's dose is tolerated, 160 mg will be given for the remainder of the trial as outlined below. From day 0 onward, any dose modifications will follow the dose adjustment algorithm outlined in section 5.3.4 below.

If two or more of the first five patients enrolled do not tolerate dose escalation to a stable daily dose of 160 mg by day 0, the trial will be paused, and the remaining volunteers will be enrolled following the same 5-day dose escalation procedure to establish a day 0-100 dose for each subject. All other study safety and endpoints, and dose adjustments will remain as described herein.

If four or more of the first five subjects tolerate 160 mg daily dosing by day 0, the remaining subjects enrolled will begin telmisartan on day -2 at 160 mg/day, as described below.

Subjects will receive 160 mg Micardis brand telmisartan (Boehringer-Ingelheim) in the form of two 80 mg pills, taken together, once daily, starting 2 days prior to HCT (day -2). All subjects will receive telmisartan in addition to the standard GvHD prophylaxis and treatment regimens prescribed by their transplant physician (see section 5.3.5). Systolic and Diastolic Blood pressure will be monitored at least every 4 hours while in the hospital. Patients who develop severe hypotension ($\geq$ grade 3) will have their doses adjusted accordingly (see section 5.3.4 for dose modification schedule). Once the patient is discharged post-HCT, treatment will continue in the outpatient setting. Treatment will continue through Day +98 post-HCT for a total of 104 days (day -5 to day +98) in the first five enrollees, and 101 days (Day -2 to Day +98) in the subsequent subjects, assuming 160 mg is well tolerated in 4 or more of the first 5 subjects. After treatment

discontinuation on or before day +98 post-HCT, subjects will be followed for up to 6 months (Day +180) for primary and secondary endpoints.

5.2 Study Endpoints

5.2.1 Efficacy Endpoints

- Grade II GVHD as per 1994 Consensus Conference on Acute GVHD Grading criteria [104]
- Grade III-IV GVHD as per 1994 Consensus Conference on Acute GVHD Grading

5.2.2 Safety Endpoints

- Grade III-IV hypotension as per the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0 (CTCAE)
- Relapse/progression of disease
- Primary graft delay, defined as ANC < 500 x 3 consecutive draws at 28 days, and < 5% donor CD3 T cell chimerism at day 28 and/or day 56.
- Primary graft failure, defined as ANC < 500 at 56 days x 3 consecutive draws and < 5% donor CD3 T cell chimerism as determined by standard of care.
- Secondary graft failure (loss of engraftment), defined as loss of ANC > 500 after 3 days of ANC > 500, or loss of chimerism subsequent to detection of chimerism
- Non-relapse mortality: Occurrence of unexpected, study drug-related

5.2.3 Exploratory Endpoints

- ST2 EIA (Presage, Critical Diagnostics)
- Reg3a EIA
- Ceruloplasmin EIA
- elafin EIA
- endotoxin activity assay (EAA, Spectral Diagnostics)
- Gut and urine microbiome sequencing of 16S rRNA DNA, to generate Shannon Diversity index and ration of Clostridia : Lactobacilli, and *firmicutes* vs. *bacteroidetes* vs. *Enterococci* species
- PBMC ROCK activity as measured by ratio of Phosphorylated : Total MBS in cryopreserved PBMCs, ROCK enzyme protein levels by WB
- Percentages of CD4+ and CD8+ cells expressing FoxP3 and CD73 or CD39, percentages of CD4+ CD146+CCR5+ cells, and percentages of a4b7 cells expressing CD39 or CD73.

5.3 Treatment Schedule

5.3.1 Standard GvHD Prophylaxis

All subjects will receive a standard GvHD prophylaxis regimen selected at the discretion of their transplant physician. The standard GvHD prophylaxis utilized at the study site includes:

- Tacrolimus 0.03 mg/kg/day by continuous infusion starting on day -2 pre-transplantation, adjusted as needed to achieve a serum level of 10-15 mcg/mL, and
- Methotrexate 5 mg/m2 given IV on days +1, 3, 6, and 11 after transplantation.

While a tacrolimus/methotrexate-based regimen is used after a variety of transplant conditioning regimens and for a variety of diseases, alternate standard regimens may be used. The standard GvHD prophylaxis regimen is not dictated by this protocol. GvHD prophylaxis regimens utilizing an investigational agent (other than telmisartan) are prohibited.

5.3.2 Telmisartan Dosing and Discontinuation of Other Anti-Hypertensive Drugs.

The first five subjects enrolled will discontinue any anti-hypertensive medications they are taking at least 24 hours prior to beginning the telmisartan dose escalation on day -5. For subsequent enrollees, subjects will be asked to discontinue any antihypertensive medications they may be taking at least 48 hours prior to first dose of study medication.

For the purposes of this study, a commercial supply of Micardis brand (Boehringer Ingelheim) of telmisartan tablets will be used due to the superior release kinetics of Micardis compared to several generic brands [105], and to ensure uniformity of product during the trial. All subjects (other than the first 5 enrollees, discussed above) will receive 160 mg telmisartan p.o. once daily starting two days prior to HCT (Day -2) and continuing for 101 days (until Day +98 post-HCT), unless discontinued early from treatment. Subjects will be instructed to take their study medication at approximately the same time every day, before bedtime, or upon waking, but there are no strict requirements for the timing of administration. Downward dosing adjustments will be made according to the steps outlined in section 5.3.4.

5.3.3 Treatment Compliance

Most patients will be discharged within 2- 3 weeks following HCT. Upon discharge patients will be given drug diaries to complete as they take their daily study medication at home. Subjects will be instructed to bring their study medication and drug diaries to all follow-up appointments at the John Theurer Cancer Center at HackensackUMC. Compliance will be monitored by the study team during the treatment period. Missed days of medication will be noted. Five missed doses over any 2 week period for reasons other than hypotension or other side effects, will be considered non-compliance. Patients who are non-compliant ***prior to*** 14days post-HCT will be withdrawn from the study. Subjects who are non-compliant ***after*** 14 days post-HCT will be allowed to continue on study treatment with counseling on compliance.

5.3.4 Dose Modifications

Blood pressure will be monitored every 4 hours, as per standard operating practice for HCT patients, during the initial hospital stay, including pre- and post-transplant periods. If hypotension does occur during the initial treatment phase in the hospital, the patient will be placed in the supine or reverse Trandelenburg position and, if necessary, given an intravenous

infusion of normal saline. A transient hypotensive response is not a contraindication to further treatment, which usually can be continued without difficulty once the blood pressure has stabilized.

Prior to discharge, patients are taught how to use an automated blood pressure cuff, and are given such a cuff upon discharge from the hospital with instructions to take their blood pressure at home twice a day. Patients are instructed to keep a diary of their systolic and diastolic blood pressure readings and to bring the diary with them to their clinic visits. They are also instructed to contact their transplant clinical caretaker for any symptoms of low blood pressure, as per SOP of the Blood and Marrow Transplantation Program of Hackensack University Medical Center.

Subjects who develop grade 3 or 4 hypotension as defined by CTCAEv4, while receiving telmisartan as their only anti-hypertensive medication will skip one daily dose of telmisartan and resume dosing the following day at 80 mg/day. Subjects who experience grade 3 or 4 hypotension on 80 mg/day will again skip one daily dose of telmisartan and resume dosing the following day at 40 mg/day. Subjects who experience grade 3 or 4 hypotension on 40 mg/day, and cannot tolerate re-initiation of 40 mg/day after a one day drug hiatus, will be discontinued from study treatment.

Subjects who develop grade 3 or 4 hypotension while on telmisartan plus one or more additional anti-hypertensive drugs will follow the same algorithm for telmisartan dose reduction described above, and the non-telmisartan anti-hypertensive drug(s) will not be re=started with the next doses of telmisartan unless additional anti-hypertensive treatment is required even after the 160 mg daily dose of telmisartan has been re-established.

In addition to the above adjustments for hypotension, telmisartan will be dose adjusted by the same guidelines for grade 3 or greater drug related adverse events and will be stopped for angioedema.

Whenever a dose reduction has occurred, patients who tolerate the reduced dose for one week will be increased to the next highest dose. If that dose is also tolerated for one week, the dose will again be increased until the original 160 mg/ day dose is achieved. If a re-escalation results in reappearance of hypotension or other drug-related side effects, the medication will be stopped for one day, and the subject will receive the previous tolerated dose (as detailed above) for the remainder of the trial dosing period.

5.3.5 Occurrence of GvHD

Subjects who experience GvHD during the study treatment period will continue to receive telmisartan through Day +98 post HCT or until treatment discontinuation (see section 5.5). Standard GvHD treatment should be given at the discretion of the subject's transplant physician. While GvHD, especially Grade III-IV GvHD, should be treated accordingly, the specific treatment regimen is not dictated by this protocol. Patients with Grade I GVHD of the skin without any other organ involvement may be treated with a topical corticosteroid cream. Patients with $\geq$ Grade II acute GVHD will be treated with high-dose, systemic corticosteroids. The following

general guidelines promote consistency in our clinical practice but may be modified for individual patients as clinical circumstances warrant:

a. Grade 0-I GVHD
   (i) Topical corticosteroids (usually 0.1% triamcinolone; 1% hydrocortisone to face) applied to rash BID.
b. Grade II-IV GVHD
   (i) Methylprednisolone or prednisone, 1 mg/kg, BID for 10-14 consecutive days.
   (ii) If response, taper by 20% every 5 days until off.
   (iii) If GVHD worsens during taper, steroids should be increased to previous dose. During steroid taper, maintain therapeutic tacrolimus levels.
   (iv) Patients who fail to respond to corticosteroids will be considered for standard second-line immunosuppressive therapy, e.g., mycophenolate mofetil, antithymocyte globulin photopheresis.

5.4 Restricted Medications and Measures

5.4.1 Antihypertensives

For the first five subjects enrolled, antihypertensives other than telmisartan should be discontinued at least 24 hours prior to the initiation of telmisartan. For subsequent patients initiating telmisartan at the 160 mg dose, anti-hypertensives other than study drug telmisartan should be discontinued 48 hours prior to initiation of study drug. However, subjects (including initial enrollees receiving dose escalation of telmisartan) who are already being treated for hypertension with three or more anti-hypertensive agents, will have a cardiology consult to determine if one or more of these agents should be continued during the period of telmisartan study drug treatment. When medically indicated, patients will be permitted to continue their non-telmisartan medications (other than potassium sparing agents such as ACE inhibitors, ARBs, spironolactone, or direct renin inhibitors) without the 24 or 48 hr. washout period prior to starting telmisartan.

Furthermore, patients who remain hypertensive after 4 doses of 160 mg telmisartan (4 days) as monotherapy will have appropriate additional antihypertensive medications (such as hydrochlorothiazide or amlodipine) introduced for the period of the study. These non-telmisartan drugs will be adjusted, as needed, to maintain appropriate BP. If telmisartan and hydrochlorothiazide or amlodipine are insufficient to control hypertension during the study, additional compatible antihypertensive medications may be added. Aliskiren is associated with increased risks of hypotension, hyperkalemia, and changes in renal function (including acute renal failure) compared to monotherapy. Patients cannot be on Aliskiren while on the study.

5.4.2 Agents That Increase the Risk of Renal Failure

Hyperkalemia may occur in patients on telmisartan, particularly in patients with advanced renal impairment, heart failure, on renal replacement therapy, or on potassium supplements, potassium-sparing diuretics, potassium-containing salt substitutes or other drugs that increase potassium levels. Potassium-sparing diuretics such as spironolactone, and angiotensin-

converting-enzyme (ACE) inhibitors will be discontinued prior to start of study and will not be used in conjunction with telmisartan during the study unless deemed necessary by the HCT clinical physicians, due to potential interactions leading to renal toxicity. Potassium supplements will also be discontinued, and re-started gradually if need is indicated by electrolyte monitoring. Foods high in potassium will be limited to safe levels during the period of telmisartan administration except as specifically allowed by the physician monitoring the patient's electrolyte status.

In patients who are elderly, volume-depleted (including those on diuretic therapy), or with compromised renal function, co-administration of NSAIDs, including selective COX-2 inhibitors, with telmisartan, may result in deterioration of renal function, including possible acute renal failure. These effects are usually reversible. Caution will be taken when administering NSAIDs to study subjects, and renal functions will be monitored.

5.4.3 Impaired Hepatic Function

As the majority of telmisartan is eliminated by biliary excretion, patients with biliary obstructive disorders or hepatic insufficiency can be expected to have reduced clearance. Hepatic function will be closely monitored as part of the standard of care for HCT patients, and increased LFTs or bilirubin will be carefully followed. Careful consideration should be taken before administering any medications that have known hepatic toxicity, especially in patients who experience hepatic insufficiency pre- or post-HCT (e.g., as a result of grade III or IV GVHD).

5.4.4 Continuation of Telmisartan and other antihypertensives following cessation of study dosing

Subjects who are hypertensive and wish to continue on some dose of telmisartan as their primary antihypertensive therapy beyond the study treatment period (Day +98 post-HCT) will be permitted to do so after consultation with their treating physician. The subject will be switched from study supply of telmisartan (Micardis) to the appropriate commercial telmisartan agent. If they require anti-hypertensives other than, or in addition to, telmisartan after study dosing is discontinued, appropriate medication will be prescribed. Telmisartan and other anti-hypertensive drugs are not paid for by the study after the period of telmisartan administration required by the study protocol.

5.5 Subject Discontinuation

5.5.1 Discontinuation from Study Treatment

Any subject who prematurely discontinues from study treatment on or after day 14 should still be followed through Day +180 Post HCT for primary, secondary, and exploratory outcomes. Adverse events (AEs) leading to treatment discontinuation of a subject will be followed until resolution, return to baseline, or until the event is considered chronic.

A subject should be withdrawn from the study treatment if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the subject. However every effort should be made to keep the subject on study treatment, especially in cases where the dose or schedule of study treatment can be modified (see section 5.3.3 Dose Modifications). Subjects who discontinue from study treatment on or after day 14 should still complete end of treatment study procedures (clinical evaluations, blood, stool, and urine samples, if they are willing to do so). Subjects may be discontinued from study treatment for any of the following reasons:

- ≥ grade 3 hypotension at the 40 mg/day dose level after one attempt at restarting this dose following a 1 day drug hiatus.
- Onset or persistence of any treatment related adverse events (AEs) that, in the opinion of the study physician, warrant discontinuation.
- Non-compliance, defined as five missed doses over any 2 weeks period, for reasons other than hypotension or other side effects, within 14 days post-HCT.
- Significant protocol deviation that would render the subject unevaluable
- Voluntary Withdrawal of consent
- Pregnancy or plan to become pregnant

If a subject wishes to stop study drug or withdraw from the study, their routine standard of care (SOC) medical data to obtain information on the critical safety and efficacy endpoints of the study, including occurrence of aGVHD, engraftment, adverse effects, relapse, and NRM, as well as other recoverable data indicated on Table 3 that is obtained as part of routine SOC will be reviewed on a regular basis up to 180 days post transplant. This will allow us to obtain useful data without requiring any additional telmisartan treatment or sampling.

In addition to safety, a reason for doing this is that one possible beneficial effect of telmisartan is the establishment of protective immune regulatory networks within the first 1 – 2 weeks of treatment. In that scenario, continuous treatment for the full intended period might not be necessary to see major benefit. This is useful information to have.

5.5.2 Replacement of Subjects

The study will enroll a total of 60 evaluable subject defined as subjects who remain on telmisartan treatment through Day + 14 post-HCT. Patients who discontinue telmisartan ***before*** day +14 post-HCT will be replaced by an additional subjects until a total of 60 evaluable subjects is reached. However, for safety reasons, we will continue to follow their medical progress for up to 180 days post-transplant. Those who discontinue telmisartan ***after*** day + 14 post-HCT will not be replaced and will be continued to be followed through the 180 day study period. Subjects who discontinue telmisartan at any time will continue all other study assessments and time points unless they choose to discontinue the study.

6.0 STUDY EVALUATIONS AND ASSESSMENTS

The study design is outlined below in **Table 3** Schedule of Events. HCT patients are typically discharged prior to the first weekly visit after Day +7 (i.e., sometime before day 14). If a subject remains hospitalized post-HCT for any visit after Day +7, or is readmitted after discharge, all attempts should be made to complete study required assessments at the appropriate time points. Discharge procedure to be performed when appropriate. Day +98 only indicates end of treatment and does not constitute a study visit. No additional assessments are required on Day +98 unless the Day +100 visit occurs 2 days early, as permitted.

For the initial five patients enrolled on the dose-escalation schedule described in section 5.1, there are additional events on days -5 through -2, as indicated in Table 3A. These additions appear on the "administration of study drug," (fifth line from the bottom).

Table 3: Schedule of Events: Please note, all days are **in relation to day of HCT** and NOT in relation to initiation of study drug

	Screening	Treatment Period												Follow-Up Period	
		Inpatient					Inpatient or Outpatient[1]								
Study day (relative to HCT)	Day -30 to Day -3	Day -2	Day -1	Day 0 (HCT)	Day +7	At Discharge	Weekly[2]	Day +28[2]	Weekly[2]	Day +56[2]	Day +70[2]	Day +84[2]	Day +98	Day +100[2]	Day +180[3]
Informed Consent	X														
Confirm Eligibility	X														
Demographics	X														
Complete Medical History	X														
Physical Exam	X	DAILY DURING HOSTPIALIZATION					X	X	X	X	X	X		X	X
Height and weight	X														
Vital Signs (BP,respirations, heart rate, temp)	X														
Blood pressure monitoring		Q 4 hrs as per SOP during hospitalization					Morning and evening (daily) while on telmisartanX	X	X	X	X	X	X	X	X
Hematology[4]	X	DAILY DURING HOSTPIALIZATION					X	X	X	X	X	X		X	X
Serum Chemistries[5]	X				X		X	X	X	X	X	X		X	X
CD3 Chimerism (blood)								X		X		X			
CD34 Chimerism (bone marrow)[8]												X[8]	X[8]	X[8]	X[8]
GVHD Assessment							X	X	X	X	X	X		X	X
Endotoxin activity assay		X[6]	X[6]				X	X	X	X		X			
Blood samples for exploratory studies		X[6]	X[6]		X		X	X	X	X	X	X		X	X
Stool sample for exploratory studies[7]		X[6]	X[6]		X		X	X	X	X	X	X		X	X
Urine sample for exploratory studies[7]		X[6]	X[6]		X		X	X	X	X	X	X		X	X
HCT				X											
Dispense study drug						X		X		X		X			
Administer study drug		Daily from Day -2 to Day +98													
Provide subject diary						X									
Review treatment compliance							X	X	X	X	X	X	X		
Review Concomitant Medications	X	Continuous													
Record Adverse Events		Continuous													

1 All Post-HCT outpatient visits are approximate and should coincide with the subject's standard post-transplant follow-up visits. If subject is still or re-hospitalized, all attempts should be made to complete study required assessments at the appropriate time points
2 ± 3 days
3 ± 7 days
4 CBC w/diff and platelets
5 Chemistry panel with Magnesium
6 Prior to telmisartan administration or any day prior to day 0, including in first 5 patients receiving dose escalation
7 If subject is able to provide sample
8 Bone marrow CD34 chimerism will be assessed any time after Day +75 if a standard of care specimen is collected. No bone marrow aspirate will be performed solely for the purpose of this Telmisartan study.

Table 3A: Schedule of Events For Initial Five Subjects: Please note, all days are **in relation to day of HCT** and NOT in relation to initiation of study drug

	Screening				Treatment Period													Follow-Up Period	
					Inpatient					Inpatient or Outpatient[1]									
Study day (relative to HCT)	Day -30 to Day -5	D -5	Day -4	Day -3	Day -2	Day -1	Day 0 (HCT)	Day +7	At Dis-charge	Weekly[2]	Day +28[2]	Weekly[2]	Day +56[2]	Day +70[2]	Day +84[2]	Day +98	Day +100[2]	Day +180[3]
Informed Consent	X																	
Confirm Eligibility	X																	
Demographics	X																	
Complete Medical History	X																	
Physical Exam	X				DAILY DURING HOSTPIALIZATION					X	X	X	X	X	X		X	X
Height and weight	X																	
Vital Signs (BP,respirations, heart rate, temp)	X																	
Blood pressure monitoring					Q 4 hrs as per SOP during hospitalization					Morning and evening (daily) while on telmisartan X	X	X	X	X	X	X	X	X
Hematology[4]	X				DAILY DURING HOSTPIALIZATION					X	X	X	X	X	X		X	X
Serum Chemistries[5]	X							X		X	X	X	X	X	X		X	X
CD3 Chimerism (serum)											X		X		X			
CD34 Chimerism (bone marrow)[8]															X[8]	X[8]	X[8]	X[8]
GVHD Assessment										X	X	X	X	X	X		X	X
Endotoxin activity assay					X[6]	X[6]		X		X	X	X	X		X			
Blood samples for exploratory studies					X[6]	X[6]		X		X	X	X	X	X	X		X	X
Stool sample for exploratory studies[7]					X[6]	X[6]		X		X	X	X	X	X	X		X	X
Urine sample for exploratory studies[7]					X[6]	X[6]		X		X	X	X	X	X	X		X	X

HCT							X											
Dispense study drug									X		X		X		X			
Administer study drug		40 mg	80 mg	80 mg	160 mg	160 mg Daily from Day -1 to Day +98												
Provide subject diary									X									
Review treatment compliance										X	X	X	X	X	X	X		
Review Concomitant Medications	X	Continuous																
Record Adverse Events		Continuous																

6.1 Screening

Prior to commencement of the screening assessments, the patient must have given full informed consent and have signed the study informed consent forms. Once the consent has been signed and dated by the patient, a qualified member of the study team investigator will confirm the subjects meets all eligibility criteria. Screening assessments, including complete medical history, physical exam, height and weight assessment, vital signs (including blood pressure, heart rate, respiratory rate, and temperature), hematology, serum chemistries, and serum pregnancy test, must occur within 28 days prior to initiation of study treatment. Most, screening assessments will be part of the patient's routine workup for HCT as per SOP of the bone and marrow transplantation program of Hackensack UMC. Therefore, any study required procedures already performed as part of the subject's routine medical care, even those done before informed consent was signed, may be used to fulfill screening requirements as long as they were performed within the 28 day window.

6.2 Telmisartan Dispensing and Administration

Dosing with telmisartan 160 mg will begin on Day -2 prior to HCT (see section 5.3 for complete dosing instructions). Prior to hospital discharge, a month supply of study drug will be dispensed and the subject will be instructed how to take study drug at home. The subject will also be given a drug diary to denote when study drug was taken at home. Additional supply of study drug will be dispensed at Day +28, Day +56, and Day +84 visits. The supply of study drug at discharge, Day +28, and Day +56 visits will include enough tablets to last until the subsequent dispensing visit (28 days plus 3 extra days to account for $\pm$ 3 day variation in timing of visits). If the subject has tablets of study drug remaining at a dispensing visit, they will be instructed to finish their prior bottle of study drug before beginning the next bottle. At the Day +84 visit, just enough study drug will be dispensed to last until Day +98, taking into account any remaining tablets from the Day +56 visit.

The subject will be instructed to bring current supply of study drug and drug diary to each visit.

6.3 Safety Evaluations

Physical exam and CBC with differential and platelets will be performed daily while in the hospital and at all subsequent visits. Chemistry panel plus magnesium will be performed at Day +7 and all subsequent study visits. Blood pressure will assessed every 4 hours, per SOP while the subject is hospitalized, morning and evening by the patients at home using an automated blood pressure monitor, and at all subsequent clinic visits. Additional assessments may be performed both pre- and post-discharge as per standard medical care for patients undergoing HCT. The results of these assessments will be recorded in patient charts only if they constitute an adverse event. Serum CD3 chimerism will be assessed at Day +28, Day +56, and Day +84. Bone marrow CD34 chimerism will be assessed any time after Day +75 if a standard of care specimen is collected. No bone marrow aspirate will be performed solely for the purpose of this Telmisartan study. GVHD will be assessed both as a safety (grades III and IV) and as an efficacy (grades II-IV) evaluation.

6.4 Efficacy Assessments

GVHD status will be assessed at all study time points after Day +7 according to 1994 Consensus Conference on Acute GVHD Grading (see section 7.1.1). Additional GVHD assessments may be performed at clinic visits not required by the study as per standard medical care for patients undergoing HCT. During these visits, positive GVHD findings will be recorded and graded as per study criteria.

6.5 Exploratory Studies

All exploratory studies will be performed in the research laboratories of David Schwartz, MD, PhD and Sujatha Iyengar, PhD in the David and Alice Jurist Research Institute at Hackensack University Medical Center. In some cases, coded stool and urine samples, without traceable identifiers, may be sent to core facilities or commercial laboratories for sequencing and generation of phylogeny trees of bacterial !6sRNA DNA. Code assignments will be maintained in locked files as hard copy and on secure computer files of the P.I. with access limited to the minimum number of investigators required to perform the research and analyses. The results of these analyses will be correlated retrospectively with patients' outcomes. These exploratory studies will not contribute to the management of patients in this study. For any questions regarding sample collection, shipment, or storage for exploratory studies, please contact the research lab at (551) 996-2368.

6.5.1 Blood

Approximately **2 ml** of whole blood will be collected in **EDTA anticoagulated** tubes for Endotoxin Activity Assay (EAA) on two separate days between Day -5 and Day 0. Additional samples will be drawn onDay +7, prior to discharge from the hospital, and samples will be collected at all subsequent study visits prior to Day +56 (excluding day of discharge). The 2 ml blood sample for EAA **must be delivered to the research laboratory within 1 hour of being drawn**.

Approximately **5 ml** of whole blood will be collected in **serum collection (red top)** tubes for ST2, Reg3a, Elafin, and Ceruloplasmin EIA samples. Another ~ **30 ml of EDTA anticoagulated** blood will be obtained for ROCK substrate phosphorylation, lymphocyte subset proportions, and T regulatory and T effector subsets analysis at all study time points (excluding Day 0 and day of discharge). Samples will be sent to the research labs for processing and batch storage within 2 hours, and preferably at the time of the EAA transport (i.e., within one hour of draw).

6.5.2 Stool and Urine Samples

Stool and clean-catch urine samples for microbiome analysis will be collected twice between Day -5, and Day 0, again on Day +7, and all subsequent clinic visits until Day +100, and once more on day 180. Kits for stool and urine collection will be provided to subjects prior to each visit. While all attempts should be made to collect these samples at all required time points, a subject can

forego collection at an individual collection if they cannot provide a stool or urine sample due to decreased performance status, malnutrition/dehydration, or other medical reasons.

7.0 METHODS FOR ASSESSING ENDPOINTS

7.1 Efficacy Endpoints

7.1.1 Graft vs. Host Disease

Acute GVHD will be scored using the clinical criteria codified at the 1994 Consensus Conference on Acute GVHD Grading [104], summarized in **Table 4**. Note that Grade I involves only skin, Grade II can involve liver and bowel, and need not involve skin, Grade III involves skin and liver, and/or gut, and Grade IV involves skin, liver, and gut.

Table 4: GVHD grading and staging extent of organ involvement

Stage	Skin	Liver	Gut
1	Rash on <25% of skin[a]	Bilirubin 2-3 mg/dl[b]	Diarrhea > 500 ml/day[c] or persistent nausea[d]
2	Rash on 25-50% of skin	Bilirubin 3-6 mg/dl	Diarrhea >1000 ml/day
3	Rash on >50% of skin	Bilirubin 6-15 mg/dl	Diarrhea >1500 ml/day
4	Generalized erythroderma with bullous formation	Bilirubin >15 mg/dl	Severe abdominal pain with or without ileus
Grade [e]			
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	—	Stage 2-3 or	Stage 2-4
IV[f]	Stage 4	Stage 4	—

a. Use "Rule of Nines" or burn chart to determine extent of rash.
b. Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
c. Volume of diarrhea applies to adults. Downgrade one stage if an additional cause of diarrhea has been documented.
d. Persistent nausea with histological evidence of GVHD in the stomach or duodenum.
e. Criteria for grading given as minimum degree of organ involvement required to confer that grade.
f. Grade IV may also include lesser organ involvement with an extreme decrease in performance

7.2 Safety Endpoints

7.2.1 Exams and laboratory assessments

Physical exam, vital signs, CBC, and Chemistry panels reflect the standard of care for patients undergoing HCT. Exams and tests will be performed according to John Theurer Cancer Center SOPs. Any deviations from baseline measurements will be noted and assessed for severity and causality. Hematologic abnormalities determined to be related to the subject's disease or HCT will not be recorded as adverse events.

7.2.2 Non-relapse mortality (NRM)

Death due to any cause, in the absence of evidence of recurrent cancer, will be scored as NRM. This would include grade V GVHD, which would be noted.

7.2.3 Engraftment

Appearance and rise in peripheral blood neutrophils will be monitored on a daily basis for the first 2 weeks, and then twice weekly. Absolute neutrophil counts that never reach 500 in the first 28 days and/or platelet counts that drop below 20,000 and remain below that number during the 28 day period will be considered "late engraftment" until repeat evaluation at days 56 and 84 and scored as a primary failure to engraft if no engraftment occurs by those time points. Chimerism analysis of peripheral blood CD3+ cells is routinely assessed at 28, 56, and 84 days after transplantation, and will also be used in the definition of engraftment (5% donor CD3 cells), as described above (see 5.2.2). These data will be collected for retrospective analysis.

7.2.4 Cancer Relapse / Progression

Depending on the cancer for which HCT is performed, appropriate monitoring for reappearance or progression of tumor will be employed by the transplant teams caring for the patients enrolled into this study. Cancer relapse in the first months post-transplantation has been rare both in our patients and in the reported literature. Most relapse occurs after 6 months post-transplant, which is beyond the formal period of data collection for this trial, although we attempt to follow all transplant patients at our clinic indefinitely as routine standard of care.

7.3 Exploratory Endpoints

Endotoxin activity assay, ST2, reg3a, elafin, ceruloplasmin are all potential biomarkers for imminent or early GVHD. They will be analyzed retrospectively for correlations with GVHD. Thus, they will not contribute to the management of patients in this study.

Endotoxin Activity (EA) will be monitored by the whole blood EA assay (EAA™) from Spectral Diagnostics (Toronto, CA), which has been CE (European Community) and FDA approved for the detection of endotoxin in blood of patients admitted to rule out sepsis (validated and approved for day of admission only). *Note:* unlike the other serum proteins being assayed, the EAA will be performed in fresh whole blood in our laboratory, within 2 hours of being drawn.

ST2 will be measured by batch assay of stored serum, using the Presage™ EIA kit from Critical Diagnostics (San Diego, CA), that has received CE (European Community) and FDA approval for monitoring cardiac disease [87]. It has very recently been shown to correlate strongly with the imminent onset of GVHD as well [86], although it is not FDA approved as yet for this indication.

Reg3a will be measured by batch assay of stored serum by EIA (AbNova) or Luminex multiplex, depending on relative costs at time of batch assay. Elafin (also called Trappin 2 and Skalp) will be assayed by EIA (R&D) or Luminex multiplex, depending on relative costs at time of batch assay. Ceruloplasmin will be assayed by EIA (Abcam). ROCK substrate phosphorylation (myosin phosphatase myosin binding subunit Threonine 853): We will compare the ration of P-MBS to total MBS in lymphocytes of patients.

Lymphocyte subset proportions: The subsets outlined previously will be measured by flow cytometry after staining with appropriate monoclonal Abs. T regulatory and T effector subsets will be enumerated by standard 4- color flow cytometry.

Stool and Urine microbiome samples will be processed for amplification of 16S rRNA coding DNA and sequenced by NexGen technology with subsequent analysis of diversity and phylogeny proportions based on this sequencing. We will test the hypotheses that diversity decreases in association with GVHD, and that there is a decrease in Firmicutes and an increase in Lactobacilli species.

8.0 STATISTICAL CONSIDERATIONS

8.1 Data Management

Data pertaining to the subject's medical history, HCT course and outcome, and clinical follow-up will be abstracted from multiple sources including medical records (paper and electronic records in EPIC), the Tumor Registry at HackensackUMC, and the HCT database maintained by the Blood and Marrow Transplantation Program at the John Theurer Cancer Center. Any study required data not available from an existing source will be recorded on study specific source document worksheets. All data will be entered into a study specific, password-protected database accessible only by qualified members of the research team. Periodic audits of study data will be performed in conjunction with Corporate Compliance at HackensackUMC. Coded data from exploratory studies will be recorded in laboratory notebooks prior to inclusion in the study database.

All subjects will be assigned a study specific identification number (ID#). All study data will be recorded using the subject's ID#. A key linking ID#'s with subject identities, accessible only by qualified members of the research team, will be kept separate from study data.

8.2 Sample Size

Enrollment of 60 subjects for this pilot study is based on the available patient population undergoing allogeneic HCT at the John Theurer Cancer Center (JTCC), expected rates of enrollment, and the historical data set from the past 3 years' allogeneic HCT experience at the JTCC, as well as the published literature from many centers performing allogeneic HCT for the same underlying diseases, and using similar preparative regimens. Statistical power of 60 subjects was considered adequate to detect fairly dramatic reductions of GVHD, as described in the Statistical Plan, below. Reductions of ~ 25% in grade II GVHD, and of ~ 62% in grade III-IV GVHD should be detected with 0.8 power, as discussed below. The similarly designed Maraviroc study [17] enrolled 38 patients, and reached statistical significance due to almost complete elimination of Grade III-IV GvHD. As we do not anticipate equally strong suppression of GVHD with telmisartan, we estimated that the somewhat larger number of 60 subjects would be appropriate to detect 60 -70% reduction. However, even a 20- 30% decrease in severe GVHD would have a real impact in the clinic, and this pilot study is not powered to detect this. Since grade II GVHD is more common, we are powered to detect a roughly 25% reduction in its

occurance. This would be clinically beneficial, and would support further study of telmisartan for this indication.

8.3 Statistical Analysis Plan

8.3.1 Efficacy Endpoints for Grade III-IV and Grade II GVHD.

The statistical test used for endpoints (Z test of proportions) takes the currently available 3 year JTCC data as the historical control data set for comparison. In reviewing these data, as currently available, it appears that the JTCC rates of severe GVHD, NRM, and cancer relapse are somewhat lower (i.e. better patient outcomes) than the national averages from published studies. For our final determinations of significance, we will use updated JTCC 3 year data as they become available at time of study completion.

Outcome variables examined include engraftment, GVHD, relapse, and NRM, however the efficacy hypotheses are constructed in relation to GVHD only.

Primary endpoint analysis

Historic GVHD data are available on 178 allogeneic transplants, of whom 46 (26)% experienced grade III or IV acute GVHD. All the other allogeneic transplantees (74%) had grade II GVHD. Based on these data, if 8 or fewer of the total 60 study participants experience grade III - IV acute GVHD during the course of the study, we will have demonstrated a protective effect for this criterion, with a $p < 0.05$, based on a one-way Z test for a decreased proportion.

Sample Size and Power Calculation Considerations

If we use the proportion P=0.26 as the rate of Grade III - IV GVHD in the current standard treatment, then using a one-sided exact test of the proportion and significance level of 0.05, testing if 9/60 (15%) rate of Grade III-IV GVHD is achievable with telmisartan treatment will have 58.8% power to detect this effect, if it exists. Testing for a 12% rate would achieve 82.2% power. Using the recommended level of significance, 0.025, for a one-sided test, testing if the grade III-IV GVHD by day 100 is 0.15 , would have power of 44.4% of detecting this effect. At the 2.5 % level, testing if the rate is less than 0.11, will have 79.0 % power of detecting. Testing if the rate of grade III-IV in telmisartan treated transplantees is 0.10, will have 85.8% power of detecting this effect using the 2.5 % level of significance.

Numeric Results for testing H0: P = P0 versus H1: P < P0 using the PASS13 sample size software
Test Statistic: Exact Test

Power	N	Proportion Given H0 (P0)	Proportion Given H1 (P1)	Target Alpha	Actual Alpha	Beta	Reject H0 If R ≤ This
0.8584	60	0.2600	0.1000	0.0250	0.0140	0.1416	8
0.7899	60	0.2600	0.1100	0.0250	0.0140	0.2101	8

Incidence of Grade II GVHD

The historical data reported a rate of incidence of 74%. At 5% level of significance, a one-sided exact test of whether telmisartan can attain lower levels of 62% (37/60), would achieve 63.2 % power of detecting this if it is indeed efficacious. A lower incidence rate of 60% will be detected with 74.5% power.

Using the recommended level of significance, 0.025, a one-sided exact test of whether Telmisartan can attain the lower incidence rate of 62% will achieve a power of 52.8% to detect this effect. A one-sided exact test of whether the grade II GVHD incidence rate of 0.50 (30/60) can be attained by Telmisartan will have 97.4% power to detect this effect. A one-sided exact test of whether the incidence rate of 60% is possible with telmisartan will achieve power of 65.1% to detect it. A one-sided exact test of whether the grade II GVHD incidence rate of 0.57 can be attained by Telmisartan will have 80.5% power to detect this effect.

Numeric Results for testing H0: P = P0 versus H1: P < P0 using the PASS13 sample size software
Test Statistic: Exact Test

Power	N	Proportion Given H0 (P0)	Proportion Given H1 (P1)	Target Alpha	Actual Alpha	Beta	Reject H0 If R ≤ This
0.9741	60	0.7400	0.5000	0.0250	0.0242	0.0259	37
0.8448	60	0.7400	0.5600	0.0250	0.0242	0.1552	37
0.8047	60	0.7400	0.5700	0.0250	0.0242	0.1953	37
0.7587	60	0.7400	0.5800	0.0250	0.0242	0.2413	37
0.6507	60	0.7400	0.6000	0.0250	0.0242	0.3493	37
0.5904	60	0.7400	0.6100	0.0250	0.0242	0.4096	37
0.5275	60	0.7400	0.6200	0.0250	0.0242	0.4725	37

Thus, with respect to grade 2 acute GVHD, if 37 or fewer patients of the 60 have grade 2 or lower acute GVHD, we will have demonstrated a drug effect at $p < 0.05$, using the one-way Z test of proportions.

To summarize, with only 26% of JTCC patients during the past 3 years exhibiting grade III or IV acute GVHD, we would have to see no more than 7 cases of grade III or IV acute GVHD (~ 12%) by the completion of the study to conclude that severe aGVHD had been reduced. However, with 74% of JTCC patients experiencing grade II aGVHD, we need only reduce that to 60%, or 36 of 60 patients having no more than grade II acute GVHD, in order to achieve significance.

8.3.2 Analysis of Exploratory Data

For exploratory endpoint biomarkers other than microbiota 16S rRNA sequences, multivariate modification of cumulative incidence statistics of Fine and Gray will be used to evaluate the univariate and multiple effects of primary markers or secondary markers on endpoints. The cumulative incidence of primary endpoints of Grade III-IV GVHD at any time among all patients in the study will be based on the initial systemic diagnosis and treatment. Cox regression

analyses will be used to identify exploratory study parameters as risk factors for Grade III-IV GVHD. Exploratory assays with a *P* value ≤ .05 for association with Grade III-IV GVHD in univariate testing will be entered in a multivariate Cox regression model.

Additionally, for each paratmeter, pairwise comparisons will be made between pre-transplant and each post-transplant time point. Statistical comparisons between GVHD(+) and GVHD(-) groups will be performed using a χ^2 or Fisher's exact test for categorical variables or the non-parametric Mann-Whitney *U* test for continuous variables. Mean values obtained from ELISA assays performed on pre- and post-transplant samples will be compared with the non-parametric Mann-Whitney *U* test for continuous variables. Next, the change in each blood factor at a given time relative to before the conditioning regimen was initiated will be compared using a paired t-test in GVHD (+) and GVHD (-) groups.

For 16S rRNA data, statistical analysis will follow the methods outlined above and detailed in Jenq et al. [99], briefly as follows:

Determining diversity, phylogenetic classification, dissimilarity, microbial chaos, and UniFrac PCoA. Operational Taxonomic Unit (OTU)-based microbial diversity will be estimated by calculating the Shannon diversity index (Magurran, A.E. 2004. Measuring Biological Diversity. Blackwell Pub., Malden, Ma.2004) using MOTHUR open software. Phylogenetic classification will be performed for each sequence, using the Bayesian classifier algorithm described (Wang, Q., G.M. Garrity, J.M. Tiedje, J.R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73:5261–5267. doi:10.1128/AEM.00062-07) with bootstrap cutoff at 60%. A phylogenetic tree will be inferred using clearcut on the 16S sequence alignment generated by MOTHUR. Microbial chaos is quantified by mean Bray-Curtis time index, calculated as follows: Bray-Curtis dissimilarity index (Magurran, 2004) between temporally adjacent samples will be quantified using MOTHUR and divided by the length of the time interval (in days) between samples, starting with the samples obtained before the transplant and all samples obtained until end of study. Unweighted UniFrac on the resulting tree (Lozupone, C., M. Hamady, R. Knight. 2006. UniFrac—an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics. 7:371. doi:10.1186/1471-2105-7-371) will be analyzed by Principal Coordinates Aanalysis performed on the resulting matrix of distances between each pair of samples.

Statistical comparisons. Shannon diversity index for intervals will be compared using unpaired two-sided Student's *t* tests with a more stringent cut-off of 0.0125 given multiple comparisons, by the Bonferroni correction for multiple time periods of independent comparisons. Comparisons of bacterial populations will use paired two-sided Wilcoxon matched pairs test for individual patients. Other comparisons will employ two-sided Mann-Whitney tests. However, given the final sample size (dependent on patient compliance with request for samples) rigorous statistical inference may be limited by confounding factors that can affect microbiome readouts in addition to GVHD. These include the exact combination and dosage of antibiotics, the underlying disease, patient nutrition, diarrhea, etc.. We will have to make this

assessment *post hoc*. It may be that, to avoid over-interpretation of data, we will limit the analysis to data description and visualization. This is acceptable for a pilot study.

8.4 Interim Safety Analysis

We have generated stopping rules based on the three year historical data from the JTCC for 5 criteria: NRM, Relapse, Graft Failure or Delay, Drug Intolerance, and Grade III-IV GVHD. These rules and the operating characteristics for the algorithms are presented below.

Stopping rules

In this section, the stopping rules for each of the adverse events non-relapse mortality, relapse, graft failure, intolerance to Telmisartan and Grade 3 or 4 GVHD by day 100 are described. Each of the 5 toxicities is monitored at 1% Type I error, i.e., the probability of stopping by chance the trial when in fact the toxicity rate observed in historical data is true. Thus, the repeated significance test maintains the level of the toxicity through all the monitoring points to be 1%.
Further, jointly all 5 toxicities have probability $(1-(1- 0.01)^{5)}=0.049$ probability of stopping the trial by chance when the null hypotheses are true.

Non-Relapse Mortality (NRM) at 100 days

If the non-relapse mortality in patients receiving Telmisartan the rate of NRM exceeds 22% then the study will be stopped. The operating characteristics of the stopping rule are described below. Based on historical data from the cancer center registry, it was assumed that 15% incidence of NRM was considered expected and incidence greater than 22% would be considered unacceptable.

Using repeated significance testing (Jennison and Turnbull) with 15.0% as lower proportion and 22.0% as higher proportion, 1% alpha level, and 80% power for early termination, shape parameter of the boundary, delta=0.2, with priority on alternative hypothesis and continuous monitoring, the following stopping guidelines were computed by the *toxbdry* function in the Clinfun package in R 3.2.0.

Table 5. Stopping Boundaries for NRM at 100 days using continuous monitoring

Monitoring Look	Number of Patients at Monitoring Look	Stop if Number of Toxicities is at least
1	2	2
2	7	3
3	12	4
4	18	5
5	24	6
6	29	7
7	35	8
8	41	9
9	47	10
10	54	11
11	60	12

The trial will be terminated if 2 patients out of the first 2 patients receiving experimental drug, Telmisartan, experience NRM by day 100. If out of the first 7 patients, 3 or more have NRM by day 100 then trial will be stopped. The boundaries were obtained such the Type I error, fixed at 1%, was spent over the multiple looks, hence the probability of stopping the trial prematurely by chance is 0.01.

Table 6. Operating characteristics for the stopping boundaries for NRM Day 100

Probability Of Toxicity	Probability of crossing low bndry	Probability of stopping low bndry	Expected sample size low bndry	Probability of crossing high bndry	Probability of stopping high bndry	Expected sample size high bdnry
0.15	0.343	0.338	47.4	0.340	0.335	47.5
0.164	0.443	0.436	44.0	0.440	0.433	44.0
0.178	0.544	0.536	40.4	0.541	0.533	40.4
0.192	0.640	0.631	36.7	0.638	0.629	36.7
0.206	0.727	0.718	33.1	0.725	0.715	33.1
0.220	**0.800**	**0.792**	**29.7**	**0.798**	**0.790**	**29.7**

Bndry, boundary.

From the operating characteristics, the probability of stopping the trial due to NRM if the level of toxicity is at high level of 22% is 0.79. The expected sample size at termination of the trial is 29.7, if Telmisartan in fact more toxic than standard treatment.

Relapse at Day 100

If the incidence of relapse by day 100 in patients receiving Telmisartan exceeds 19% then the study will be stopped. The operating characteristics of the stopping rule are described below. Based on historical data from the cancer center registry, it was assumed that 12% incidence of relapse was considered expected and incidence greater than 19% would be considered unacceptable.

Using repeated significance testing (Jennison and Turnbull) with 12.0% as lower proportion and 19.0% as higher proportion, 1% alpha level, with priority given to the alternative hypothesis and 80% power to for early termination, shape parameter of the boundary, delta=0.2, for continuous monitoring, the following stopping guidelines were computed by the *toxbdry* function in the Clinfun package in R 3.2.0.

Table 7. Stopping Boundaries for Relapse at Day 100

Monitoring Look	Number of Patients at Monitoring Look	Stop if Number of Toxicities is at least
1	3	2
2	8	3
3	15	4
4	22	5
5	29	6
6	37	7
7	44	8
8	51	9
9	59	10
10	60	11

For this continuous monitoring scheme, the trial will be terminated if 2 or more patients out of the first 3 patients receiving experimental drug, Telmisartan, experience relapse by day 100. If out of the first 8 patients, 3 or more have relapse by day 100 then trial will be stopped, and so forth. Out of all the 15 patients that receive Telmisartan, if 4 or more patients have relapse by day 100 then the drug will be considered more toxic than standard treatment. The boundaries were obtained such the Type I error, fixed at 1%, was spent over the multiple looks, hence the probability of stopping the trial prematurely by chance is 0.01.

Table 8. Operating characteristics for the stopping boundaries for Relapse by Day 100

Probability Of Toxicity	Probability of crossing low bndry	Probability of stopping low bndry	Expected sample size low bndry	Probability of crossing high bndry	Probability of stopping high bndry	Expected sample size high bdnry
0.120	0.312	0.312	48.4	0.307	0.307	48.8
0.134	0.418	0.418	44.8	0.414	0.414	45.2
0.148	0.527	0.527	40.8	0.523	0.523	41.3
0.162	0.631	0.631	36.9	0.628	0.628	37.3
0.176	0.724	0.724	33.0	0.721	0.721	33.4
0.19	**0.801**	**0.801**	**29.4**	**0.799**	**0.799**	**29.8**

Bndry, boundary.

From the operating characteristics, the probability of stopping the trial due to grade 3 or 4 GVHD if the level of toxicity is at high level of 36% is 0.79. The expected sample size at termination of the trial is 31.8, if Telmisartan in fact more toxic than standard treatment.

Graft Failure at Day 100 (includes delayed engraftment and failure)

If the incidence of graft failure by day 100 in patients receiving Telmisartan exceeds 7.0% then the study will be stopped. The operating characteristics of the stopping rule are described below. Based on historical data from the cancer center registry, it was assumed that 7.0% incidence of relapse was considered expected and incidence greater than 12.0% would be considered unacceptable.

Using repeated significance testing (Jennison and Turnbull) with 7.0% as lower proportion and 12.0% as higher proportion, 1% alpha level, and 80% power to for early termination, shape parameter of the boundary, delta=0.2, giving priority to the alternative hypothesis, and continuous monitoring the following stopping guidelines were computed by the *toxbdry* function in the Clinfun package in R 3.2.0.

Table 9. Stopping Boundaries for Graft Failure at Day 100

Monitoring Look	Number of Patients at Monitoring Look	Stop if Number of Toxicities is at least
1	9	2
2	21	3
3	33	4
4	45	5
5	58	6
6	60	7

The trial will be terminated if 2 or more patients out of the first 9 patients receiving experimental drug, Telmisartan, experience graft failure by day 100. If out of the first 21 patients, 3 or more have graft failure by day 100 then trial will be stopped. The boundaries were obtained such the Type I error, fixed at 1%, was spent over the multiple looks, hence the probability of stopping the trial prematurely by chance is 0.01.

Table 10. Operating characteristics for the stopping boundaries for Graft Failure Day 100

Probability Of Toxicity	Probability of crossing low bndry	Probability of stopping low bndry	Expected sample size low bndry	Probability of crossing high bndry	Probability of stopping high bndry	Expected sample size high bdnry
0.07	0.373	0.373	46.1	0.365	0.365	46.2
0.08	0.477	0.477	42.4	0.467	0.466	42.5
0.09	0.576	0.576	38.7	0.565	0.565	38.8
0.10	0.666	0.666	35.0	0.655	0.654	35.1
0.11	0.744	0.744	31.6	0.734	0.733	31.7
0.12	**0.809**	**0.809**	**28.5**	**0.799**	**0.798**	**28.6**

Bndry, boundary.

From the operating characteristics, the probability of stopping the trial due to graft failure if the level of toxicity is at high level of 12% is 0.798. The expected sample size at termination of the trial is 28.6, if Telmisartan in fact more toxic than standard treatment.

Telmisartan Intolerance (includes angioedema and grade 3 or greater drug related adverse events after dose adjustments)

We use 5% as our acceptable rate of intolerance for drug treatment. Using repeated significance testing (Jennison and Turnbull) with 5.0% as lower proportion and 11.0% as higher proportion, 1% alpha level, and 80% power to for early termination, shape parameter of the boundary, delta=0.2, giving priority to the alternative hypothesis and continuous monitoring, the following stopping guidelines were computed by the *toxbdry* function in the Clinfun package in R 3.2.0.

Table 11. Stopping Boundaries for Intolerance to Telmisartan at Day 100

Monitoring Look	Number of Patients at Monitoring Look	Stop if Number of Toxicities is at least
1	8	2
2	22	3
3	38	4
4	55	5
5	60	6

The trial will be terminated if 2 or more patients out of the first 8 patients receiving experimental drug, Telmisartan, experience intolerance by day 100. If out of the first 22 patients, 3 or more have intolerance by day 100 then trial will be stopped. The boundaries were obtained such the Type I error, fixed at 1%, was spent over the multiple looks, hence the probability of stopping the trial prematurely by chance is 0.01.

Table 12. Operating characteristics for the stopping boundaries for Intolerance Day 100

Probability Of Toxicity	Probability of crossing low bndry	Probability of stopping low bndry	Expected sample size low bndry	Probability of crossing high bndry	Probability of stopping high bndry	Expected sample size high bdnry
0.050	0.222	0.221	52.3	0.219	0.218	52.4
0.062	0.350	0.350	48.1	0.346	0.345	48.2
0.074	0.483	0.483	43.5	0.480	0.477	43.6
0.086	0.608	0.608	38.9	0.604	0.601	39.0
0.098	0.714	0.711	34.4	0.712	0.708	34.6
0.11	**0.800**	**0.797**	**30.4**	**0.798**	**0.794**	**30.6**

From the operating characteristics, the probability of stopping the trial due to intolerance if the level of toxicity is at high level of 11% is 0.794. The expected sample size at termination of the trial is 30.6, if Telmisartan in fact more toxic than standard treatment.

Grade 3 or 4 GVHD at 100 days

If the Grade 3 or 4 GVHD in patients receiving Telmisartan the rate of NRM exceeds 36% then the study will be stopped. The operating characteristics of the stopping rule are described below. Based on historical data from the cancer center registry, it was assumed that 26% incidence of NRM was considered expected and incidence greater than 36% would be considered unacceptable.

Using repeated significance testing (Jennison and Turnbull) with 26.0% as lower proportion and 36.0% as higher proportion, 1% alpha level, and 80% power for early termination, shape parameter of the boundary, delta=0.2, with priority on alternative hypothesis and continuous monitoring, the following stopping guidelines were computed by the *toxbdry* function in the Clinfun package in R 3.2.0.

Table 13. Stopping Boundaries for NRM at 100 days using continuous monitoring

Monitoring Look	Number of Patients at Monitoring Look	Stop if Number of Toxicities is at least
1	3	3
2	5	4
3	8	5
4	11	6
5	14	7
6	18	8
7	21	9
8	24	10
9	28	11
10	31	12
11	35	13
12	38	14
13	42	15
15	45	16
16	49	17
17	52	18

18	56	19
19	59	20
20	60	21

The trial will be terminated if 3 patients out of the first 3 patients receiving experimental drug, Telmisartan, experience Grade 3 or 4 GVHD by day 100. If out of the first 5 patients, 4 or more have NRM by day 100 then trial will be stopped. The boundaries were obtained such the Type I error, fixed at 1%, was spent over the multiple looks, hence the probability of stopping the trial prematurely by chance is 0.01.

Table 14. Operating characteristics for the stopping boundaries for NRM Day 100

Probability Of Toxicity	Probability of crossing low bndry	Probability of stopping low bndry	Expected sample size low bndry	Probability of crossing high bndry	Probability of stopping high bndry	Expected sample size high bdnry
0.26	0.253	0.247	51.5	0.247	0.247	51.5
0.28	0.359	0.351	48.1	0.351	0.351	48.1
0.30	0.478	0.467	44.3	0.467	0.467	44.3
0.32	0.597	0.695	40.2	0.585	0.585	35.2
0.34	0.708	0.695	35.9	0.695	0.695	35.9
0.36	**0.801**	**0.790**	**31.8**	**0.790**	**0.790**	**31.8**

Bndry, boundary.

From the operating characteristics, the probability of stopping the trial due to grade 3 or 4 GVHD if the level of toxicity is at high level of 36% is 0.79. The expected sample size at termination of the trial is 31.8, if Telmisartan in fact more toxic than standard treatment.

9.0 Safety Monitoring

9.1 Adverse Events

For the purposes of this research study, an "adverse event" (AE) is any untoward medical occurrence associated with the use of a study drug, whether or not considered drug related. An AE can be a clinical event in the form of signs, symptoms, disease, or laboratory or physiological observations occurring in a study participant, regardless of causal relationship. A "pre-existing" condition is one that is present prior to study drug administration and is reported as part of the patient's medical history. A pre-existing condition should be reported as an AE only if the frequency, intensity, or character of the pre-existing condition worsens during the course of the study.

Laboratory abnormalities associated with subjects underlying disease or related to the subject's HCT will not be considered adverse events. However, a laboratory abnormality (e.g. a clinically significant change detected on clinical chemistry or hematology) that is independent from the underlying medical condition and/or HCT that requires medical or surgical intervention, or leads to study drug discontinuation, will be considered an AE.

9.2 Recording Adverse Events

All AE's will be graded according to the NCI's Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. All grade 3, 4, and 5 non-hematologic adverse events will be recorded. Grade 1 and 2 adverse events will be recorded if they are: 1) potentially associated with GvHD, or 2) potentially attributable to the administration of telmisartan during the period of administration. All AEs should be recorded and, whenever possible, followed until resolution. Documented AEs should contain the following information:

- Severity grade according to CTCAEv4
- Duration: including start and end dates or if the event is ongoing
- Relationship to the study treatment: unrelated, possibly related, related
- Action taken with regard to study treatment
- Whether other medication or therapies were needed and initiated
- Outcome: resolved, not resolved, resolved with sequelae, fatal, or unknown
- Whether it constitutes a serious adverse event (SAE)

9.3 Serious Adverse Events

An adverse event or suspected adverse reactions is considered serious if, in the view of the investigator or sponsor, it results in any of the following outcomes:

- Death

- Life-threatening AE: Places the patient at immediate risk of death at the time of the event as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Inpatient hospitalization or prolongation of hospitalization
- Congenital anomaly or birth defect

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition above. All SAEs will be recorded and reported to the IRB and FDA according to institutional and federal policy.

9.4 Data and Safety Monitoring Board (DSMB)

This study will be approved and monitored by the HackensackUMC DSMB. The DSMB is responsible for the monitoring of investigator initiated research studies to ensure the safety of participants, the integrity of the data, and the appropriate termination of studies in the event that undue risks have been uncovered, or it appears that trials cannot be conducted successfully. The DSMB provides multidisciplinary, independent oversight of research studies conducted by HackensackUMC staff and or affiliates. The DSMB has the ability to require protocol modifications related to participant safety and to recommend suspension of termination to the IRB and institutional official of any research protocols that fall within its jurisdiction. The DSMB may request that HackensackUMC's Corporate Compliance Department conduct periodic audits to assure that data are being collected and recorded according to the protocol.

The investigator is required to submit monthly monitoring reports which include current enrollment data, adverse event summary data and any other data requested by the DSMB. The DSMB will meet monthly to review the trial progress, adverse event data, and any relevant information such as significant amendments or reviews from IRB submitted by the principal investigator. If significant concerns are raised by the DSMB, the concerns will be forwarded to the PI as well as recommendations made to the IRB, and Institutional Official. Recommendations may include modifying, suspending, or terminating the protocol.

10.0 RISKS AND BENEFITS ASSESSMENT

10.1 Potential Benefits

The primary benefit to patients enrolled into this study is a reduction in the incidence or severity of acute GvHD, without a diminution in the desired graft-versus-cancer effect of allogeneic HSC transplantation. A reduction in the incidence or severity of acute GvHD will result in a decrease in the use of corticosteroids used in the management of acute GvHD, and a resulting decrease in

complications of corticosteroid use including immunosuppression, opportunistic viral and fungal infections, steroid myopathy, cataract formation, and avascular necrosis of the bone.

10.2 Risk/Benefits

Patients undergoing allogeneic hematopoietic stem cell transplantation using myeloablative regimens and cells from related or unrelated donors face considerable risks associated with this treatment. These risks include prolonged periods of marrow hypoplasia requiring blood component and antibiotic support. Even after engraftment, the immunological dysfunction persisting for months after transplantation can lead to opportunistic infections. A small proportion of patients will fail to achieve sustained donor cell engraftment, requiring re-conditioning and a second transplant. The primary causes of treatment failure, however, arise from acute and/or chronic GvHD and from relapse of disease.

In phase III and post-marketing safety studies, telmisartan, 80 or 160 mg per day, was found to have a very good safety profile, with the incidence of reported “poor tolerability” of only 0.5% [106]. In theory, due to its anti-inflammatory actions, telmisartan could increase the risks of primary or secondary graft failure, delayed engraftment, or early post-transplant relapse of disease. However, immunosuppression or increased risk of infection were not drug related adverse events, either in controlled Phase III trials or in post-marketing review.

Other serious complications of allogeneic HSC transplantation could occur through unforeseen interactions with other medications or as a result of chemotherapy administration. It is unlikely, but theoretically possible, that successful reduction of acute IT GvHD could redirect allo-reactive T cells to the skin or lungs to produce higher levels of GVHD at those sites. In addition to telmisartan, subjects will be receiving standard GvHD prophylaxis as ordered by their transplant physician.

The Transplantation Program team of physicians, advanced-practice nurses, nursing staff, pharmacists, social workers, nutritionists, and physical therapists manages patients undergoing allogeneic HSC transplantation at HackensackUMC. Patients undergo medical care in dedicated inpatient and outpatient units. The resources of the Transplantation Program ameliorate the potential risks of allogeneic HCT in the treatment of malignant diseases. These same resources will apply to patients enrolled into this clinical study. In addition, a research nurse coordinator will monitor patients while receiving telmisartan. All adverse events will be recorded and scored per CTCAEv4.

Patients will be educated regarding their responsibility in the use of the experimental agent. Telmisartan will be stored and dispensed by the research pharmacy. Patients will keep a diary of drug administration, accounting for all the doses. Patients requiring emergent care will be managed by members of the Transplantation Program, in conjunction with any other physicians/resources of HackensackUMC necessary to respond to the patient’s immediate needs.

11.0 Ethical and Regulatory Standards

11.1 Ethics

This study will be conducted in compliance with the Declaration of Helsinki, the Belmont Report, International Conference on Harmonization (ICH) guidelines (E6), US FDA Regulations, principles included in current Good Clinical Practice Guidelines, and all applicable state and local regulations.

11.2 Informed Consent

Voluntary, written informed consent will be obtained from each subject's parents or legal guardians in accordance with GCP and federal and institutional regulations. Should any study amendments require changes to the informed consent document that would affect subject's decision to continue participating, ongoing subjects will be reconsented for their continued participation. This will be at the discretion of the PI with approval by the IRB.

11.3 Investigator Responsibilities

The co-PIs will conduct the study according to the current protocol, obtain IRB approval to conduct the study, will obtain informed consent from each study participant, will maintain and supply auditors and regulatory agencies adequate and accurate records of study activity, will report serious adverse events to the IRB, will personally conduct or supervise the study, and will ensure that colleagues participating in the study are informed about their obligations in meeting the above commitments.

11.4 Institutional Review Board Approval

The PI will submit this protocol, the consent form, any other relevant supporting information to the Hackensack University Medical Center IRB for review and approval before study initiation. Amendments to the protocol and ICF must also be approved by the IRB before the implementation of changes in this study. The Investigator is also responsible for providing the IRB any required information before or during the study, such as serious adverse event expedited reports or study progress reports.

11.5 Confidentiality

All samples and study data will be coded with each subject's ID#. The list linking subject ID# to subject identity will be accessible only to authorized members of the study team. All other study data at Hackensack University medical Center will be kept in files locked in the office of the PI or another member of the study team (eg: research coordinator). Data will be accessible only to the PI and other authorized members of the study team. All subjects will be asked to sign a HIPAA Authorization before they begin participation in this research study.

11.6 Study Termination

The PI reserves the right to terminate the study at any time. Conditions that may warrant termination of the study include, but are not limited to:

- Unsatisfactory enrollment
- Serious and/or persistent non-compliance with the protocol and/or applicable regulatory guidelines in conducting the study
- IRB or DSMB decision to terminate or suspend approval
- Investigator fraud (altered data, omitted data, or manufactured data)
- The incidence/severity of AEs indicates a potential health hazard to patients
- Discovery of an unexpected, serious, or unacceptable risk to subjects

11.7 IND-Exempt Determination

As per 21 CFR part 312, this study qualifies as exempt from IND requirements. Telmisartan (Micardis) is an FDA-approved, marketed drug in the United States. 21 CFR part 312 states that "The clinical investigation of a drug product that is lawfully marketed in the United States is exempt from the requirements of this part if all the following apply:

i) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

(ii) If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;

(iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

(iv) The investigation is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and

(v) The investigation is conducted in compliance with the requirements of 312.7.

After careful consideration, the PI and sub-investigators have determined that all of the above criteria have been met for this study. The standard, oral route of administration for telmisartan (Micardis) will be used. The dose of 160 mg used in this study has been assessed in multiple clinical studies and has shown no statistical increase in anti-hypertensive effect or adverse side effects [107-112]. The co-PI who is expert in the field of hematopoietic stem cell transplantation, believes that giving telmisartan (Micardis) to HCT patients enrolled in this study at the proposed dose and schedule should not increase the risk or decrease the acceptability of risk. An IND application along with the protocol, consent form, and required forms was submitted to the FDA and an IND exemption was granted in writing. Any revisions made to the protocol and consent

have been resubmitted to the FDA to ensure that the changes made do not affect the IND exempt status.

References

1. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. Blood. 2000 May 1;95(9):2754-9. Review. PubMed PMID: 10779417.
2. Chen X, Dodge J, Komorowski R, Drobyski WR. A critical role for the retinoic acid signaling pathway in the pathophysiology of gastrointestinal graft-versus-host disease. Blood. 2013 May 9;121(19):3970-80. doi:10.1182/blood-2012-08-445130. Epub 2013 Mar 25. PubMed PMID: 23529927; PubMed Central PMCID: PMC3650708.
3. Eriguchi Y, Takashima S, Oka H, Shimoji S, Nakamura K, Uryu H, Shimoda S, Iwasaki H, Shimono N, Ayabe T, Akashi K, Teshima T. Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. Blood. 2012 Jul 5;120(1):223-31. doi: 10.1182/blood-2011-12-401166. Epub 2012 Apr 24. PubMed PMID: 22535662.
4. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol. 2012 May 11;12(6):443-58. doi: 10.1038/nri3212. Review. PubMed PMID: 22576252; PubMed Central PMCID: PMC3552454.
5. Wolf D, von Lilienfeld-Toal M, Wolf AM, Schleuning M, von Bergwelt-Baildon M, Held SA, Brossart P. Novel treatment concepts for graft-versus-host disease. Blood. 2012 Jan 5;119(1):16-25. doi: 10.1182/blood-2011-08-339465. Epub 2011 Sep 26. Review. PubMed PMID: 21948301
6. Choi S, Reddy P. Graft-versus-host disease. Panminerva Med. 2010 Jun;52(2):111-24. Review. PubMed PMID: 20517195.
7. Ringdén O, Shrestha S, da Silva GT, Zhang MJ, Dispenzieri A, Remberger M, Kamble R, Freytes CO, Gale RP, Gibson J, Gupta V, Holmberg L, Lazarus H, McCarthy P, Meehan K, Schouten H, Milone GA, Lonial S, Hari PN. Effect of acute and chronic GVHD on relapse and survival after reduced-intensity conditioning allogeneic transplantation for myeloma. Bone Marrow Transplant. 2012 Jun;47(6):831-7. doi: 10.1038/bmt.2011.192. Epub 2011 Sep 26. PubMed PMID:21946381; PubMed Central PMCID: PMC3369112.
8. Lee SJ, Klein JP, Barrett AJ, Ringden O, Antin JH, Cahn JY, Carabasi MH, Gale RP, Giralt S, Hale GA, Ilhan O, McCarthy PL, Socie G, Verdonck LF, Weisdorf DJ, Horowitz MM. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. Blood. 2002 Jul 15;100(2):406-14. PubMed PMID: 12091329.
9. Signori A, Crocchiolo R, Oneto R, Sacchi N, Sormani MP, Fagioli F, Rambaldi A, Ciceri F, Bacigalupo A. Chronic GVHD is associated with lower relapse risk irrespective of stem cell source among patients receiving transplantation from unrelated donors. Bone Marrow Transplant. 2012 Nov;47(11):1474-8. doi:10.1038/bmt.2012.58. Epub 2012 Apr 2. PubMed PMID: 22465976.
10. Patterson AE, Korngold R. Infusion of select leukemia-reactive TCR Vbeta+ T cells provides graft-versus-leukemia responses with minimization of graft-versus-host disease following murine hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2001;7(4):187-96. PubMed PMID:11349805.

11. Fanning SL, Zilberberg J, Stein J, Vazzana K, Berger SA, Korngold R, Friedman TM. Unraveling graft-versus-host disease and graft-versus-leukemia responses using TCR Vβ spectratype analysis in a murine bone marrow transplantation model. J Immunol. 2013 Jan 1;190(1):447-57. doi: 10.4049/jimmunol.1201641. Epub 2012 Nov 30. PubMed PMID: 23203931; PubMed Central PMCID: PMC3529851.

12. Komanduri KV. Targeting neovascularization in GVHD. Blood. 2013 Apr 25;121(17):3303-4. doi: 10.1182/blood-2013-01-480483. PubMed PMID: 23620570.

13. Leonhardt F, Grundmann S, Behe M, Bluhm F, Dumont RA, Braun F, Fani M, Riesner K, Prinz G, Hechinger AK, Gerlach UV, Dierbach H, Penack O, Schmitt-Gräff A, Finke J, Weber WA, Zeiser R. Inflammatory neovascularization during graft-versus-host disease is regulated by αv integrin and miR-100. Blood. 2013 Apr 25;121(17):3307-18. doi: 10.1182/blood-2012-07-442665. Epub 2013 Jan 17. PubMed PMID: 23327924.

14. Penack O, Socié G, van den Brink MR. The importance of neovascularization and its inhibition for allogeneic hematopoietic stem cell transplantation. Blood. 2011 Apr 21;117(16):4181-9. doi: 10.1182/blood-2010-10-312934. Epub 2011 Jan 21. PubMed PMID: 21258010.

15. Penack O, Henke E, Suh D, King CG, Smith OM, Na IK, Holland AM, Ghosh A, Lu SX, Jenq RR, Liu C, Murphy GF, Lu TT, May C, Scheinberg DA, Gao DC, Mittal V, Heller G, Benezra R, van den Brink MR. Inhibition of neovascularization to simultaneously ameliorate graft-vs-host disease and decrease tumor growth. J Natl Cancer Inst. 2010 Jun 16;102(12):894-908. doi: 10.1093/jnci/djq172. Epub 2010 May PubMed PMID: 20463307; PubMed Central PMCID: PMC2886094.

16. Petrovic A, Alpdogan O, Willis LM, Eng JM, Greenberg AS, Kappel BJ, Liu C, Murphy GJ, Heller G, van den Brink MR. LPAM (alpha 4 beta 7 integrin) is an important homing integrin on alloreactive T cells in the development of intestinal graft-versus-host disease. Blood. 2004 Feb 15;103(4):1542-7. Epub 2003 Oct 16. PubMed PMID: 14563643.

17. Reshef R, Luger SM, Hexner EO, Loren AW, Frey NV, Nasta SD, Goldstein SC, Stadtmauer EA, Smith J, Bailey S, Mick R, Heitjan DF, Emerson SG, Hoxie JA, Vonderheide RH, Porter DL. Blockade of lymphocyte chemotaxis in visceral graft-versus-host disease. N Engl J Med. 2012 Jul 12;367(2):135-45. doi: 10.1056/NEJMoa1201248. PubMed PMID: 22784116; PubMed Central PMCID: PMC3568501.

18. Engelhardt BG, Jagasia M, Savani BN, Bratcher NL, Greer JP, Jiang A, Kassim AA, Lu P, Schuening F, Yoder SM, Rock MT, Crowe JE Jr. Regulatory T cell expression of CLA or α(4)β(7) and skin or gut acute GVHD outcomes. Bone Marrow Transplant. 2011 Mar;46(3):436-42. doi: 10.1038/bmt.2010.127. Epub 2010 May 31. PubMed PMID: 20577222; PubMed Central PMCID: PMC3217583.

19. Engelhardt BG, Sengsayadeth SM, Jagasia M, Savani BN, Kassim AA, Lu P, Shyr Y, Yoder SM, Rock MT, Crowe JE Jr. Tissue-specific regulatory T cells: biomarker for acute graft-vs-host disease and survival. Exp Hematol. 2012 Dec;40(12):974-982.e1. doi: 0.1016/j.exphem. 2012.08.002. Epub 2012 Aug 10. PubMed PMID: 22885125; PubMed Central PMCID: PMC3611587.

20. Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, Defor T, Levine BL, June CH, Rubinstein P, McGlave PB, Blazar BR, Wagner JE. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood. 2011 Jan 20;117(3):1061-70. doi: 10.1182/blood-2010-07-293795. Epub 2010 Oct 15. PubMed PMID: 20952687; PubMed Central PMCID: PMC3035067.

21. Liu Z, Zhao Y, Wei F, Ye L, Lu F, Zhang H, Diao Y, Song H, Qi Z. Treatment with telmisartan/rosuvastatin combination has a beneficial synergistic effect on ameliorating Th17/Treg functional imbalance in hypertensive patients with carotid atherosclerosis. Atherosclerosis. 2014 Mar;233(1):291-9. doi:10.1016/j.atherosclerosis.2013.12.004. Epub 2014 Jan 10. PubMed PMID: 24495792.

22. Mihaescu A, Santén S, Jeppsson B, Thorlacius H. Rho kinase signalling mediates radiation-induced inflammation and intestinal barrier dysfunction. Br J Surg. 2011 Jan;98(1):124-31. doi: 10.1002/bjs.7279. Epub 2010 Sep 29. PubMed PMID:20882561.

23. Arab HH, Al-Shorbagy MY, Abdallah DM, Nassar NN. Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. PLoS One. 2014 May 15;9(5):e97193. doi: 10.1371/journal.pone.0097193. eCollection 2014. PubMed PMID: 24831514; PubMed Central PMCID: PMC4022743.

24. Kobayashi N, Ohno T, Yoshida K, Fukushima H, Mamada Y, Nomura M, Hirata H, Machida Y, Shinoda M, Suzuki N, Matsuoka H. Cardioprotective mechanism of telmisartan via PPAR-gamma-eNOS pathway in dahl salt-sensitive hypertensive rats. Am J Hypertens. 2008 May;21(5):576-81. doi: 10.1038/ajh.2008.27. Epub 2008 Mar 20. PubMed PMID: 18437150.

25. Iyengar S, Zhan C, Lu J, Korngold R, Schwartz DH. Treatment with a Rho Kinase Inhibitor Improves Survival from Graft-Versus-Host Disease in Mice after MHC-Haploidentical Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 2014 May 2. pii: S1083-8791(14)00269-9. doi: 10.1016/j.bbmt.2014.04.029. [Epub ahead of print] PubMed PMID: 24796280.

26. Song EK, Yim JM, Yim JY, Song MY, Rho HW, Yim SK, Jeon SY, Kim HS, Yhim HY, Lee NR, Kwak JY, Sohn MH, Park HS, Jang KY, Yim CY. Rosiglitazone prevents graft-versus-host disease (GVHD). Transpl Immunol. 2012 Oct;27(2-3):128-37. doi: 10.1016/j.trim.2012.09.001. Epub 2012 Sep 13. PubMed PMID: 22982856.

27. Walcher D, Hess K, Heinz P, Petscher K, Vasic D, Kintscher U, Clemenz M, Hartge M, Raps K, Hombach V, Marx N. Telmisartan inhibits CD4-positive lymphocyte migration independent of the angiotensin type 1 receptor via peroxisome proliferator-activated receptor-gamma. Hypertension. 2008 Feb;51(2):259-66. Epub 2007 Dec 24. PubMed PMID: 18158351.

28. Cianchetti S, Del Fiorentino A, Colognato R, Di Stefano R, Franzoni F, Pedrinelli R. Anti-inflammatory and anti-oxidant properties of telmisartan in cultured human umbilical vein endothelial cells. Atherosclerosis. 2008 May;198(1):22-8. Epub 2007 Oct 22. PubMed PMID: 17950296.

29. Siragusa M, Sessa WC. Telmisartan exerts pleiotropic effects in endothelial cells and promotes endothelial cell quiescence and survival. Arterioscler Thromb Vasc Biol. 2013 Aug;33(8):1852-60. doi: 10.1161/ATVBAHA.112.300985. Epub 2013 May 23. PubMed PMID: 23702662; PubMed Central PMCID: PMC3818114.

30. Usui T, Sugisaki K, Iriyama A, Yokoo S, Yamagami S, Nagai N, Ishida S, Amano S. Inhibition of corneal neovascularization by blocking the angiotensin II type 1 receptor. Invest Ophthalmol Vis Sci. 2008 Oct;49(10):4370-6. doi:10.1167/iovs.07-0964. PubMed PMID: 18829859.

31. Nagai N, Noda K, Urano T, Kubota Y, Shinoda H, Koto T, Shinoda K, Inoue M, Shiomi T, Ikeda E, Tsubota K, Suda T, Oike Y, Ishida S. Selective suppression of pathologic, but not physiologic, retinal neovascularization by blocking the angiotensin II type 1 receptor. Invest Ophthalmol Vis Sci. 2005 Mar;46(3):1078-84. PubMed PMID: 15728568.

32. Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. Nat Rev Mol Cell Biol. 2003 Jun;4(6):446-56. Review. PubMed PMID: 12778124.

33. Yoneda A, Multhaupt HA, Couchman JR. The Rho kinases I and II regulate different aspects of myosin II activity. J Cell Biol. 2005 Aug 1;170(3):443-53. Epub 2005 Jul 25. PubMed PMID: 16043513; PubMed Central PMCID: PMC2171463.

34. Wang Y, Zheng XR, Riddick N, Bryden M, Baur W, Zhang X, Surks HK. ROCK isoform regulation of myosin phosphatase and contractility in vascular smooth muscle cells. Circ Res. 2009 Feb 27;104(4):531-40. doi: 10.1161/CIRCRESAHA.108.188524. Epub 2009 Jan 8. PubMed PMID: 19131646; PubMed Central PMCID: PMC2649695.

35. Vicari RM, Chaitman B, Keefe D, Smith WB, Chrysant SG, Tonkon MJ, Bittar N, Weiss RJ, Morales-Ballejo H, Thadani U; Fasudil Study Group. Efficacy and safety of fasudil in patients with stable angina: a double-blind, placebo-controlled, phase 2 trial. J Am Coll Cardiol. 2005 Nov 15;46(10):1803-11. Epub 2005 Oct 19.PubMed PMID: 16286163.

36. Shimokawa H, Hiramori K, Iinuma H, Hosoda S, Kishida H, Osada H, Katagiri T,Yamauchi K, Yui Y, Minamino T, Nakashima M, Kato K. Anti-anginal effect of fasudil, a Rho-kinase inhibitor, in patients with stable effort angina: a multicenter study. J Cardiovasc Pharmacol. 2002 Nov;40(5):751-61. PubMed PMID:12409984.

37. Fukumoto Y, Mohri M, Inokuchi K, Ito A, Hirakawa Y, Masumoto A, Hirooka Y, Takeshita A, Shimokawa H. Anti-ischemic effects of fasudil, a specific Rho-kinase inhibitor, in patients with stable effort angina. J Cardiovasc Pharmacol. 2007 Mar;49(3):117-21. PubMed PMID: 17414222.

38. Otsuka T, Ibuki C, Suzuki T, Ishii K, Kodani E, Atarashi H, Kishida H, Takano T. Vasodilatory effect of subsequent administration of fasudil, a rho-kinase inhibitor, surpasses that of nitroglycerin at the concentric coronary stenosis in patients with stable angina pectoris. Circ J. 2006 Apr;70(4):402-8. PubMed PMID: 16565555.

39. Masumoto A, Mohri M, Shimokawa H, Urakami L, Usui M, Takeshita A. Suppression of coronary artery spasm by the Rho-kinase inhibitor fasudil in patients with vasospastic angina. Circulation. 2002 Apr 2;105(13):1545-7. PubMed PMID:11927519.

40. Mohri M, Shimokawa H, Hirakawa Y, Masumoto A, Takeshita A. Rho-kinase inhibition with intracoronary fasudil prevents myocardial ischemia in patients with coronary microvascular spasm. J Am Coll Cardiol. 2003 Jan 1;41(1):15-9. PubMed PMID: 12570938.

41. Inokuchi K, Ito A, Fukumoto Y, Matoba T, Shiose A, Nishida T, Masuda M, Morita S, Shimokawa H. Usefulness of fasudil, a Rho-kinase inhibitor, to treat intractable severe coronary spasm after coronary artery bypass surgery. J Cardiovasc Pharmacol. 2004 Sep;44(3):275-7. PubMed PMID: 15475822.

42. Kishi T, Hirooka Y, Masumoto A, Ito K, Kimura Y, Inokuchi K, Tagawa T, Shimokawa H, Takeshita A, Sunagawa K. Rho-kinase inhibitor improves increased vascular resistance and impaired vasodilation of the forearm in patients with heart failure. Circulation. 2005 May 31;111(21):2741-7. PubMed PMID: 15927989.

43. Witzenrath M, Ahrens B, Schmeck B, Kube SM, Hippenstiel S, Rosseau S, Hamelmann E, Suttorp N, Schütte H. Rho-kinase and contractile apparatus proteins in murine airway hyperresponsiveness. Exp Toxicol Pathol. 2008 Jun;60(1):9-15. doi: 10.1016/j.etp.2008.03.002. Epub 2008 Apr 22. PubMed PMID: 18434112.

44. Noma K, Rikitake Y, Oyama N, Yan G, Alcaide P, Liu PY, Wang H, Ahl D, Sawada N, Okamoto R, Hiroi Y, Shimizu K, Luscinskas FW, Sun J, Liao JK. ROCK1 mediates leukocyte recruitment and neointima formation following vascular injury. J Clin Invest. 2008 May;118(5):1632-44. doi: 10.1172/JCI29226. PubMed PMID: 18414683; PubMed Central PMCID: PMC22933

45. Lämmermann T, Bader BL, Monkley SJ, Worbs T, Wedlich-Söldner R, Hirsch K, Keller M, Förster R, Critchley DR, Fässler R, Sixt M. Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature. 2008 May 1;453(7191):51-5. doi: 10.1038/nature06887. PubMed PMID: 18451854.

46. Smith A, Bracke M, Leitinger B, Porter JC, Hogg N. LFA-1-induced T cell migration on ICAM-1 involves regulation of MLCK-mediated attachment and ROCK-dependent detachment. J Cell Sci. 2003 Aug 1;116(Pt 15):3123-33. Epub 2003 Jun 10. PubMed PMID: 12799414.

47. van Buul JD, Hordijk PL. Signaling in leukocyte transendothelial migration. Arterioscler Thromb Vasc Biol. 2004 May;24(5):824-33. Epub 2004 Feb 19. Review. PubMed PMID: 14976004.

48. Benais-Pont G, Punn A, Flores-Maldonado C, Eckert J, Raposo G, Fleming TP, Cereijido M, Balda MS, Matter K. Identification of a tight junction-associated guanine nucleotide exchange factor that activates Rho and regulates paracellular permeability. J Cell Biol. 2003 Mar 3;160(5):729-40. Epub 2003 Feb 25. PubMed PMID: 12604587; PubMed Central PMCID: PMC2173357.

49. Li B, Zhao WD, Tan ZM, Fang WG, Zhu L, Chen YH. Involvement of Rho/ROCK signalling in small cell lung cancer migration through human brain microvascular endothelial cells. FEBS Lett. 2006 Jul 24;580(17):4252-60. Epub 2006 Jun 30. PubMed PMID: 16828752.

50. Nohria A, Grunert ME, Rikitake Y, Noma K, Prsic A, Ganz P, Liao JK, Creager MA. Rho kinase inhibition improves endothelial function in human subjects with coronary artery disease. Circ Res. 2006 Dec 8;99(12):1426-32. Epub 2006 Nov 9. PubMed PMID: 17095725; PubMed Central PMCID: PMC2666070.

51. Bardi G, Niggli V, Loetscher P. Rho kinase is required for CCR7-mediated polarization and chemotaxis of T lymphocytes. FEBS Lett. 2003 May 8;542(1-3):79-83. PubMed PMID: 12729902.

52. Lee JH, Katakai T, Hara T, Gonda H, Sugai M, Shimizu A. Roles of p-ERM and Rho-ROCK signaling in lymphocyte polarity and uropod formation. J Cell Biol. 2004 Oct 25;167(2):327-37. PubMed PMID: 15504914; PubMed Central PMCID: PMC2172551.

53. Xue F, Takahara T, Yata Y, Xia Q, Nonome K, Shinno E, Kanayama M, Takahara S, Sugiyama T. Blockade of Rho/Rho-associated coiled coil-forming kinase signaling can prevent progression of hepatocellular carcinoma in matrix metalloproteinase-dependent manner. Hepatol Res. 2008 Aug;38(8):810-7. doi: 10.1111/j.1872-034X.2008.00333.x. Epub 2008 May 27. PubMed PMID: 18507693.

54. Wong CC, Wong CM, Ko FC, Chan LK, Ching YP, Yam JW, Ng IO. Deleted in liver cancer 1 (DLC1) negatively regulates Rho/ROCK/MLC pathway in hepatocellular carcinoma. PLoS One. 2008 Jul 23;3(7):e2779. doi: 10.1371/journal.pone.0002779. PubMed PMID: 18648664; PubMed Central PMCID: PMC2464714.

55. Ogawa T, Tashiro H, Miyata Y, Ushitora Y, Fudaba Y, Kobayashi T, Arihiro K, Okajima M, Asahara T. Rho-associated kinase inhibitor reduces tumor recurrence after liver transplantation in a rat hepatoma model. Am J Transplant. 2007 Feb;7(2):347-55. Epub 2007 Jan 4. PubMed PMID: 17229077.

56. Wang DS, Dou KF, Li KZ, Song ZS. Enhancement of migration and invasion of hepatoma cells via a Rho GTPase signaling pathway. World J Gastroenterol. 2004 Jan 15;10(2):299-302. PubMed PMID: 14716844.

57. Vishnubhotla R, Sun S, Huq J, Bulic M, Ramesh A, Guzman G, Cho M, Glover SC. ROCK-II mediates colon cancer invasion via regulation of MMP-2 and MMP-13 at the site of invadopodia as revealed by multiphoton imaging. Lab Invest. 2007 Nov;87(11):1149-58. Epub 2007 Sep 17. PubMed PMID: 17876296.

58. Kamai T, Tsujii T, Arai K, Takagi K, Asami H, Ito Y, Oshima H. Significant association of Rho/ROCK pathway with invasion and metastasis of bladder cancer. Clin Cancer Res. 2003 Jul;9(7):2632-41. PubMed PMID: 12855641.

59. Sahai E, Marshall CJ. ROCK and Dia have opposing effects on adherens junctions downstream of Rho. Nat Cell Biol. 2002 Jun;4(6):408-15. PubMed PMID: 11992112.

60. Croft DR, Sahai E, Mavria G, Li S, Tsai J, Lee WM, Marshall CJ, Olson MF. Conditional ROCK activation in vivo induces tumor cell dissemination and angiogenesis. Cancer Res. 2004 Dec 15;64(24):8994-9001. PubMed PMID: 15604264.

61. Mizukami Y, Fujiki K, Duerr EM, Gala M, Jo WS, Zhang X, Chung DC. Hypoxic regulation of vascular endothelial growth factor through the induction of phosphatidylinositol 3-kinase/Rho/ROCK and c-Myc. J Biol Chem. 2006 May 19;281(20):13957-63. Epub 2006 Mar 16. PubMed PMID: 16543245.

62. Somlyo AV, Phelps C, Dipierro C, Eto M, Read P, Barrett M, Gibson JJ, Burnitz MC, Myers C, Somlyo AP. Rho kinase and matrix metalloproteinase inhibitors cooperate to inhibit

angiogenesis and growth of human prostate cancer xenotransplants. FASEB J. 2003 Feb;17(2):223-34. PubMed PMID: 12554701.

63. Somlyo AV, Bradshaw D, Ramos S, Murphy C, Myers CE, Somlyo AP. Rho-kinase inhibitor retards migration and in vivo dissemination of human prostate cancer cells. Biochem Biophys Res Commun. 2000 Mar 24;269(3):652-9. PubMed PMID:10720471.

64. Ying H, Biroc SL, Li WW, Alicke B, Xuan JA, Pagila R, Ohashi Y, Okada T, Kamata Y, Dinter H. The Rho kinase inhibitor fasudil inhibits tumor progression in human and rat tumor models. Mol Cancer Ther. 2006 Sep;5(9):2158-64. PubMed PMID: 16985048.

65. Bourguignon LY, Zhu H, Shao L, Zhu D, Chen YW. Rho-kinase (ROK) promotes CD44v(3,8-10)-ankyrin interaction and tumor cell migration in metastatic breast cancer cells. Cell Motil Cytoskeleton. 1999;43(4):269-87. PubMed PMID: 10423269.

66. Barreiro O, Yanez-Mo M, Serrador JM, Montoya MC, Vicente-Manzanares M, Tejedor R, Furthmayr H, Sanchez-Madrid F. Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. J Cell Biol. 2002 Jun 24;157(7):1233-45. Epub 2002 Jun 24. PubMed PMID: 12082081; PubMed Central PMCID: PMC2173557.

67. Bivalacqua TJ, Champion HC, Usta MF, Cellek S, Chitaley K, Webb RC, Lewis RL, Mills TM, Hellstrom WJ, Kadowitz PJ. RhoA/Rho-kinase suppresses endothelial nitric oxide synthase in the penis: a mechanism for diabetes-associated erectile dysfunction. Proc Natl Acad Sci U S A. 2004 Jun 15;101(24):9121-6. Epub 2004 Jun 7. PubMed PMID: 15184671; PubMed Central PMCID: PMC428483.

68. Ming XF, Viswambharan H, Barandier C, Ruffieux J, Kaibuchi K, Rusconi S, Yang Z. Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. Mol Cell Biol. 2002 Dec;22(24):8467-77. PubMed PMID: 12446767; PubMed Central PMCID: PMC139860.

69. Tominaga T, Sugie K, Hirata M, Morii N, Fukata J, Uchida A, Imura H, Narumiya S. Inhibition of PMA-induced, LFA-1-dependent lymphocyte aggregation by ADP ribosylation of the small molecular weight GTP binding protein, rho. J Cell Biol. 1993 Mar;120(6):1529-37. PubMed PMID: 7680658; PubMed Central PMCID: PMC2119740.

70. Sánchez-Madrid F, del Pozo MA. Leukocyte polarization in cell migration and immune interactions. EMBO J. 1999 Feb 1;18(3):501-11. Review. PubMed PMID: 9927410; PubMed Central PMCID: PMC1171143.

71. Alevriadou BR. CAMs and Rho small GTPases: gatekeepers for leukocyte transendothelial migration. Focus on "VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration". Am J Physiol Cell Physiol. 2003 Aug;285(2):C250-2. Review. PubMed PMID: 12842833.

72. Etienne S, Adamson P, Greenwood J, Strosberg AD, Cazaubon S, Couraud PO. ICAM-1 signaling pathways associated with Rho activation in microvascular brain endothelial cells. J Immunol. 1998 Nov 15;161(10):5755-61. PubMed PMID: 9820557.

73. Zhu F, Zhang Z, Wu G, Li Z, Zhang R, Ren J, Nong L. Rho kinase inhibitor fasudil suppresses migration and invasion though down-regulating the expression of VEGF in lung cancer cell

line A549. Med Oncol. 2011 Jun;28(2):565-71. doi: 10.1007/s12032-010-9468-5. Epub 2010 Mar 19. PubMed PMID: 20300976.

74. Nakabayashi H, Shimizu K. HA1077, a Rho kinase inhibitor, suppresses glioma-induced angiogenesis by targeting the Rho-ROCK and the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signal pathways. Cancer Sci. 2011 Feb;102(2):393-9. doi: 10.1111/j.1349-7006.2010.01794.x. Epub 2010 Dec 19. PubMed PMID: 21166955.

75. Washida N, Wakino S, Tonozuka Y, Homma K, Tokuyama H, Hara Y, Hasegawa K, Minakuchi H, Fujimura K, Hosoya K, Hayashi K, Itoh H. Rho-kinase inhibition ameliorates peritoneal fibrosis and angiogenesis in a rat model of peritoneal sclerosis. Nephrol Dial Transplant. 2011 Sep;26(9):2770-9. doi: 10.1093/ndt/gfr012. Epub 2011 Mar 4. PubMed PMID: 21378147.

76. Kuno M, Takai S, Matsushima-Nishiwaki R, Minamitani C, Mizutani J, Otsuka T, Harada A, Adachi S, Kozawa O, Tokuda H. Rho-kinase inhibitors decrease TGF-beta-stimulated VEGF synthesis through stress-activated protein kinase/c-Jun N-terminal kinase in osteoblasts. Biochem Pharmacol. 2009 Jan 15;77(2):196-203. doi: 10.1016/j.bcp.2008.10.014. Epub 2008 Oct 19. PubMed PMID: 18996357.

77. Takata K, Morishige K, Takahashi T, Hashimoto K, Tsutsumi S, Yin L, Ohta T, Kawagoe J, Takahashi K, Kurachi H. Fasudil-induced hypoxia-inducible factor-1alpha degradation disrupts a hypoxia-driven vascular endothelial growth factor autocrine mechanism in endothelial cells. Mol Cancer Ther. 2008 Jun;7(6):1551-61. doi: 10.1158/1535-7163.MCT-07-0428. PubMed PMID: 18566226.

78. Hata Y, Miura M, Nakao S, Kawahara S, Kita T, Ishibashi T. Antiangiogenic properties of fasudil, a potent Rho-Kinase inhibitor. Jpn J Ophthalmol. 2008 Jan-Feb;52(1):16-23. doi: 10.1007/s10384-007-0487-5. Epub 2008 Mar 28. PubMed PMID: 18369695.

79. Liu C, Li Y, Yu J, Feng L, Hou S, et al. (2013) Targeting the Shift from M1 to M2 Macrophages in Experimental Autoimmune Encephalomyelitis Mice Treated with Fasudil. PLoS ONE 8(2): e54841. doi:10.1371/journal.pone.0054841

80. Ozeki K, Tanida S, Morimoto C, Inoue Y, Mizoshita T, Tsukamoto H, Shimura T, Kataoka H, Kamiya T, Nishiwaki E, Ishiguro H, Higashiyama S, Joh T. Telmisartan inhibits cell proliferation by blocking nuclear translocation of ProHB-EGF C-terminal fragment in colon cancer cells. PLoS One. 2013;8(2):e56770. doi: 10.1371/journal.pone.0056770. Epub 2013 Feb 22. PubMed PMID: 23451083; PubMed Central PMCID: PMC3579940.

81. Sharpe M, Jarvis B, Goa KL. Telmisartan: a review of its use in hypertension. Drugs. 2001;61(10):1501-29. Review. PubMed PMID: 11558835.

82. Smith DH, Matzek KM, Kempthorne-Rawson J. Dose response and safety of telmisartan in patients with mild to moderate hypertension. J Clin Pharmacol.2000 Dec;40(12 Pt 1):1380-90. PubMed PMID: 11185637

82a. Stangier J, Su CA, Roth W. Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. J Int Med Res. 2000 Jul-Aug;28(4):149-67. PubMed PMID:11014323.

82b. Forclaz A, Maillard M, Nussberger J, Brunner HR, Burnier M. Angiotensin II receptor blockade: is there truly a benefit of adding an ACE inhibitor? Hypertension. 2003 Jan;41(1):31-6. PubMed PMID: 12511526.

83. Levine JE, Paczesny S, Sarantopoulos S. Clinical applications for biomarkers of acute and chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2012 Jan;18(1 Suppl):S116-24. doi: 10.1016/j.bbmt.2011.10.019. Review. PubMed PMID: 22226094; PubMed Central PMCID: PMC3282925.

84. Paczesny S. Discovery and validation of graft-versus-host disease biomarkers. Blood. 2013 Jan 24;121(4):585-94. doi: 10.1182/blood-2012-08-355990. Epub 2012 Nov 19. Review. PubMed PMID: 23165480; PubMed Central PMCID: PMC3557644.

85. Levine JE, Logan BR, Wu J, Alousi AM, Bolaños-Meade J, Ferrara JL, Ho VT, Weisdorf DJ, Paczesny S. Acute graft-versus-host disease biomarkers measured during therapy can predict treatment outcomes: a Blood and Marrow Transplant Clinical Trials Network study. Blood. 2012 Apr 19;119(16):3854-60. doi: 10.1182/blood-2012-01-403063. Epub 2012 Mar 1. PubMed PMID: 22383800; PubMed Central PMCID: PMC3335389.

86. Vander Lugt MT, Braun TM, Hanash S, Ritz J, Ho VT, Antin JH, Zhang Q, Wong CH, Wang H, Chin A, Gomez A, Harris AC, Levine JE, Choi SW, Couriel D, Reddy P, Ferrara JL, Paczesny S. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. N Engl J Med. 2013 Aug 8;369(6):529-39. doi:10.1056/NEJMoa1213299. PubMed PMID: 23924003.

87. Mueller T, Dieplinger B. The Presage(®) ST2 Assay: analytical considerations and clinical applications for a high-sensitivity assay for measurement of soluble ST2. Expert Rev Mol Diagn. 2013 Jan;13(1):13-30. doi: 10.1586/erm.12.128. Review. PubMed PMID: 23256700.

88. Ferrara JL, Harris AC, Greenson JK, Braun TM, Holler E, Teshima T, Levine JE, Choi SW, Huber E, Landfried K, Akashi K, Vander Lugt M, Reddy P, Chin A, Zhang Q, Hanash S, Paczesny S. Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease. Blood. 2011 Dec 15;118(25):6702-8. doi: 10.1182/blood-2011-08-375006. Epub 2011 Oct 6. PubMed PMID: 21979939; PubMedCentral PMCID: PMC3242723

89. Holler E, Landfried K, Meier J, Hausmann M, Rogler G. The role of bacteria and pattern recognition receptors in GVHD. Int J Inflam. 2010 Oct 31;2010:814326. doi: 10.4061/2010/814326. PubMed PMID: 21188220; PubMed Central PMCID:PMC3003997.

90. Penack O, Holler E, van den Brink MR. Graft-versus-host disease: regulation by microbe-associated molecules and innate immune receptors. Blood. 2010 Mar 11;115(10):1865-72. doi: 10.1182/blood-2009-09-242784. Epub 2009 Dec 30. Review. PubMed PMID: 20042727.

91. Eriguchi Y, Takashima S, Oka H, Shimoji S, Nakamura K, Uryu H, Shimoda S, Iwasaki H, Shimono N, Ayabe T, Akashi K, Teshima T. Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. Blood. 2012 Jul 5;120(1):223-31. doi: 10.1182/blood-2011-12-401166. Epub 2012 Apr 24. PubMed PMID: 22535662.

92. Cooke KR, Gerbitz A, Crawford JM, Teshima T, Hill GR, Tesolin A, Rossignol DP, Ferrara JL. LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia

activity after experimental bone marrow transplantation. J Clin Invest. 2001 Jun;107(12):1581-9. PubMed PMID: 11413166; PubMed Central PMCID: PMC200193.

93. Lv M, Ye HG, Zhao XS, Zhao XY, Chang YJ, Liu DH, Xu LP, Huang XJ. Ceruloplasmin is a potential biomarker for aGvHD following allogeneic hematopoietic stem cell transplantation. PLoS One. 2013;8(3):e58735. doi:10.1371/journal.pone.0058735. Epub 2013 Mar 7. PubMed PMID: 23505556; PubMed Central PMCID: PMC3591372

94. Romaschin AD, Klein DJ, Marshall JC. Bench-to-bedside review: Clinical experience with the endotoxin activity assay. Crit Care. 2012 Dec 3;16(6):248.[Epub ahead of print] PubMed PMID: 23206992; PubMed Central PMCID: PMC3672550.

95. Hilmi I, Kellum J, Planinsic R, Foster D, Abdullah A, Damian D, Gligor S, Klein D, Abu-elmagd K. Endotoxemia is common following abdominal organ transplantation and is associated with reperfusion and rejection.Journal of Organ Dysfunction. Dec 2009, Vol. 5 Issue 4, p254-260.

96. Sanada Y, Mizuta K, Urahashi T, Ihara Y, Wakiya T, Okada N, Yamada N, Ushijima K, Otomo S, Sakamoto K, Yasuda Y, Kawarasaki H. Impact of endotoxin measured by an endotoxin activity assay during liver transplantation. J Surg Res. 2013Apr;180(2):349-55. doi: 10.1016/j.jss.2012.05.004. Epub 2012 May 24. PubMed PMID:22677614.

97. Marshall JC, Foster D, Vincent JL, Cook DJ, Cohen J, Dellinger RP, Opal S, Abraham E, Brett SJ, Smith T, Mehta S, Derzko A, Romaschin A; MEDIC study. Diagnostic and prognostic implications of endotoxemia in critical illness: results of the MEDIC study. J Infect Dis. 2004 Aug 1;190(3):527-34. Epub 2004 Jul 2. PubMed PMID: 15243928

98. Ikeda T, Ikeda K, Suda S, Ueno T. Usefulness of the endotoxin activity assay as a biomarker to assess the severity of endotoxemia in critically ill patients. Innate Immun. 2014 Jan 7. [Epub ahead of print] PubMed PMID: 24398861.

99. Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA, Liu C, West ML, Singer NV, Equinda MJ, Gobourne A, Lipuma L, Young LF, Smith OM, Ghosh A, HanashAM, Goldberg JD, Aoyama K, Blazar BR, Pamer EG, van den Brink MR. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. J Exp Med. 2012 May 7;209(5):903-11. doi: 10.1084/jem.20112408. Epub 2012 Apr 30. PubMed PMID: 22547653; PubMed Central PMCID: PMC3348096.

100. Fricke WF, Maddox C, Song Y, Bromberg JS. Human microbiota characterization in the course of renal transplantation. Am J Transplant. 2014 Feb;14(2):416-27. doi: 10.1111/ajt.12588. Epub 2013 Dec 26. PubMed PMID: 24373208.

101. Arck P, Handjiski B, Hagen E, Pincus M, Bruenahl C, Bienenstock J, Paus R. Is there a 'gut-brain-skin axis'? Exp Dermatol. 2010 May;19(5):401-5. doi:10.1111/j.1600-0625.2009.01060.x. Epub 2010 Jan 25. PubMed PMID: 20113345.

102. Gomez A, Hammer S, Braun T, Hanash S, Ferrara J, Greenson J, Wang H, Zhang Q, Vander Lugt M, Pongtornpipat P, Lamiman K, Colon M, Chang L, Choi S, Levine J, Harris A, Couriel D, Reddy P, Paczesny S. A novel CD4+CD146+CCR5+ T-cell population is a biomarker of intestinal graft-versus-host disease. 39th meeting of the European Group for Blood and Marrow Transplantation Wednesday, April 10, 2013, 09:30 - 09:42

103. Cheng CI, Lin YC, Tsai TH, Lin HS, Liou CW, Chang WN, Lu CH, Yuen CM, Yip HK. The prognostic values of leukocyte Rho kinase activity in acute ischemic stroke. Biomed Res Int. 2014;2014:214587. doi: 10.1155/2014/214587. Epub 2014 Feb 27. PubMed PMID: 24716192; PubMed Central PMCID: PMC3955656.

104. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995 Jun;15(6):825-8. Review. PubMed PMID: 7581076.

105. Patel PA, Patravale VB, Commercial Telmisartan Tablets: A Comparative Evaluation with Innovator Brand Micardis, International Journal of Pharma Sciences and Research (IJPSR) 2010, 1(8): 282-292.

106. Michel MC, Bohner H, Köster J, Schäfers R, Heemann U. Safety of telmisartan in patients with arterial hypertension : an open-label observational study. Drug Saf. 2004;27(5):335-44. PubMed PMID: 15061687.

107. Forclaz, A., Maillard, M., Nussberger, J., Brunner, H. R., & Burnier, M. (2003). Angiotensin II Receptor Blockade Is There Truly a Benefit of Adding an ACE Inhibitor?. Hypertension, 41(1), 31-36.

108. Schumacher H, Mancia G. The safety profile of telmisartan as monotherapy or combined with hydrochlorothiazide: a retrospective analysis of 50 studies. Blood Press Suppl. 2008 Jun;1:32-40. PubMed PMID: 18705533.

109. Sharpe M, Jarvis B, Goa KL. Telmisartan: a review of its use in hypertension. Drugs. 2001;61(10):1501-29. Review. PubMed PMID: 11558835.

110. Bähr IN, Tretter P, Krüger J, Stark RG, Schimkus J, Unger T, Kappert K, Scholze J, Parhofer KG, Kintscher U. High-dose treatment with telmisartan induces monocytic peroxisome proliferator-activated receptor-γ target genes in patients with the metabolic syndrome. Hypertension. 2011 Oct;58(4):725-32. doi:10.1161/HYPERTENSIONAHA.111.173542. Epub 2011 Aug 29. PubMed PMID: 21876071.

111. Chetty VT , Damjanovic S, Gerstein H, Singh N, Yusuf S, Anand SS, Sharma AM. Metabolic effects of telmisartan in subjects with abdominal obesity: A prospective randomized controlled trial Blood Pressure, February 2014, Vol. 23, No. 1: Pages 54-60 (doi: 10.3109/08037051.2013.791411)

112. White, W. B. (2002), Comparative Effects of Telmisartan in the Treatment of Hypertension. The Journal of Clinical Hypertension, 4: 20–25. doi: 10.1111/j.1524-6175.2002.01585