

Senescence, Frailty, and
Mesenchymal Stem Cell
Functionality in Chronic Kidney
Disease: Effect of Senolytic
Agents

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***SENESCENCE, FRAILITY, AND MESENCHYMAL STEM CELL
FUNCTIONALITY IN CHRONIC KIDNEY DISEASE: EFFECT OF
SENOLYTIC AGENTS***

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CKD	Chronic Kidney Disease
CRF	Case Report Form
DN	Diabetic Nephropathy
DSMB	Data and Safety Monitoring Board
eGFR	Estimated glomerular filtration rate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
MSC	Mesenchymal Stem Cell
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Table 1 Study Summary

Title	Senescence, Frailty, and Mesenchymal Stem Cell Functionality in Chronic Kidney Disease: Effect of Senolytic Agents
Running Title	Senescence and Frailty in CKD
Protocol Number	15-005843
Phase	Phase II
Methodology	Single blind with blinding on the study analyses, open label, randomized study.
Overall Study Duration	18 months
Subject Participation Duration	12 months
Single or Multi-Site	Single Site Study
Objectives	To compare the effect of senolytic drugs on cellular senescence, physical ability or frailty, and adipose tissue-derived MSC functionality in patients with chronic kidney disease.
Number of Subjects	30
Diagnosis and Main Inclusion Criteria	Chronic kidney disease, diabetes mellitus
Study Product, Dose, Route, Regimen and Duration	This study will involve a single 3-day oral treatment regimen with dasatinib 100 mg daily and quercetin 1000 mg total daily
Statistical Methodology	<p>For the analyses, categorical data will be described by counts and percents and quantitative data by means and standard deviations.</p> <p>Power calculations were based on senescence by SA-β-galactoside measurement which has a mean of 5% (i.e., 5% of MSCs have the SA-β-gal marker) in healthy adult and is 3-4 fold higher in DN kidney tissue. In addition, we will explore the relationship between patient laboratory studies (primarily creatinine, proteinuria, blood glucose, hemoglobin A1c), vital measurements (e.g., BMI), frailty index score, and senescence (or MSC function) using linear regression.</p>

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

As the global epidemic of obesity and diabetes mellitus spreads, an exponential rise in incident chronic kidney disease (CKD) is predicted, leaving healthcare systems overwhelmed worldwide¹⁻³. In 2012, 29.1 million people (9.3% of the population) were living with diabetes in the U.S., with estimated costs of \$249 billion⁴. Diabetes is the most common cause of end stage renal disease (ESRD), a costly condition with high morbidity and mortality⁵. Moreover, a **frailty phenotype**, often regarded as distinct clinical syndrome of physiologic vulnerability to stress, is highly prevalent in the CKD population⁶⁻⁸. As recently highlighted in a systematic review, frailty in CKD is associated with high risk for adverse events^{6,8-11}. Hence, containing the high cost and heightened cardiovascular morbidity of the CKD population is key and novel efforts to minimize the progression to ESRD must be rigorously pursued.

CKD, particularly diabetic nephropathy (DN), is characterized by vascular damage resulting from chronic sterile inflammation, increased reactive oxygen species generation, advanced glycation end product accumulation, steatosis, insulin resistance, and altered renin-angiotensin-aldosterone system (RAAS) activation. At present, there is no effective intervention to ameliorate progression to ESRD beyond that of RAAS blockade, healthy lifestyle modifications, and optimal diabetes and blood pressure control^{2,12-20}. In the diabetic kidney, intrinsic regenerative capacity is limited and unable to prevent the development of chronic glomerulosclerosis and tubulointerstitial fibrosis. However, recent advances in regenerative medicine applying **mesenchymal stem cell (MSC) transplantation offer hope for DN** and several other kidney diseases²¹⁻²⁴. MSCs are non-embryonic stem cells with anti-fibrotic, anti-inflammatory, and pro-angiogenic paracrine activity²⁵⁻²⁷. In preclinical studies, MSC reparative properties reduce glomerulosclerosis and microalbuminuria, and improve kidney function in DN^{20,28-38}. There are no ongoing clinical trials applying MSC for DN in the U.S. However, results from clinical trials using stem cell therapy for type 1 and 2 diabetes mellitus indicate improved glycemia and safety of the approach³⁹⁻⁴².

To minimize risk of alloimmunization, autologous (rather than allogeneic) MSC transplantation is often preferred. However, despite encouraging results of preclinical trials, patient-associated risk factors appear to alter the biologic characteristics of MSC and reduce their reparative ability⁴³⁻⁴⁵. Common characteristics in DN such as older age, uremia, *frailty*, and obesity may impair functionality of MSC thereby limiting the efficacy of autologous MSC transplantation. **Diabetes is a major risk factor for frailty and impaired MSC function**, and functional impairment has previously been described in MSC isolated from diabetic patients⁴⁴⁻⁴⁹.

An important cause underpinning MSC dysfunction is a milieu of increased **cellular senescence**, an irreversible cell cycle arrest caused by potentially oncogenic, metabolic, or other insults⁵⁰⁻⁵³. Affected cells develop a senescence-associated secretory phenotype (SASP), generating increased inflammatory cytokines, extracellular matrix-modifying proteases, and reactive oxygen

species, which impair neighboring cell function and alter tissue structure⁵³⁻⁵⁶. Patients with diabetes have increased markers of senescence and inflammatory cytokines in fat and kidney tissues^{47,57-60}. Hence, increased senescent cell burden in DN may substantially compromise MSC function and become a barrier to cellular therapy. Our exciting new data reveal that **cellular senescence may be treatable with drugs, which do not require in-vitro cell manipulation**⁶¹. We recently identified and validated a new class of **senolytic** drugs that selectively eliminate senescent cells⁶². Our **preliminary data** show that in pre-diabetic animal models, which exhibit increased senescence, eradicating senescent cells by senolytic treatment improves stem cell function. In addition, clearance of the senescent cell burden in mice with doxorubicin-induced senescence was associated with *functional improvement in running endurance therein modifying the frailty profile*⁶¹. Given these findings, we aim to examine senolytic therapy as a potential in vivo preconditioning method to improve mesenchymal stem cell function (**Figure 1**), minimize the burden of cellular senescence, and optimize frailty parameters in patients with CKD.

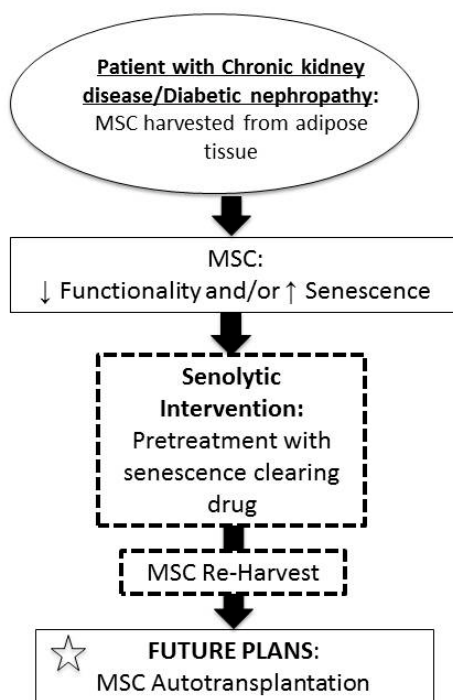


Figure 1: Rationale: Schematic illustration of a preconditioning process to optimize opportunities for future autologous stem cell transplantation with mesenchymal stem cells (MSC) from patients with chronic kidney disease.

In planning for cellular-based therapy for CKD (or DN), patients with increased senescence burden or impaired MSC function would be offered a pre-conditioning protocol with senolytic drugs to improve MSC function and the microenvironment, thereby allowing for autologous transplantation. Moreover, removal of a high senescent cell burden in the body may allow for positive changes in kidney function and bodily physical ability (reduced frailty).

The proposed studies will be the first of its kind which will compare cellular senescence, frailty, and MSC function in CKD across a range of estimated glomerular filtration rate (eGFR), and examine the effect of senolytic agents on CKD-MSC senescence and function both in vitro and in vivo. Additionally, we will examine the effect on frailty markers and cellular senescence (in the skin) before and after senolytic

therapy. **Our overall goal is to change the trajectory of kidney disease. The early step in this mission is to characterize and optimize the functional properties of MSC and their microenvironment in CKD, to allow these patients to benefit from future enrollment in clinical trials using stem cell transplantation.** In doing so, we will investigate an innovative approach for preconditioning MSC as a critical first step toward the application of cellular therapy for CKD. These studies will advance the knowledge of the effects of cellular senescence, frailty, and MSC functionality in CKD. In addition, they will help develop pre-screening protocols to optimize enrollment in trials using autologous MSC transplantation for CKD.

1.2 Investigational Agent

An investigational product is defined as a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical study, including products already with a marketing authorization but used or assembled (formulated or packaged) differently than the authorized form, or used for an unauthorized indication, or when used to gain further information about the authorized form. The investigational product(s) will be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product(s) will be dispensed only by authorized personnel according to local regulations. In this protocol, investigational products are Sprycel® (dasatinib), and quercetin.

1.2.1 Sprycel® (dasatinib)

Sprycel® (dasatinib) is an FDA approved product (NDA #021986). SPRYCEL is a kinase inhibitor indicated for the treatment of:

- newly diagnosed adults with Philadelphia chromosome-positive (Ph⁺) chronic myeloid leukemia (CML) in chronic phase. The trial is ongoing and further data will be required to determine long-term outcome.
- adults with chronic, accelerated, or myeloid or lymphoid blast phase Ph⁺ CML with resistance or intolerance to prior therapy including imatinib.
- adults with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph⁺ ALL) with resistance or intolerance to prior therapy.

Commercially available dasatinib will be purchased for the purposes of this trial. Dasatinib will be supplied as 100 mg tablet white to off-white, biconvex, oval, film-coated with “BMS 100” debossed on one side and “852” on the other side.

1.2.2 Quercetin

Quercetin is a flavonoid present in many fruits, vegetables, and grains and is also used as an ingredient in supplements, beverages, or various types of foods. Typical dietary intakes are between 5 mg and 40 mg per day, but intakes of 200-500 mg/day are possible with high consumption of fruits and vegetables, especially when the peel is consumed. Quercetin has been safely used in amounts up to 500 mg twice daily for up to 8 weeks. Up to date, no reports of significant toxicity have been reported, and weight of the current evidence from multiple in vitro studies demonstrates safety of this product as a food additive. Our anticipation of any risks of toxicity in CKD patients which will receive this drug for only three days is <1%. Quercetin phytosome dehydrate capsules equating to 1000 mg total daily dosage will be administered for 3 consecutive days orally for the CKD patients randomized to the Intervention group. This will be obtained commercially from the Thorne research company.

Quercetin will be supplied as quercetin phytosome (sophora japonica concentrate (leaf) / phosphatidylcholine complex from Sunflower) 250 mg by Thorne Research. Quercetin Phytosome is a “00” hypromellose (vegetarian cellulose) capsule filled with a pale yellow powder containing 250 mg quercetin phytosome. Microcrystalline cellulose, leucine, and silicon dioxide are added as manufacturing aids.

1.3 Preclinical Data

Insulin resistance in obese hypertensive pigs is associated with increased MSC senescence.

Similar to findings in diabetic patients^{44,45}, our preliminary data in obese hypertensive pigs, which develop insulin resistance, suggest that pre-diabetes may contribute to premature senescence of adipose-derived MSC (Figure 2).

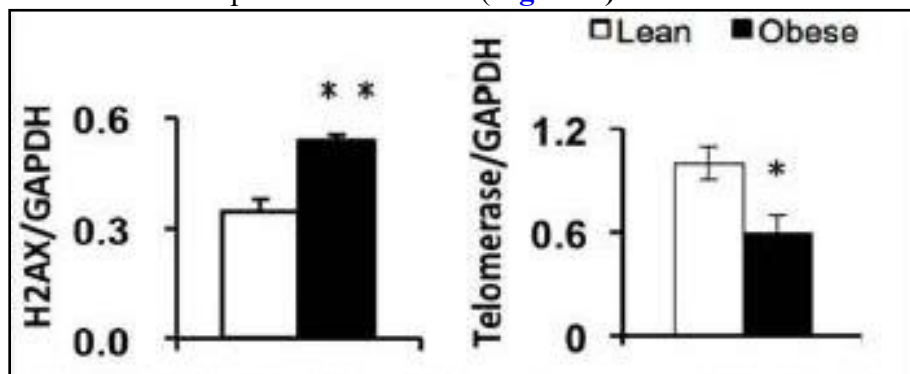


Figure 2: MSC obtained from obese pre-diabetic pigs show increased senescence, demonstrated by H2AX upregulation and decreased telomerase expression by quantitative PCR. * $P < 0.05$, ** $P < 0.01$ vs. Lean.

Relevance: These data show increased senescence in disease models of obesity and hypertension. As kidney function declines, superimposition of a ‘uremic state’ may further impair MSC function, thereby limiting the reparative capacity of MSCs and serving as a barrier to autologous transplantation in patients with advanced DN.

Senolytic drugs clear senescent cells and improve MSC function

A. Senolytic drugs clear senescent cells.

After screening 46 agents that target gene products which protect senescent cells from apoptosis *in vitro*, we recently identified 2 agents, dasatinib and quercetin, which are effective in selectively clearing senescent cells (Figure 3).

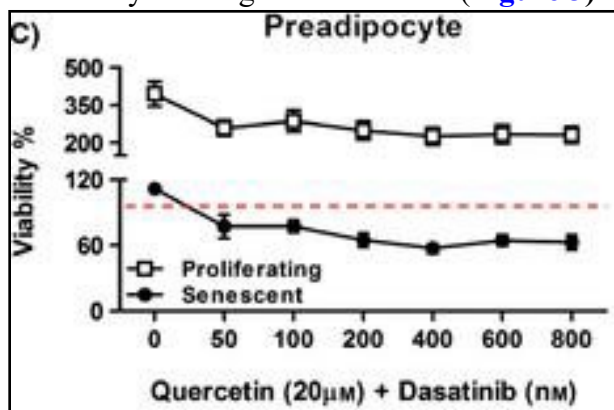


Figure 3: Newly identified senolytic drugs (dasatinib and quercetin) reduce the viability (ATPLite) of senescent

This landmark work was recently published⁶¹, and these drugs will be available for our pilot studies testing senolytic agents in DN-MSC. Further evidence is found in our preliminary *in vivo* studies of dasatinib and quercetin in skin biopsies from old, diabetic female Rhesus monkeys. Studies revealed a reduction in the expression of p16, a reliable marker of senescent cell burden (from 54.8 to 45.1 arbitrary units; $P < 0.009$; T test) as early as 5 days after 3 daily doses of dasatinib and quercetin. In murine models, this effect was sustained for several weeks.

B. MSC function improves with clearance of senescent cells.

In preliminary studies, we examined function of MSC from old mice after senolytic treatment (oral quercetin and dasatinib, 10 weeks). Gene expression (quantitative PCR) of key adipogenesis markers, including CEBP α and PPAR γ , was found to be increased in the senolytic-treated mice versus vehicle-treated mice, **Figure 4**.

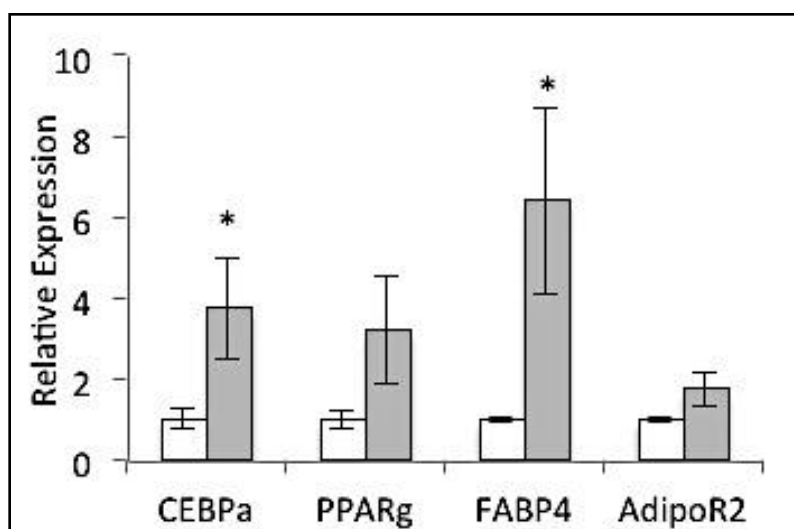


Figure 4: MSC function improves with clearance of senescent cells. MSCs were isolated from inguinal white adipose tissue of 27-month old mice that had been treated with 3 doses of senolytics (1x/month). Key markers of adipogenesis were more robustly expressed in MSC from dasatinib and quercetin (gray bars, n=3) than in vehicle (white bars, n=4) treated mice. * $P < 0.05$

Given that CEBP α and PPAR γ are both necessary for insulin sensitivity to develop in the maturation of preadipocytes⁶³, these data show that senolytic treatment improves the function of these progenitor cells by enhancing their ability to undergo transdifferentiation.

Relevance: These novel studies provide the framework for preconditioning adipose-derived MSC with senolytic drugs. Our observations that 1) senolytic drugs selectively eradicate senescent cells, 2) clearance of senescent cells is evident within 5 days of senolytic treatment and persists for several weeks in animal models, and 3) impairment in MSC function is reversible when treated with senolytics *in vitro*, all strongly support the hypotheses for the AIMS in the proposed study.

1.4 Clinical Data to Date

The senolytic drugs used in this study include dasatinib and quercetin. Dasatinib has previously been approved for the treatment of chronic myeloid leukemia (CML) by the FDA. This proposal

outlines a new indication for use and will be provided in conjunction with the dietary supplement quercetin for a 3-day period.

To our knowledge, there are no published studies utilizing dasatinib or quercetin or the combination of both drugs in the chronic kidney disease (CKD) population independent of a diagnosis of CML. Dasatinib is a tyrosine kinase inhibitor used as an oral daily treatment for CML with fewer side effects and improved survival compared to older treatment agents for this disease⁶⁴. Dasatinib is generally well-tolerated with the more common adverse effects consisting of rash, abdominal pain, diarrhea, nausea, myelosuppression, fluid retention, and headache. Fortunately, one recent publication by Yilmaz et al⁶⁵ conducted a historical cohort review of 468 newly diagnosed patients with chronic-phase chronic myeloid leukemia at MD Anderson who were treated long-term with tyrosine kinase inhibitors (imatinib, dasatinib, and nilotinib). The primary goal was **to examine the incidence of changes in kidney function over time**. Among this study cohort, 99 of 468 patients received dasatinib dosed at 100 mg daily or 50 mg twice daily for more than 3 months and were followed for a median of 39 months. Among the dasatinib treated patients, only one of 99 patients developed acute kidney injury defined as a rise in serum creatinine of 0.3 mg/dL. Among the 93 dasatinib-treated patients with normal baseline kidney function, the mean estimated glomerular filtration rate (eGFR) initially declined and then stabilized after 4 years. Among the 6 dasatinib-treated patients with CKD (eGFR <60 ml/min) at baseline, there were no significant changes in eGFR over time within this small group. The overall findings were that long-term dasatinib-treated patients with normal baseline kidney function did experience a decline in eGFR but no association was found with development of acute kidney injury. Additionally, those with CKD at baseline did not experience any further decline in eGFR (for either of the tyrosine kinase inhibitors studied).

Dasatinib has rarely been associated with acute kidney injury among patients treated for leukemia. The injury may occur in the context of tumor lysis syndrome which is of minimal to no risk for CKD patients who lack the underlying hematological disease process. Among patients treated for leukemia, available case reports also describe a variety of kidney lesions including: acute tubular necrosis from adverse effects such as gastroenteritis and nephrotic syndrome from thrombotic microangiopathy occurring in patients receiving dasatinib therapy⁶⁶⁻⁶⁸. These events generally resolve with interruption of drug therapy and conservative management. Based on a case report and review by Wallace et al in the American Journal of Kidney Disease⁶⁸ a possible mechanism for dasatinib-induced kidney injury is through inhibition of the vascular endothelial growth factor (VEGF) signaling pathway, a specific tyrosine kinase signaling pathway. However, there are no package insert recommended dosing adjustments for either kidney disease or reductions in kidney function for patients receiving dasatinib therapy.

Quercetin is a naturally-occurring flavonoid known to inhibit PI3Kinase, other kinases, and mTOR pathways. Quercetin is present in many fruits, vegetables, and grains and is also used as an ingredient in supplements, beverages, or various types of foods. Quercetin is a supplement and not FDA approved for any indication. The primary contraindication/warning for this drug is a hypersensitivity to Quercetin. Adverse effects associated with quercetin include emesis, dyspnea, and nephrotoxicity or kidney injury. Kidney injury from this drug has not been substantiated in recent reports and overall appears safe in clinical application⁶⁹, and several studies involve use of quercetin for its anti-oxidative and anti-apoptotic effects in animal models of kidney disease, including diabetic nephropathy (DN). In fact, there are numerous preclinical studies in acute kidney injury or DN suggesting that quercetin may reduce oxidative stress and

1.5 Dose Rationale and Risk/Benefits

Drugs that eliminate senescent cells are known as “senolytic(s)”. We discovered senolytics that selectively eliminate senescent cells including tyrosine kinase inhibitors (TKI). Dasatinib is a TKI that is currently used for certain cancer treatments and has been approved for treatment of chronic myeloid leukemia (CML). Dasatinib initially induces senescence followed by necrosis in cancer cells. In a recent study evaluating the effect of dasatinib against thyroid cancer cell lines in vitro and a xenograft model in vivo, dasatinib-treated cells (BHP2-7 and Cal62) exhibited a characteristic clumped appearance on biopsy and most of these cells were noted to be senescent using a SA- β -Galactosidase “SABG” assay. In this study, the average number of senescent cells per high powered field (hpf) were: BHP2-7, control 0/hpf; BHP2-7, dasatinib 20/hpf; Cal62, control 0/hpf; Cal62, dasatinib 25/hpf. When these senescent cells were followed, they were noted to become necrotic after 10 days of dasatinib treatment.

Senescent cell eradication (or reduction) by activating a drug-inducible “suicide” gene can delay multiple age-related phenotypes in genetically-modified progeroid mice. We screened 46 agents/drugs that could theoretically induce apoptosis preferentially of senescent cells in vitro. Then we evaluated these agents and different combinations to determine those agents that are most effective at eliminating senescent cells⁶¹. Dasatinib preferentially reduced viability and caused cell death in senescent human preadipocytes (also known as mesenchymal stem cells or MSCs), but was much less effective on senescent human umbilical vein cells (HUVEC) (**Figure 5**). After 3 days of exposure, proliferating preadipocytes increased by 2-5 fold in number compared to day 0 in the presence of dasatinib. The viability of non-dividing senescent preadipocytes from the same subjects decreased by 30-40% in the presence of dasatinib, demonstrating selective reduction.

The second agent that we discovered as a promising senolytic drug was quercetin, a naturally occurring flavonoid known to inhibit PI3Kinase, other kinases, and mTOR pathways. In contrast to dasatinib, at low concentrations quercetin reduced the viability and caused cell death of senescent HUVECs to a greater extent than proliferating cells, but was less effective on preadipocytes (**Figure 6**). The combination of dasatinib and quercetin [D+Q] afforded selective killing of both senescent preadipocytes and endothelial cells (**Figure 7** and **Figure 8**). By day 3, the viability of non-dividing senescent preadipocytes exposed to both these agents was reduced by ~70% compared to day 0, while non-senescent, proliferating cells had increased by 2-4 fold, suggesting that the combination of dasatinib and quercetin selectively targets a broader range of senescent cell types than either agent alone, hence forming the basis of our hypothesis for the proposed study. This combination of senolytics (D+Q) was shown not only to be helpful in decreasing senescent cell burden, but we also observed functional improvement in running endurance in mice with doxorubicin-induced senescence (**Figure 9**). We also found that D+Q alleviated **age-related cardiac and vascular dysfunction, improved gait in mice disabled by exposing a leg to ionizing radiation, and delayed frailty, neurological dysfunction, and osteoporosis** in mice with an accelerated aging syndrome⁶¹.

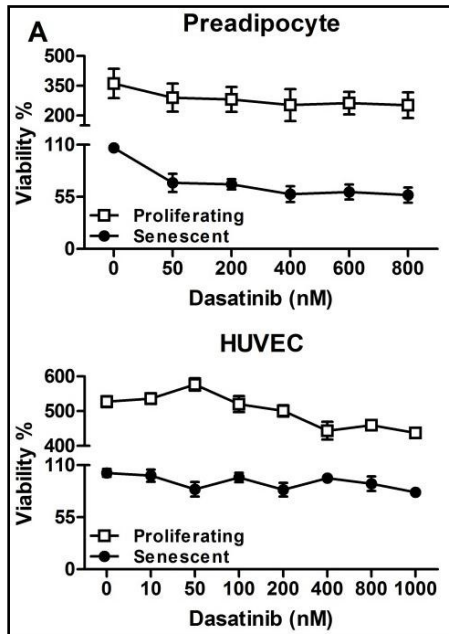


Figure 5: Effect of dasatinib on preadipocytes (also known as mesenchymal stem cells) and HUVEC

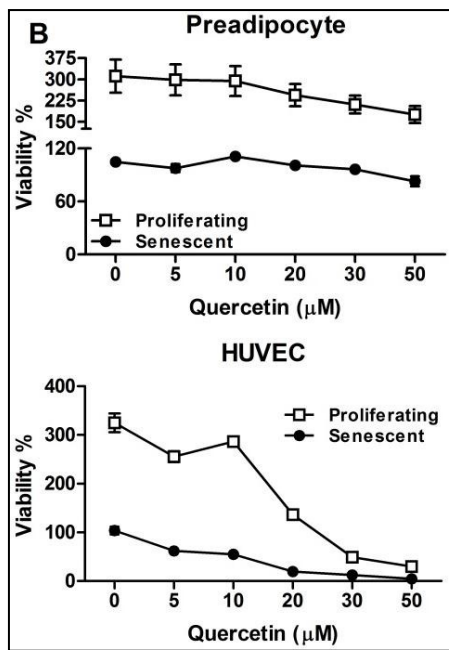


Figure 6: Effect of quercetin on preadipocytes (also known as mesenchymal stem cells) and HUVEC

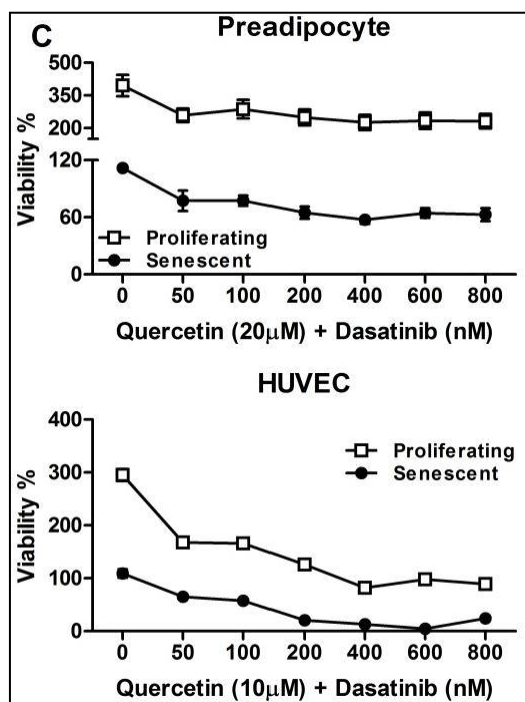


Figure 7: Effect of quercetin on preadipocytes (also known as mesenchymal stem cells) and HUVEC.

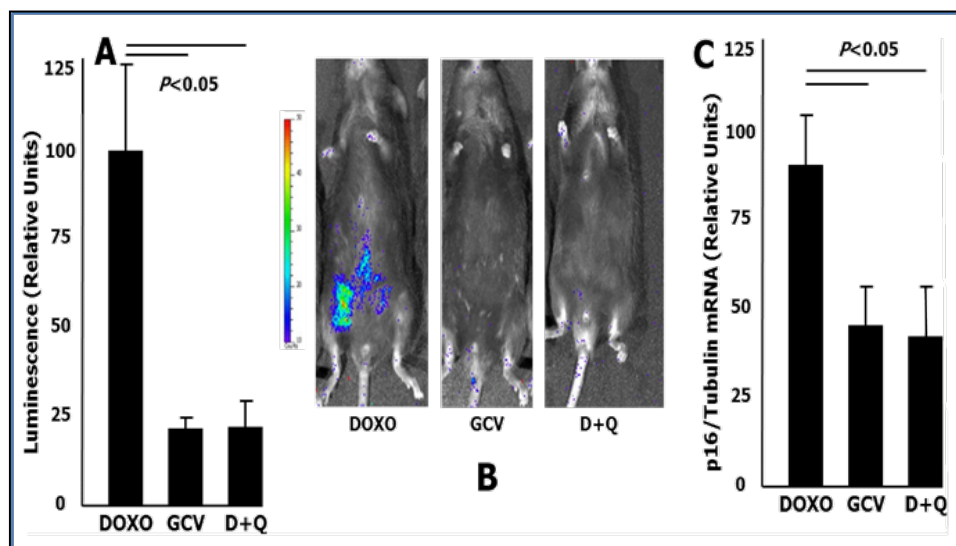


Figure 8: Combination of dasatinib with quercetin (D+Q) removes senescent cells induced by chemotherapy.

Senescence was induced by doxorubicin 10mg/kg in 5 month old p16-3MR mice. After 10 days, mice were given vehicle (DOXO), ganciclovir (GCV), which activates the thymidylate kinase “suicide” gene in p16-expressing senescent cells, or our pilot senolytic compounds (D+Q). After another 5 days, animals were imaged for luminescence (B; quantified in A; N=9 mice/ group). C: Skin biopsies were collected pre- and post-drug treatment (control, GCV, or D+Q). p16 mRNA values (RT-PCR) are % p16 expression post-treatment vs. pre-treatment (each animal is normalized to itself; N=4/group).

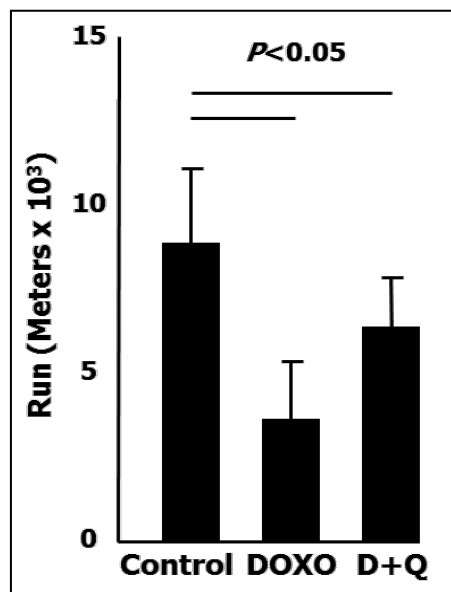


Figure 9: Senescent cell clearance ameliorates impairment of running endurance due to chemotherapy.

Doxorubicin induces widespread cellular senescence. Treadmill running endurance was tested in 5 month old p16-3MR mice that had been treated 15 days before with vehicle (Control) or doxorubicin to induce senescence. Ten days later, the mice treated with doxorubicin were given either vehicle (DOXO) or D+Q. Five days after that, treadmill testing was done. Despite only a single treatment with D+Q 5 days before, exercise endurance improved (D&Q half-life is <12 hrs; N=4; T tests).

Justification of the selection of an intervention's dose, frequency and administration:

Two drugs, dasatinib and quercetin, will be utilized as “senolytic agents” in the intervention arm of the study:

To our knowledge, dasatinib has not been previously used in the management of chronic kidney disease (CKD), alteration of frailty markers, or for preconditioning of mesenchymal stem cells (MSC). Dasatinib has been used clinically for the treatment of cancers. Based on our preclinical experience with 46 agents/drugs examined, the combination of dasatinib and quercetin induces apoptosis of senescent cells in vitro with minimal effect on non-senescent or healthy cells⁶¹. Removal of these senescent cells has broad potential implications including optimization of impaired MSC function, improvement in the frailty phenotype parameters, and improvement in kidney function in patients with CKD.

Particularly, in this study we will examine the effects of senolytics which have immense potential as a **preconditioning treatment for MSC**. This would serve as a critical first step toward the application of cellular therapy for CKD. As an off-target effect, removal of highly abundant senescent cells in the CKD kidney, may allow for functional improvement of neighboring tubule cells and structures therein allowing for enhancement in kidney function. This finding alone may provide an un-tapped opportunity to alter the trajectory of kidney failure. Frailty, generally described as a distinct biological syndrome of decreased reserve and resistance to stressors (illness, surgery) which results from cumulative declines across multiple physiologic systems, is associated with increased risk for mortality, morbidity, disability, and health care

utilization. This phenotype is common in the CKD population, particularly patients with diabetes; therefore an improvement in frailty phenotype may lead to improved survival, quality of life, and healthcare expenditures.

Acknowledging the potential benefits gained from using dasatinib and quercetin, the **risks** of use of these drugs in combination for 3 days are likely to be minimal in relation to the anticipated benefits and the knowledge that may be gained from these clinical investigations.

2 Study Objectives

Our preliminary data reveal increased MSC senescence in pre-diabetic animal models. Importantly, we recently discovered and successfully demonstrated the utility of *senolytic drugs* that selectively eliminate senescent cells in other disease models, and demonstrated in animal models that eradicating senescent cells improves stem cell function. Our **hypothesis** underlying the proposed studies is that **adipose tissue-derived MSC obtained from patients with DN show increased senescence and decreased functionality, which can be ameliorated, both in vitro and in vivo, using drugs that clear senescent cells.** This hypothesis will be pursued in 3 primary specific aims:

Primary Aims:

Aim 1: To compare cellular senescence and functionality in adipose tissue-derived MSC from patients with DN (diabetic nephropathy) [estimated glomerular filtration rate (eGFR) 15-60 mL/min/1.73m²] to MSC from age- and gender- matched controls. MSC senescence will be assessed by specific markers of senescence. MSC functionality will be assessed by (a) multilineage differentiation potential, (b) proliferation, migration, and tube-formation capacity, and (c) paracrine anti-inflammatory and pro-angiogenic activities.

- Hypothesis 1a: MSC senescence and dysfunction are increased in DN compared to healthy controls.
- Hypothesis 1b: The proportion of senescent cells in patients with DN correlates with impairments in MSC function.

Aim 2: To determine the reversibility of DN-MSC dysfunction utilizing incubation with senolytic agents in vitro.

- Hypothesis 2: Incubation of MSC obtained from DN patients with senolytic agents will decrease the fraction of senescent MSC and improve their function relative to MSC from healthy controls.

Aim 3: To examine the effect of senolytic agents on MSC function in a pilot study of patients with DN (eGFR 15-60 mL/min/1.73m²). MSC senescence and function will be measured at baseline and day 14 in both DN patients treated or untreated with senolytic agents.

- Hypothesis 3: DN patients treated with senolytic agents will have a decrease in the fraction of senescent MSC and alleviation of MSC dysfunction.

Secondary Aims:

Aim 4: To examine the effect of senolytic agents on markers of frailty (frailty phenotype) in a pilot study of study of patients with DN (eGFR 15-60 mL/min/1.73m²). Frailty index will be determined at baseline, day 14, and month 4 in DN patients treated or untreated with senolytic agents.

- Hypothesis 4: DN patients treated with senolytic agents will have an improvement in the frailty phenotype at 14 days and 4 months compared to baseline examination.

Aim 5: To examine the effect of senolytic agents on eGFR in a pilot study of study of patients with DN (eGFR 15-60 mL/min/1.73m²). eGFR will be measured at baseline, day 14, month 4 and 12 in DN patients treated or untreated with senolytic agents.

- Hypothesis 5: DN patients treated with senolytic agents will have an improvement in the eGFR at 14 days and 4 months compared to baseline examination.

Aim 6: To assess the safety and tolerability of a single 3-day treatment regimen with dasatinib 100 mg daily and quercetin 1000 mg total daily in subjects with DN-CKD.

Aim 7: To examine the molecular characteristics of MSC (or DN-MS) before and after senolytic therapy by using high throughput RNA sequencing and semi-automated real time quantitative polymerase chain reaction (qPCR) platform with gene biomarkers for proliferation, survival, quiescence, senescence, differentiation, metabolic activity.

These novel studies will advance the knowledge of the effects of cellular senescence on MSC functionality and physical ability or frailty, and help develop pre-selection protocols to optimize enrollment in trials using autologous MSC transplantation for DN and CKD. Furthermore, the proposed studies explore an innovative approach for preconditioning MSC and aid in developing a completely novel therapeutic strategy to delay the progression of CKD.

MSC from patients enrolled in AIM 3 (Senolytic study) will be sent to Dr. Andre van Wijnen's lab for RNA sequencing. Samples will be studied before and after senolytic intervention.

The molecular characteristics of MSCs will be examined using high throughput RNA sequencing and semi-automated real time quantitative polymerase chain reaction (qPCR) platform with gene biomarkers for proliferation, survival, quiescence, senescence, differentiation, metabolic activity (1. before & after senolytics drug therapy and 2. by GFR groupings).

Primary Objectives

To assess the efficacy of a single 3-day treatment regimen with dasatinib and quercetin (senolytic drugs) on clearing senescent adipose-derived MSC in patients with CKD.

To assess the efficacy of a single 3-day treatment regimen with dasatinib and quercetin (senolytic drugs) on improving adipose-derived MSC functionality in patients with CKD.

Secondary Objective

To assess the short-term effect of a single 3-day treatment regimen with dasatinib and quercetin (senolytic drugs) on frailty index score in patients with CKD.

To assess the short-term effect of a single 3-day treatment regimen with dasatinib and quercetin (senolytic drugs) on kidney function in patients with CKD.

To assess the safety and tolerability of a single 3-day treatment regimen with dasatinib 100 mg daily and quercetin 1000 mg total daily in subjects with CKD.

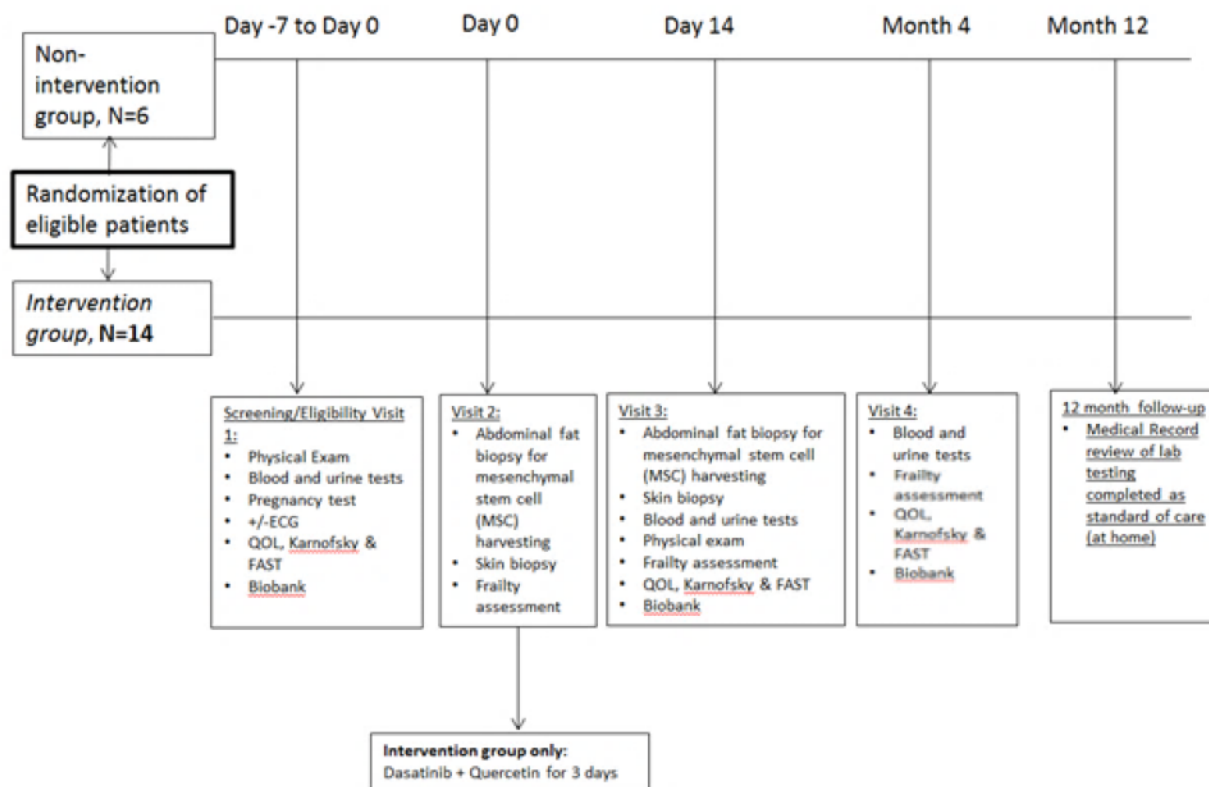
3 Study Design

3.1 General Design

Subjects will be screened at outpatient visits in the nephrology, hypertension, diabetes, family medicine and internal medicine clinic visit appointments at Mayo Clinic. To increase community engagement and minority enrollment, we will expand recruitment into the region by providing Mayo-approved recruitment materials in the community (library, churches, public areas, community-sponsored health seminars). Other forms of advertisement may include: radio and/or newspaper advertisements, website postings, podcast interviews of the principal investigator, etc. Interested, qualified subjects will be consented and offered participation in this trial. In this initial proof of concept study, we will evaluate 30 subjects (20 Intervention and 10 Non-Intervention). Once consent has been obtained baseline values will be established and subjects in the intervention group will receive a single 3-day course of dasatinib plus quercetin (senolytic agents). The non-intervention group will be observed for the study duration. Subjects will be followed for a total of 12 months. At enrollment/screening baseline laboratory tests will include measurements of kidney function, diabetes and cholesterol, inflammatory markers. Following randomization, patients will undergo an abdominal fat biopsy for harvesting of mesenchymal stem cells and a 6-mm punch skin biopsy for assessment of senescence markers at time zero and day 14. Blood and urine tests will be obtained at day -7 to day 0, day 14, month 4 and month 12 (by medical record review). Comprehensive examinations performed at the day 0 visit will also consist of screening for lifestyle factors (smoking, alcohol, obesity, physical activity), and cardiovascular risk assessment. Frailty phenotyping through creation of a frailty index score will also be conducted at visits (day 0, day 14, month 4). If screening/eligibility tests were performed clinically up to 7 days prior to enrollment visit (Day 0), then subjects will undergo modified testing to include only those tests not previously completed.

Subjects will be screened at outpatient visits in the nephrology, hypertension, diabetes, family medicine and internal medicine clinic appointments at Mayo Clinic. To increase community engagement and minority enrollment, we will expand recruitment into the region by providing Mayo-approved recruitment materials in the community (library, churches, public areas, community-sponsored health seminars). Other forms of advertisement may include: radio and/or newspaper advertisements, website postings, podcast interviews of the principal investigator, etc. Due to lack of access to electronic medical records in the non-Mayo Clinic patients, an additional telephone or in-person screening interview may be necessary to confirm eligibility prior to the formal study screening/enrollment date. Interested and qualified subjects will be consented and offered participation in this trial. Once consent has been obtained baseline values will be established and subjects in the intervention group will begin a single 3- day treatment with the senolytics, dasatinib and quercetin.

Figure 10: Diagram of Study Events



*The 12 month visit will be completed via medical record review. Subjects will be asked to provide a release of information so that we are only to request a copy their laboratory data.

*If screening/eligibility tests were performed clinically up to 7 days prior to enrollment visit (Day 0), then subjects will undergo modified testing to include only those tests not previously completed.

3.2 Primary Study Endpoints

The primary endpoints include:

- Proportion of senescent mesenchymal stem cells (and senescent skin cells) present at day 14 compared to baseline (day 0).
- Mesenchymal stem cell functionality (proliferation, migration, tube formation), paracrine activities (secretion of angiogenic and growth factors), and anti-inflammatory activities at day 14 compared to day 0.

3.3 Secondary Study Endpoints

The secondary endpoints include:

- Change in estimated glomerular filtration rate from baseline to period of short-term (0-4 months) and long-term (12 months) follow up.
- Change in frailty index score from baseline to day 14.
- Safety of combination therapy using dasatinib and quercetin.

3.4 Primary Safety Endpoints

Safety assessments are undertaken with the measurement of safety laboratory tests and

procedures, vital signs and recording of adverse events

4 Subject Selection Enrollment and Withdrawal

Informed Consent:

Trained clinical study coordinators will contact subjects, assist in recruitment and carry-through of the protocols. Description of the studies and review of the informed consent forms with each patient will be conducted in person directly with the clinical coordinators and/or the responsible principle and co-investigators involved in the study. Written informed consent will be obtained with details of the procedures to be followed, the number of subjects to be included, identification of the risks and benefits, and alternative procedures. Subjects will be required to give appropriate consent or have an appropriate representative available to do so.

Subject selection:

Diabetic nephropathy (DN) will be defined clinically as CKD (estimated glomerular filtration rate; eGFR 15-60 mL/min/1.73m²) in the setting of diabetes without other overt etiologies of CKD beyond concomitant hypertension.

4.1 Inclusion Criteria

1. Age 40-80 years
2. Chronic kidney disease estimated glomerular filtration rate (eGFR) 15-45 ml/min/1.73m²
3. Diabetes mellitus and taking diabetes medications

4.2 Exclusion Criteria

1. Concomitant glomerulonephritis,
2. Nephrotic syndrome,
3. Solid organ transplantation,
4. Autosomal dominant or recessive polycystic kidney disease,
5. Known renovascular disease,
6. Pregnancy,
7. Active immunosuppression therapy,
8. Hemoglobin A1c $\geq 10\%$ at screening,
9. History of active substance abuse (including alcohol) within the past 2 years,
10. Current alcohol abuse (>3 alcoholic beverages/day or >21 per week),
11. Body weight >150 kg or body mass index (BMI) >50
12. Human immunodeficiency virus infection
13. Active hepatitis B or C infection
14. Tyrosine kinase inhibitor therapy
15. Known hypersensitivity or allergy to dasatinib or quercetin
16. Inability to give informed consent
17. Uncontrolled systemic lupus erythematosus
18. Uncontrolled pleural/pericardial effusions or ascites
19. New invasive cancer except non-melanoma skin cancers
20. Invasive fungal or viral infection

21. Inability to tolerate oral medications
22. Total bilirubin > 2x upper limit of normal
23. Subjects taking medications that are sensitive to substrates or substrates with a narrow therapeutic range for CYP3A4, CYP2C8, CYP2C9, or CYP2D6 or strong inhibitors or inducers of CYP3A4 (e.g. cyclosporine, tacrolimus or sirolimus). If antifungals are absolutely necessary from an infectious disease perspective, then they will be allowed only if the levels are therapeutic.
24. Strong inhibitors of CYP3A4. See [Appendices 1-3](#).
25. Subjects on therapeutic doses of anticoagulants (Warfarin (Coumadin); Rivaroxaban (Xarelto); Apixaban (Eliquis); Dabigatran (Pradaxa, Prazaxa) or Other).
26. Subjects on antiplatelet agents ((Clopidogrel (Plavix); Dipyridamole + Aspirin (Aggrenox); Ticagrelor (Brilinta); Prasugrel (Effient); Ticlopidine (Ticlid) or Other) who are unable or unwilling to reduce or hold therapy prior to and during the 2-day drug dosing. Subjects may continue their previous regimen on day 3.
27. Subjects on quinolone antibiotic therapy for treatment or for prevention of infections within 10 days.
28. Subjects taking H2-antagonists or proton pump inhibitors and *unwilling* to discontinue therapy for 1 week before and 2 weeks following enrollment.
29. QTc > 450 msec
30. Presence of any condition that the Investigator believes would put the subject at risk or would preclude the patient from successfully completing all aspects of the trial.

*Active immunosuppression therapy may include common systemic drugs such as tacrolimus, sirolimus, cyclosporin, rituximab (or other monoclonal antibodies), mycophenolate mofetil. Most potential subjects on these medication therapies will be identified through the exclusion criteria outlined above.

Involvement of special vulnerable populations:

We will not involve special vulnerable populations, such as fetuses, neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered vulnerable populations.

4.3 Subject Recruitment, Enrollment and Screening

An accrual feasibility study was performed for patients residing within 150 miles of Rochester, MN that were seen at Mayo Clinic within the last 24 months and matched study inclusion criteria. We identified 1900 chronic kidney disease (CKD) patients among which 750 patients had diabetic nephropathy (DN). With a 20% accrual rate expected for a study that involves fat and skin biopsies, we would easily be able to recruit the target patients for the senolytic study over the course of the award period. Recruitment sources include: 1) the CKD clinic in which the Principal Investigator, Dr. Hickson serves as Co-Director (minimum of 250 CKD Clinic patients per quarter), 2) Nephrology clinics, and 3) Endocrine and general/family medicine clinics at Mayo Clinic in Rochester. To increase community engagement and minority enrollment, we will expand recruitment into the region by providing Mayo-approved recruitment materials in the community (library, churches, public areas, community-sponsored health seminars). Other forms of advertisement may include: radio and/or newspaper advertisements, website postings, podcast interviews of the principal investigator, etc. Due to lack of access to electronic medical records

in the non-Mayo Clinic patients, an additional telephone or in-person screening interview may be necessary to confirm eligibility prior to the formal study screening/enrollment date. Further support will come from our institution's well-established electronic and Survey Research Center clinical trial recruitment resources.

Troubleshooting for unmet targets for minority recruitment:

After 6-9 months of recruitment efforts we are unsuccessful in recruiting African Americans (goal 30% of subjects), then we will capitalize on our ongoing collaborative relationship with Mayo Clinic Jacksonville in Jacksonville, Florida. Duval county, Florida has a more diverse representation of minorities with African Americans comprising 30.3% of the population in 2016. In addition, we continue to partner with the University of Minnesota/Hennepin County in Minneapolis, Minnesota and Emory University in Atlanta, Georgia for successful, targeted recruitment of African American subjects. As needed, we will adapt study protocols and obtain IRB approval accordingly. Minority subjects recruited from off-site sites will be reimbursed for airfare, hotel accommodations, and food up to an allowable maximum amount.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

All subjects will be assessed during the 3 days of medication administration, day 14 visit, and subsequent study visits. If a severe adverse event (SAE) occurs at any time during administration of the 3-day drug regimen, a formal review will occur and subsequent patients will be enrolled one at a time using the same regimen. If three or more events accrue, the pilot study will be held and either a potential dosing regimen revisited or discontinuation of the study protocol will occur. Other interventions will be as per the direction of the Food and Drug Administration and Mayo Institutional Review Board.

Study Completion:

For each subject in the study, the end of study will be reached when treatment and post-treatment safety follow-up periods have been completed.

Subject withdrawal:

A subject may be withdrawn from the study prior to that subject completing all of the study related procedures. Some reasons may include:

- Subject safety issues
- Failure of subject to adhere to protocol requirements
- Disease progression
- Subject decision to withdraw from the study (withdrawal of consent)

Withdrawn subjects may not reenter the study.

Premature withdrawal from study:

Subjects may voluntarily withdraw from the study for any reason at any time. Subjects are considered withdrawn if they state an intention to withdraw further participation in all components of the study, die or are lost to follow-up for any other reason. The investigator may withdraw a subject from the study (without regard to the subject's consent) if they believe that continued participation in the study would be contrary to the best interests of the patient.

Subjects are considered as lost to follow-up if all reasonable attempts by the investigator to communicate with the individual fail. The investigator will take preventive measures to avoid a subject being lost to follow-up (e.g., document different ways of contact such as telephone number, home address, e-mail address, person to be contacted in case the subject cannot be reached). If the subject cannot be reached, the investigator will make a reasonable effort to contact the subject, document all attempts and enter the loss of follow-up information into the Case Report Form (CRF). The following methods will be used: at least two telephone calls will be placed to the last available telephone number (each call on different days) and one registered letter will be sent by post to the last available home address. If the subject is still unreachable after all contact attempts listed above, he/she will be considered to be lost to follow-up.

If premature withdrawal occurs for any reason, the reason for premature withdrawal from the study, along with who made the decision (subject, investigator) will be recorded in the CRF.

Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study subject and then complete the Study Adverse Event Worksheet and log. The investigator will evaluate the event and determine the necessary follow-up and reporting required.

Subject replacement:

Subjects withdrawn from the study will be replaced by newly recruited subjects meeting inclusion criteria matching similar baseline characteristics (age, gender, race and kidney function).

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

- For withdrawn subjects not undergoing any study intervention, no additional follow-up will be done
- For withdrawn subjects only undergoing fat biopsy: For safety monitoring telephone contact will be attempted up to 14 days beyond biopsy procedure.
- For withdrawn subjects receiving dasatinib and quercetin (Intervention). For safety monitoring telephone contact will be attempted and subjects will be encouraged to return to complete the laboratory evaluations at 14 day and 4 month visit. No follow up phone call needed beyond 4 months. It will be highly recommended that withdrawn subjects return for clinical blood (CBC, creatinine) and urine (urinalysis) studies as part of safety monitoring. Research data will not be collected on subjects after they are withdrawn from the study, any additional evaluation will be for subject safety only.

5 Study Drugs

5.1 Description

Commercially available dasatinib will be purchased for the purposes of this trial. Dasatinib will be supplied as 100 mg tablet white to off-white, biconvex, oval, film-coated with “BMS 100” debossed on one side and “852” on the other side.

Quercetin will be supplied as quercetin phytosome (sophora japonica concentrate (leaf) / phosphatidylcholine complex from Sunflower) 250 mg by Thorne Research. This drug product

will be dispensed through the research pharmacy in child resistant containers. Quercetin Phytosome is a “00” hypromellose (vegetarian cellulose) capsule filled with a pale yellow powder containing 250 mg quercetin phytosome. Microcrystalline cellulose, leucine, and silicon dioxide are added as manufacturing aids.

5.2 Treatment Regimen

- 1) Dasatinib is a tyrosine kinase inhibitor used to treat cancer, particularly Philadelphia chromosome-positive (Ph⁺) chronic myeloid leukemia (CML) or Ph⁺ acute lymphoblastic leukemia (ALL).
 - a. Dose: Dasatinib in the form of Sprycel (Bristol Myers Squibb) 100 mg orally daily for 3 consecutive days. One (100 mg) tablet will be taken in the morning for each of the 3 days.
- 2) Quercetin is a naturally-occurring flavonoid known to inhibit PI3Kinase, other kinases, and mTOR pathways. Quercetin is present in many fruits, vegetables, and grains and is also used as an ingredient in supplements, beverages, or various types of foods.
 - a. Dose: Quercetin dehydrate capsules equating to 1000 mg total daily dosage will be administered orally for 3 consecutive days. Two (250 mg) capsules will be taken each morning and two (250 mg) capsules will be taken in the evening for each of the 3 days.

This protocol is based on current package inserts and recommended dosing regimens, in addition to preliminary preclinical studies and recent investigations published in *Aging Cell*⁶¹. There are no recommended dosing adjustments for kidney dysfunction. There are no specific contraindications to using these drugs.

Dasatinib:

Dasatinib is currently FDA approved. Precautions include: cardiac adverse events, avoidance of use of H₂ blockers and proton pump inhibitors (affects dasatinib absorption), fluid retention, hemorrhage, myelosuppression, dermatologic reactions, and avoidance in pregnancy/breast feeding. The drug interferes with cytochrome P450 and may require drug adjustments to agents such as calcineurin inhibitors ([See Appendices](#)). Unlike standard treatment regimens for CML (lasting several months), this medication will only be administered for 3 days. Elimination half-life is 3-5 hours in adults. Similarly, in our animal models, drug clearance was observed within 72 hours. Yet, based on animal studies, senescent cell clearance persists for approximately 2-4 weeks.

Quercetin:

Quercetin is a supplement and not FDA approved for any indication. The recommended dose ranges from a total of 750-1500 mg per day. The primary contraindication/warning is hypersensitivity to Quercetin. Listed drug interactions include: cyclosporine, digoxin, and fluoroquinolones. Adverse effects include emesis, dyspnea, and nephrotoxicity. The nephrotoxicity has not been substantiated in recent reports, and several studies^{72-80,83,84} involve use of quercetin for its anti-oxidative and anti-apoptotic effects in kidney disease models, including DN.

5.3 Method for Assigning Subjects to Treatment Groups

Subject randomization will be by chance in a ratio of 14 subjects to the intervention arm and 6 subjects in the non-intervention arm.

5.4 Subject Compliance Monitoring

Patient adherence to study treatment will be monitored by drug accountability (pill counts). The study coordinator will do pill counts.

5.5 Prior and Concomitant Therapy

For the intervention group, drugs listed as part of the exclusion criteria are not permitted during the 3-day course of treatment with dasatinib and quercetin. If patients are required to initiate these medications within the 3-day period then they will be removed from the study primarily due to risk of drug-drug interaction.

5.6 Masking/Blinding of Study

In order to minimize the study bias, the laboratories analyzing the collected samples and initial data will not have access as to which group the samples came from.

Since this is an open label study the investigator and subject will be aware of which group they are in.

5.7 Receiving, Packaging, Storage, Dispensing and Return

5.7.1 Receipt of Drug Supplies

The investigational products for this study will be delivered to and managed by the Research Pharmacy according to their established standard procedures.

5.7.2 Packaging

Subjects randomized to the intervention arm will be provided with one bottle containing 3 tablets of dasatinib, and one bottle containing 12 capsules of quercetin. The bottles will be prepared and dispensed by the Research Pharmacy with appropriate labeling to include a statement that these products are for investigational use only.

5.7.3 Storage

Investigational products should be stored at room temperature 68° to 77°F (20° to 25°C).

5.7.4 Dispensing of Study Drug

The study drug is to be used exclusively in the clinical study according to the instructions of this protocol and directions for use. The Investigator's designee is responsible for providing subjects with the study drug and instructions for dosing and proper storage of the study drug. Subjects will be instructed to return unused product to the research center.

The Investigator's designee will record the amount of study drug dispensed, date of dispensing as well as the amount of drug returned and drug remaining.

5.7.5 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be

documented in the study files.

6 Study Procedures

6.1 Visit 1 – Screening and Eligibility

Patients meeting the entry criteria and consenting to participate in the protocol will undergo a complete physical exam and the following laboratory tests:

- Eligibility confirmation and informed consent discussion and documentation
- Comprehensive Exam
- Pregnancy test
 - Point of Care test for all premenopausal women
- Blood Tests
- Spot Urine Tests
- 24 hour Urine
- Electrocardiogram (EKG)
- Karnovsky and Functional Assessment Staging (FAST) score
- SF 36 Health Survey
- Plasma – urine – Cell Biobank for future research

If screening/eligibility tests were performed clinically up to 7 days prior to enrollment visit (Day 0), then subjects will undergo modified testing to include only those tests not previously completed.

6.2 Visit 2 – Enrollment Time Zero

This visit will be recorded as “Day 0” and will require a visit to the research center where the following tests will be performed:

- Fat Biopsy
- Skin Biopsy
- Frailty assessment
- Interval Assessment
- Dispensing of 3 day supply of investigational product to the Intervention group only.

6.3 Study Days 1, 2 and 3 – Investigational Product Administration

The Intervention group will be provided with dasatinib + quercetin on day 1 (or before). Dasatinib and quercetin will be taken for 3 consecutive days only. If the dasatinib or quercetin drugs are started in the evening, then they will be required to complete the 72 hour course (unless AEs occur, see stopping rules), thus the subjects may need to take drug(s) the fourth day as well. Subjects will be asked to record the time/date each dose is taken in a drug diary form and will be instructed to return unused product and the diary to the research center.

6.4 Visit 3 – Study Day 14 (+/- 3 days)

This visit will be recorded as “day 14”. A summary of the events during this day are below:

- Interval Assessment
- Fat Biopsy
- Skin Biopsy
- Frailty assessment
- Blood Tests

- Spot Urine Tests
- 24 hour Urine
- Karnovsky and Functional Assessment Staging (FAST) score
- SF 36 Health Survey
- Plasma – Urine - Cell Biobank for future research

For this visit, a ± 3 day window will be allowed.

6.5 Visit 4 – Follow-up Visit at 4 months (+/-2 weeks)

This visit will be recorded as “4-month” visit. During this visit, the following tests would occur:

- Interval Assessment
 - Frailty assessment
 - Blood Tests
 - Spot Urine Tests
 - 24 hour Urine
 - Karnovsky and Functional Assessment Staging (FAST) score
 - SF 36 Health Survey
- Plasma – Urine – Cell Biobank for future research
- For this visit, a ± 2 week window will be allowed.

6.6 Follow-up at 12 months (+/-2 months)

This visit will be completed via medical record view. Subjects will be asked to provide a release of information so that we are able to request a copy of their laboratory data.

For this visit, a ± 2 month window will be allowed.

Table 1. Schedule of visits

	Screening/ Eligibility Day -7 to Day 0	Enrollment, Time Zero	Day 1- Rx	Day 2- Rx	Day 3- Rx	Day 14 (+/-3)	4 months	12 months
Visits	[V1]	[V2]				[V3]	[V4]	
Eligibility Confirmation & Consent	X							
Comprehensive Exam ^(1, 2)	X ⁽¹⁾							
Vital Signs and Interval Assessment	X	X				X	X	
Pregnancy Test	X							
Fat Biopsy (2 total) ⁽³⁾		X				X		
Skin biopsy (2 total) ⁽⁴⁾		X				X		
Intervention group RX: Dasatinib+Quercetin			X	X	X			
Drug Diary			X	X	X			
Blood Tests ⁽⁵⁾	X					X	X	X ^A
Spot Urine Tests ⁽⁶⁾	X					X	X	X ^A

24 hr Urine (1)	X					X	X	X ^A
Electrocardiogram	X							
Frailty assessment (3 total) (8)		X				X	X	
Plasma-Urine-Cell biobank(2)	X					X	X	
SF 36 QOL	X					X	X	
Karnofsky& FAST Performance Assessment	X					X	X	

^A=clinically performed test.

¹If screening/eligibility tests were performed clinically up to 7 days prior to enrollment visit (Day 0), then subjects will undergo modified testing to include only those tests not previously completed.

²For safety purposes the Study Investigators have the right to screen fail patients.

³Drug education will occur at screening or Day 0

All chronic kidney disease/diabetic nephropathy patients will undergo routine laboratory assessment of kidney function and of potential complications of CKD. These laboratory studies (primarily proteinuria, lipid panel, blood glucose, hemoglobin A1c) and vital measurements (primarily body mass index (BMI)) will be used to determine clinical metabolic parameters which may relate to senescence, as previously suggested in DN kidney.

(1) Comprehensive Exam (Initial)

History assessment of past medical and surgical history. Medication review or reconciliation (statin, oral hypoglycemic, insulin, anti-hypertensives, etc). Further screening for exclusion criteria. Collection of vital signs including heart rate and blood pressure, height/weight, body mass index; BMI. Physical exam consisting of visualization of the abdominal wall and assessment of lower extremity edema

(2) Vital Signs and Interval Assessment

- Interval history assessment of: new medications (since enrollment), recent hospitalizations, or treated infection, and Adverse Event Assessment. Collection of vital signs including heart rate and blood pressure, height/weight, body mass index; BMI. Physical exam consisting of visualization of the abdominal wall and assessment of lower extremity edema.

(3) Fat Biopsy

- Subjects will undergo a subcutaneous fat biopsy (0.5-2 g) by Mayo surgeons using a 1-1.5 inch incision in our outpatient surgical suite under sterile conditions. One to two dissolvable sutures may be placed to close the incision. The fat tissue will be transferred to designated study labs at Mayo Clinic, Rochester, MN.

(4) Skin Biopsy

- At the same time as the fat biopsy a 6mm punch skin biopsy will be collected by the surgeon. The skin tissue will be transferred to designated study labs at Mayo Clinic, Rochester, MN.

(5) Blood Tests

- Kidney function: serum creatinine with estimated glomerular filtration rate (eGFR), blood urea nitrogen, and cystatin C.

- **Bone mineral metabolism:** serum calcium, phosphorus, albumin, bicarbonate, parathyroid hormone, protein total, sodium, potassium, bilirubin, alkaline phosphatase, and AST.
- **Anemia of renal disease:** serum complete blood count, iron and total iron binding capacity and ferritin.
- **Diabetes:** Serum glucose, hemoglobin A1c.
- **Inflammatory and cardiovascular disease markers:** C-reactive protein, cardiac troponin T, tumor necrosis factor- α , and uric acid.
- **Other labs:** pregnancy test
- **Lipid Screen:** Cholesterol, HDL, and Triglycerides
- **Senescence:** Activin A

(6) Spot Urine Tests

- Urinalysis with microscopy.
- Urine protein: creatinine (microalbumin, urine).

(7) 24 hour urine collection

- 24 hour Creatinine clearance
- 24 hour Urine Protein

(8) Frailty Assessment

- Frailty phenotyping will consist of generation of a frailty index score from 5 measures of weakness through grip strength, walking speed, endurance and energy screening, unintended weight loss screening, and physical activity level calculations. [Appendix 4](#).

(9) Plasma-Urine-Cell Biobank

- Collection of plasma, cells and urine from subjects will enable us to evaluate and answer pertinent questions related to the biology of aging and senescence. Studies utilizing these samples will allow us to gain a greater understanding of the pathophysiology of accelerated aging and the potential for us to develop new therapies to improve patient outcomes. An aliquot of 22 mL plasma will be obtained from each subject at the same time that other blood tests are done. The specimen will be kept on ice and immediately transported to the plasma bank for processing. In addition, an aliquot of urine up to 100 mL will be obtained from each subject.

7 Statistical Plan

For the analyses, categorical data will be described by counts and percents and quantitative data by means and standard deviations.

Study personnel will remain blinded to kidney function and treatment status. A statistician, working with the pharmacist at time of analyses, will be un-blinded for interim (~50% recruitment) and final analyses. In the case of an adverse event, information related to that particular patient will be revealed to the clinician.

Sample Size and Power

Power calculations were based on senescence by SA- β -galactoside measurement which has a

mean of 5% (i.e., 5% of MSCs have the SA-β-gal marker) in healthy adult and is 3-4 fold higher in DN kidney tissue,^{57,58} **Table.** In addition, we will explore the relationship between patient laboratory studies (primarily creatinine, proteinuria, blood glucose, hemoglobin A1c), vital measurements (e.g., BMI), frailty index, and senescence (or MSC function) using linear regression.

Table.	Groups	Power analyses were based on senescence (SA-β-gal)
Analysis 1	eGFR 15-60 (n=14 DN treated with senolytics; n=6 DN treated with placebo)	The sample size (n=30; 20 senolytic; 10 placebo-treated) will have 95% power to detect a difference in means of 5.5% (eg, a pre-senolytic SABG mean, μ1, of 15% and a post-senolytic μ2 of 9.5%), assuming a standard deviation of differences of 5.0, using a paired t-test with a 0.05 two-sided significance level.
Analysis 2		We will explore the relationship between laboratory studies (primarily proteinuria, LDL cholesterol, blood glucose, hemoglobin A1c), vital measurements (primarily BMI), medication exposure (metformin, insulin dosages), frailty index, and senescence (or MSC function) using linear regression.

Treatment Compliance

Adherence to medication regimes will be described in terms of percent of medication taken (actual or reported) for dasatinib and quercetin and compared between the groups.

Feasibility Assessment

We will conduct a feasibility assessment at 50% enrollment to assess for any detectable changes in the treatment arm.

8 Safety and Adverse Events

Common Terminology Criteria for Adverse Events (CTCAE) is widely accepted throughout the cancer and HSCT community as the standard classification and severity grading scale for adverse events in cancer therapy clinical trials and other oncology settings. The National Cancer Institute published the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 on May 28, 2009 (v4.03: June 14, 2010). The toxicities in the intervention group will be assessed by this scale. The 196 page document for CTCAE V4 can be found on this website http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf (accessed 7/7/2015)

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

Any unanticipated problem or adverse event that meets the following three criteria:

- **Serious:** Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or

prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**

- **Unanticipated:** (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**
- **Related:** A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event

An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event

Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as 12 months. The adverse event reporting period ends when the subject completes the study (12 months).

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

In the CKD and diabetes patient population, symptoms associated with uremia or diabetes may be present at baseline. These may include: nausea, vomiting, diarrhea, pruritus, rash, chronic pain, hypoglycemic reactions, and headaches. We anticipate that in this patient population these may be exacerbated with many new drugs initiated for clinical or research purposes. If it is

determined by the PI that these symptoms were present prior to initiation of the drugs, they will not be collected as adverse events.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values

The following clinical laboratory abnormality should be documented as an adverse event if ...

1. Acute kidney injury on chronic kidney disease in the Intervention group:
 - Rise in serum creatinine >0.3 mg/dL within 14 days after entry in the study
 - Doubling of serum creatinine
2. New onset nephrotic syndrome (hypoalbuminemia, dyslipidemia, new onset nephrotic range proteinuria, edema, lipiduria) in the Intervention group.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

The following hospitalizations will not be considered SAE for this study:

- a visit to the emergency department or other hospital department <24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g. routine mammogram)
- medical or surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF) or in a separate adverse event worksheet. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Investigator reporting: notifying the Mayo IRB

The Investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Information collected on the adverse event worksheet (and entered in the research database):

- Subject identifier:
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research drug
- If the adverse event was expected:
- Grade and attribution
- The severity of the adverse event: (use a table to define severity scale 1-5)
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal

relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Stopping Rules

All patients will be assessed during the 3 days of drug administration and during the day 14 visit. If a severe adverse event occurs at any time during administration of the 3-day drug regimen, a formal review will occur and subsequent patients will be enrolled one at a time using the same regimen. If three or more events accrue, the pilot study will be held and either a potential dosing regimen revisited or discontinuation of the study protocol will occur. Other interventions will be as per the direction of the FDA and Mayo IRB.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Data and Safety Monitoring Board or Data Safety Monitoring Plan

There will not be a formal DSMB however we will have a DSMP and the PI will monitor.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that

the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. Case reports in the form of completed checklists will be kept to assure inclusion/exclusion criteria and review of adverse events/toxicity. All data requested on the CRF must be recorded; data will be entered directly into REDCap. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction.

Data Management

The data will be housed in both hard copy case report forms (CRFs) and eCRFs through a system called REDCap.

Data Security and Confidentiality

Source documents and CRFs and original consents will be stored in secured locations. All data will be entered into a password protected, limited access database. Individually-identifiable patient history and medical record information will be stored in a database under coded accession numbers. Clinical laboratory values are stored in the electronic medical record system, requiring protected password access. These data are monitored regularly for access and a formal policy regarding protection of personal privacy is in place throughout each institution. The key to identification of subjects will be maintained in a secure office environment under the direction of the principal investigators.

Data Quality Assurance

Manual and computerized quality checks will occur during data collection and analyses and any discrepancies will require Case Report Form (CRF) review and validation of correct data.

9.4 Records Retention

The principle investigator will maintain records and essential documents related to the conduct

of the study. These will include subject case histories and regulatory documents. The investigator will retain the specified records and reports for;

- Up to 2 years after use of the drug; or, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. Or whichever is longer.
- 2. As outlined in the Mayo Clinic Research Policy Manual –“Retention of and Access to Research Data Policy” http://mayocontent.mayo.edu/research-policy/MSS_669717 The specified records and reports are outlined in the Mayo Clinic Research Policy Manual – “Retention of and Access to Research Data Policy”_ http://mayocontent.mayo.edu/research-policy/MSS_669717

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

As a service to the sponsor-investigator, this study may be monitored during the conduct of the trial by staff from the Mayo Clinic Office of Research Regulatory Support. Clinical trial monitoring may include review of the study documents and data generated throughout the duration of the study to help ensure the validity and integrity of the data along with the protection of human research subjects. This will assist sponsor-investigators in complying with Food and Drug Administration regulations.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this

study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the 2015 DOM Research Career Development Award and provided by the National Institute of Health (NIH).

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

12.3 Subject Stipends or Payments

Subject remuneration will consist of \$175 for each fat+skin biopsy performed and an additional \$75 if they complete the four month visit. If subject screen fails during first visit and does not undergo a fat/skin biopsy, remuneration will consist of \$75. Subjects will receive parking passes for the time involved with completing the study visits or taxi reimbursement to attend study visits with the exception of month 12. Subjects will be directed where to park in order to receive the parking passes. In addition, subjects will be provided a Mayo pen for participation in the study.

Minority subjects recruited from off-site sites will be reimbursed for airfare, hotel accommodations, and food up to an allowable maximum amount as follows: Mileage per driven mile: Your reimbursement for travel is calculated on the actual round trip miles you travel from your home address to the Mayo Clinic and back as determined by a web-based mileage calculator (e.g. ,MapQuest). This distance will be documented in the subjects study file. They will receive reimbursement at the current IRS determined rate. Flight costs to and from the nearest airport to Rochester International Airport or MSP. Shuttle service or cab service from the airport (RST or MSP) to Mayo Clinic. Overnight hotel accommodations up to \$150.00 dollars per night for max 2 night stay. Airport parking costs at your home airport. Meal per diem of \$75.00 dollars.

Please see Patient Contact Material: Travel Information for details.

13 Publication Plan

The principle investigator, Dr. Hickson, holds the primary responsibility for publication of the results of the study.

We will register with ClinicalTrials.gov prior to subject recruitment and enrollment. We will post results to ClinicalTrials.gov within 12 months of final data collection for the primary

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Appendices

Appendix 1. Strong Inhibitors of CYP3A4

Boceprevir
Clarithromycin

Conivaptan
Indinavir

Itraconazole

Ketoconazole
Lopinavir/ritonavir
Nefazodone

Nelfinavir
Posaconazole

Ritonavir
Saquinavir
Telaprevir
Telithromycin
Voriconazole

Appendix 2. Strong inducers of CYP3A4

Carbamazepine
Phenytoin
Rifampin
St. John's wort

Appendix 3. Major or sensitive substrates of CYP2C8, CYP2C9, CYP2D6 and CYP3A4

	Sensitive substrate	Substrate with narrow therapeutic range
CYP2C8	Repaglinide	Paclitaxel
CYP2C9	Celecoxib	Warfarin, phenytoin
CYP3A	Alfentanil, aprepitant, budesonide, buspirone, conivaptan, darifenacin, darunavir, dasatinib, dronedarone, eletriptan, eplerenone, everolimus, felodipine, indinavir, fluticasone, lopinavir, lovastatin, lurasidone, maraviroc, midazolam, nisoldipine, quetiapine, saquinavir, sildenafil, simvastatin, sirolimus, tolvaptan, tipranavir, triazolam, vardenafil	Alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozone, quinidine, sirolimus, tacrolimus,

CYP2D6	Atomoxetine, desipramine, dextromethorphan, metoprolol, nebivolol, perphenazine, tolterodine, venlafaxine	Thioridazine
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Sensitive CYP substrates refer to drugs whose plasma AUC values have been shown to increase 5-fold or higher when co-administered with a known CYP inhibitor.

CYP substrates with narrow therapeutic range refers to drugs whose exposure-response relationship indicates that small increases in their exposure levels by the concomitant use of CYP inhibitors may lead to serious safety concerns (e.g., Torsades de Pointes).

Appendix 4: Frailty Phenotyping

The following tests will be administered to determine frailty phenotyping (index) using the following criteria:

- Weakness:** Grip strength of the dominant hand will be tested using a Jamar electronic hand held dynamometer. Three serial measurements will be taken and the mean calculated. Strength measurements will be normalized by gender and body mass index. Weakness will be defined as grip strength in the lowest 20th percentile of a community dwelling population of adults 65 years and older.
- Slow Walking Speed:** Time to walk 5 meters will be measured using a handheld ultrasonic measuring device. Subjects will be instructed to walk at their usual comfortable pace. Walking aids such as canes and walkers may be used if needed. Three separate trials will be collected. Subjects will meet the slow walking speed criteria if the average time to walk the 5-meter course is ≥ 6 seconds.
- Poor Endurance and energy:** Is indicated by self-report of exhaustion. Subjects will be asked two questions from the Center for Epidemiological Studies Depression Scale (CES-D): 1) I felt that everything I did was an effort, and 2) I could not get going. Subjects who answer “a moderate amount of time (3-4 days)” or “most of the time (5-7 days)” to either of the statements will be categorized as meeting the exhaustion criteria of the frailty scale.
- Unintended Weight Loss:** Subjects will be asked if they have unintentionally lost ≥ 10 pounds in the prior year. An affirmative response fulfills this criterion.
- Low Physical Activity Level:** A weighted score of kilocalories expended per week will be calculated at baseline using the Physical Activity Scale for the Elderly (PASE). This is an easily administered and scored instrument that measures the level of physical activity in individuals aged 65 or older. The PASE is a 10 item instrument which assesses the frequency and duration of participation in; 1) leisure activities (e.g., walking outside the home, light, moderate and strenuous sport and recreation), 2) household activities (dusting, washing dishes, vacuuming, home repairs, lawn work, gardening) and 3) work-related activities (e.g., amount of physical activity required on the job). Weekly activity level can be converted to equivalent kilocalories of expenditure, and individuals reporting a weekly kilocalorie expenditure in the lowest 20th percentile for their gender (men, <383 kcal/week; women <270 kcal/week) were classified as having

low physical activity.

Frailty criteria	Indicators
Weight loss	Loss of >10 pounds in past 12 months, unintentional.
Exhaustion	Response of "a moderate amount of the time (3–4 days)" or "most of the time" to either of two CES-D scale items: "I felt that everything I did was an effort"; "I could not get going" during the past week.
Weakness	Maximal grip strength in kg using Jamar hand-held dynamometer. Lowest 20%, stratified by gender and BMI quartiles.
Slowness	Time in seconds to walk 15 feet at usual pace. Slowest 20%, stratified by gender and standing height.
Low physical activity level	Weighted score of kilocalories expended per week in physical activities "you have done in the past 2 weeks" reported on short version of Minnesota Leisure Time Activity questionnaire. Lowest 20% for each gender.
Frailty	Presence of 3 or more of the above criteria.

BMI: body mass index. CES-D: Center for Epidemiologic Studies-Depression.