Targeting Cellular Senescence with Senolytics to Improve Skeletal Health in Older Humans: A Phase 2, Single-Center, 20-week, Open-Label, Randomized Controlled Trial

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Targeting Cellular Senescence with Senolytics to Improve Skeletal Health in Older Humans: A Phase 2, Single-Center, 20-Week, Open-Label, Randomized Controlled Trial

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Study Product:	• Sprycel® (dasatinib) and Quercetin
Protocol Number: (IRBe)	18-010546
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Table of Contents

List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
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
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Study Summary

Title	Targeting Cellular Senescence with Senolytics to Improve Skeletal Health in Older Humans: A Phase 2, Single-Center, 20-Week, Open-Label, Randomized Controlled Trial
Running Title	Effects of Senolytics on Markers of Aging and Bone Metabolism
Protocol Number	18-010546
Phase	Phase II
Methodology	Randomized, open-label trial
Overall Study Duration	20 weeks
Subject Participation Duration	20 weeks, plus screening visit
Single or Multi-Site	Single Site Study
Objectives	Test whether intermittent senolytic therapy reduces markers of biological age and improves bone turnover markers and skeletal parameters in older postmenopausal women
Number of Subjects	240 screened, 74 accrued
Diagnosis and Main Inclusion Criteria	Elderly postmenopausal women, aged ≥60years, with increased senescent cell abundance in blood
Study Product, Dose, Route, Regimen	This study will involve an oral treatment regimen with Dasatinib (D; 100 mg for two days) plus Quercetin (Q; 1000 mg total daily for three consecutive days) taken orally on an intermittent schedule (starting every 28 days) with no-therapy periods in between dosing regimens, repeated every 28 days over 20 weeks, resulting in five total dosing periods throughout the entire intervention. Note that we are modifying the protocol to complete 30 subjects in the control group, 30 in the D+Q group, and 14 who have completed the F-group but no additional subjects will be added to that group.
Duration of Administration	Five total intermittent dosing periods throughout the 20-week intervention
Reference therapy	Untreated control group
Statistical Methodology	Using previously published data, power calculations were conducted, while allowing for an estimated withdrawal rate of 10%. This calculation demonstrated that 30 subjects per group will provide the study with 90% power to detect a 19.8% difference in the bone resorption marker, CTX (17.1% with 80% power), which is our primary endpoint.

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

The Mayo Clinic Aging Group is at the forefront of discovering, developing, and optimizing therapies to eliminate senescent cells – "senolytics". With aging, DNA damage and/or other cellular stressors[1-4] cause proliferating[5,6] and terminally differentiated, non-dividing cells[7-10] to undergo senescence. Senescent cell characteristics include profound chromatin and secretome changes, increased expression of senescence biomarkers (*e.g., p16^{Ink4a}* and *p21^{Cip1}*) and resistance to apoptosis[1,11]. Senescent cells also develop the senescence-associated secretory phenotype (SASP), consisting of pro-inflammatory cytokines, chemokines, and matrix-degrading proteins that have deleterious paracrine and systemic effects[12-16]. Further, because senescent cells accumulate in multiple tissues in temporal and spatial synchrony with age-associated functional decline in animals and humans[5-7,17-24], they have been hypothesized to drive the deterioration underlying numerous chronic diseases[1]. Indeed, even a relatively low abundance of senescent cells (*e.g.,* ~10-15% in old primates[23]) is sufficient to cause tissue dysfunction. Therefore, senescent cells represent promising therapeutic targets to delay or alleviate functional deficits and chronic disorders of aging, including age-related bone loss.

Consistent with this, eliminating even a relatively small proportion (~30%) of senescent cells using a "suicide" transgene, *INK-ATTAC* (*p16^{Ink4a}-linked apoptosis through targeted activation of caspase*[25]), that permits inducible elimination of *p16^{Ink4a}*-expressing senescent cells upon administration of a drug (AP20187), extends healthspan and prevents the development of multiple age-related morbidities in both progeroid and naturally aged mice[12,25-27]. As an alternative to this genetic approach and one applicable to humans, the Kirkland group at Mayo Clinic exploited the dependence of senescent cells on specific pro-survival pathways and identified that the combination of Dasatinib (D; an FDA-approved tyrosine kinase inhibitor [TKI] in clinical use for treating hematologic disorders[28,29]) plus Quercetin (Q; a natural flavanol present in many fruits and vegetables[30] that targets BCL-2, insulin/IGF-1, and HIF-1α networks[31-33]) [D+Q], has *in vitro* senolytic activity by killing senescent cells without affecting proliferating or quiescent, differentiated cells[31].

To test this approach *in vivo*, work by our group as well as independent laboratories using mouse models of accelerated or natural aging has demonstrated that intermittent D+Q therapy delays the onset of several age-related disorders[7,26,27,31,34-41]. For example, intermittent D+Q therapy extends healthspan in mice by preventing age-related bone loss[27] and improving several aspects of physical dysfunction/frailty[39] as well as atherosclerosis[26], idiopathic pulmonary fibrosis (IPF)[36], and tau-mediated neurodegenerative disease[38]. Intermittent D+Q therapy also extended maximal lifespan in old mice as post-treatment survival was significantly increased by 36%[39]. More recent findings[34,40] as well as our unpublished data have established that Fisetin (F; a flavonol with similar chemical structure to Q[42,43]) also has potent senolytic activity both *in vitro*[34,40] and *in vivo*[40], with beneficial effects on multiple tissues in both progeroid and young wild-type (WT) mice "aged" by senescent cell transplantation. Thus, intermittently treating aged

mice with D+Q or F enhances healthspan[7,26,27,31,34-41], which sets the stage for clinical studies testing intermittent senolytic therapy in humans as proposed in this randomized controlled trial (RCT).

This study will include normal, elderly postmenopausal women, aged ≥60years, with increased senescent cell abundance in peripheral blood T-lymphocytes (PBTL) at screening by T-cell assay.

1.2 Investigational Agent

An investigational product is defined as a pharmaceutical form of an active substance 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 products will be stored in a secure area according to local regulations. Investigators will have responsibility for ensuring that investigational product is only dispensed to study subjects. The investigational products (Dasatinib plus Quercetin) will be dispensed only by authorized personnel according to regulations that apply at Mayo, Rochester. In this protocol, investigational products are Dasatinib plus 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. This will be an open label study.

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. To date, no reports of significant toxicity have been reported, and current evidence from multiple *in vitro* studies demonstrates safety of this product as a food additive. Our anticipation of any risks of toxicity in

elderly postmenopausal women who will receive this drug intermittently for only three consecutive days at a time (five total three-day dosing regimens) is <1%. Quercetin phytosome dehydrate capsules equating to 1,000 mg total daily dosage will be administered for three consecutive days orally to the elderly postmenopausal women randomized to the D+Q group. They will receive two capsules in the morning, two capsules in the evening. 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. This will be an open label study.

1.3 Preclinical Data

Discovery of senolytics by targeting the Achilles' heel of senescent cells: from transcriptome to drug identification. The class of small molecule drugs that can selectively induce death (or eliminate) senescent cells are known as "senolytic(s)" – *i.e.,* from the word "senescence" and "lytic" – destroying. Our colleagues in the Kirkland laboratory and Mayo Clinic Kogod Center on Aging discovered senolytics including the combination of Dasatinib plus Quercetin (D+Q) and Fisetin (F)[31,34]. Dasatinib is a tyrosine kinase inhibitor (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. Recent work evaluating the effects of Dasatinib against thyroid cancer cell lines *in vitro* and in a xenograft model in vivo, demonstrated that Dasatinib-treated cells (BHP2-7 and Cal62) exhibited a characteristic clumped appearance upon biopsy and most of these cells were classified as senescent using a common technique for assessing cellular senescence – *i.e.,* the senescence-associated β-Galactosidase (SA-β-Gal) assay. This work further showed that the average number of senescent cells per high powered field were: BHP2-7, control 0/hpf; BHP2-7, Dasatinib 20/hpf; Cal62, control 0/hpf; Cal62 Dasatinib 25/hpf. In addition, when these senescent cells were followed, they displayed necrotic features after 10 days of Dasatinib treatment.

Senescent cell eradication (or reduction) by activating a drug-inducible "suicide" gene (*i.e.,* via $p16^{INK4a}$-linked apoptosis through targeted activation of caspase [*INK-ATTAC*]) can delay multiple age-related phenotypes in both genetically modified progeroid mice and natural, chronologically aged mice[25,57]. In subsequent studies, the Kirkland laboratory at Mayo Clinic screened 46 agents/drugs that could theoretically induce apoptosis preferentially of senescent cells *in vitro*. They then evaluated these agents and different combinations to determine the specific subset of agents that were most effective at eliminating senescent cells[31]. Dasatinib preferentially reduced viability and caused cell death in senescent human preadipocytes (also known as mesenchymal stem cells [MSCs]), was much less effective on senescent human umbilical vein cells (HUVECs) (**Fig. 1A**). After three days of exposure, proliferating preadipocytes increased by 2-5fold in number compared to day 0 in the presence of Dasatinib. The viability of non-dividing senescent preadipocytes from the same human subjects decreased by 30-40% in the presence of Dasatinib, thus demonstrating a selective reduction.

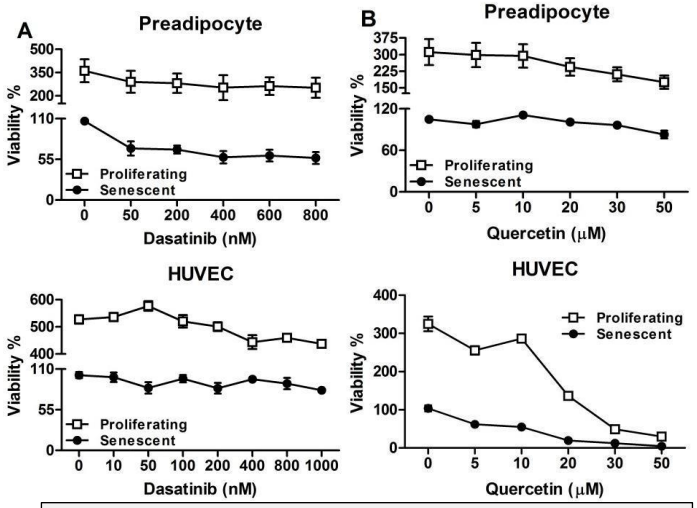

Fig. 1. Effects of (A) Dasatinib and (B) Quercetin on preadipocytes and HUVECs.

Another senolytic agent our colleagues in the Kirkland laboratory discovered was Quercetin (Q), 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 (**Fig. 1B**). The combination of D+Q displayed selective killing of both senescent preadipocytes and endothelial cells (**Fig. 2**). By day 3, the viability of non-dividing senescent preadipocytes exposed to D+Q was reduced by ~70% compared to day 0, while non-senescent, proliferating cells had increased by 2-4 fold, establishing that the combination of D+Q selectively targets a broader range of senescent cell types then either agent alone, hence forming the basis of our hypothesis in our study in mice (see below). In a previous study published in collaboration with the Kirkland laboratory[31], it was shown that the combination of D+Q not only decreased the senescent cell burden in mice *in vivo*, but also improved functional improvements in running endurance in younger mice that were "aged" by administration of doxorubicin, a chemotherapeutic drug known to cause DNA damage and induce cellular senescence. In addition, it was demonstrated that intermittent D+Q administration alleviated age-related cardiac and vascular dysfunction, improved gait in mice disabled exposing a limb to ionizing radiation,

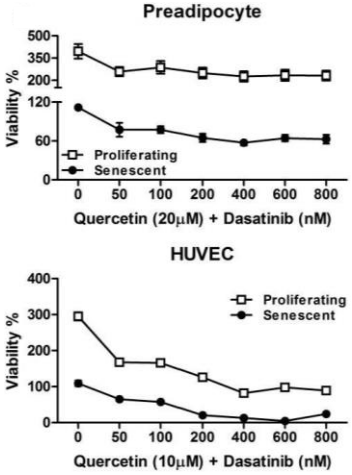

Fig. 2. Effects of Dasatinib plus Quercetin (D+Q) on preadipocytes and HUVECs.

and delayed frailty, neurological dysfunction, and **osteoporosis** in mice with an accelerated aging syndrome[31]. These findings were later further supported by studies in our laboratory more specifically examining the roles of senescent cells in mediating age-related bone loss (detailed below).

Identifying senescent cells in bone and the causal role of cellular senescence in age-related bone loss. Our group recently found[7] in mice that with aging, a subset of cells of various lineages within the bone microenvironment become senescent and that some of these senescent cell populations develop a SASP. Importantly, we found similar results in bone biopsies from older vs younger women[7], establishing that senescent cells are present at the time and location of age-related bone loss in humans, as they are in mice. To establish causality[27], we next used genetic (*INK-ATTAC*[25]) and pharmacological (D+Q[31]) approaches to target senescent cells and assessed their impact on age-related bone loss in old mice. As detailed in our publication[27] and as summarized in our recent reviews[58,59], the skeletal effects of intermittent D+Q therapy in old WT mice (**Fig. 3 A-F**) were virtually identical to those observed in the old *INK-ATTAC* mice treated with AP20187 (a synthetic drug that induces suicide transgene activation[25]), demonstrating that senescent cell clearance via either genetic or senolytic approaches prevents age-related bone loss. By histomorphometry, we found that targeting senescent cells using genetic or senolytic approaches reduced bone resorption (**Fig. 3D**). Importantly, despite lower bone resorption, bone formation was either maintained (trabecular sites) or was higher (cortical sites; **Fig. 3 E, F**) following senescent cell clearance[27]. Thus, from a therapeutic perspective, targeting senescent cells offers substantial advantages over conventional anti-resorptive therapies[58] as the latter inhibit/eliminate osteoclasts to reduce bone resorption, with a concomitant reduction in bone formation due to coupling. By contrast, senolytic therapy reduces the senescent cell burden, which in turn suppresses bone resorption with either increased (cortical bone) or maintained (trabecular bone) bone formation, leading to positive "bone balance"[27]. The studies proposed in this application are aimed at translating these novel findings in mice to humans.

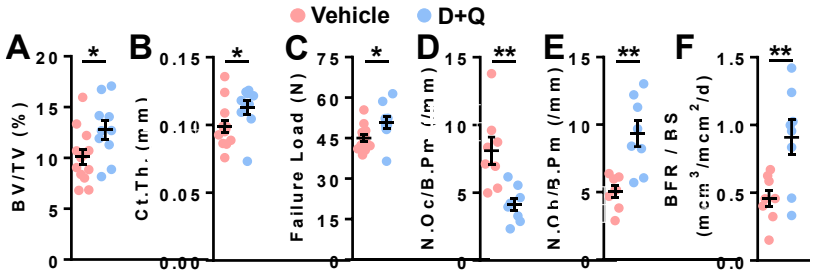

Fig. 3. D+Q (n=10) prevents age-related bone loss in old WT mice vs vehicle (n=13). (**A-C**) µCT-derived spine bone volume fraction (BV/TV), femur cortical thickness (Ct.Th.), and femur failure load (strength). (**D-F**) Histomorphometry (n=8/group) at the femoral endocortical surface: osteoclast #'s/bone perimeter (N.Oc/B.Pm), osteoblast #'s/bone perimeter (N.Ob/B.Pm), and bone formation rate/bone surface (BFR/BS). Mean ± SEM. *p<0.05; **p<0.01.

Fisetin is a senolytic that extends health- and lifespan and rescues senescent cell-induced bone loss. As noted earlier, Fisetin (**F** – 3,3',4',7-tetrahydroxyflavone) is a member of the flavonoid family of naturally occurring polyphenolic compounds present in many fruits and vegetables with high concentrations in strawberries (160 µg/g)[42,43]. To date, no adverse effects of F have been reported, even when taken at high doses[60]. The hydrophobic nature of F allows it to penetrate cell membranes and accumulate in cells to exert its effects[49,52]. *In vitro* studies in either senescent primary human umbilical vein endothelial cells or senescent primary murine embryonic fibroblasts demonstrate that F significantly reduced viability (causing apoptosis of 30-70% of senescent cells)

and senescence biomarkers relative to vehicle[34,40]. Notably, eliminating ~30% of senescent cells in *INK-ATTAC* mice was sufficient to alleviate multiple age-related conditions[25]. *In vivo* studies in both naturally aged WT mice and *Ercc1*$^{-/\Delta}$ mice, which model human XFE progeria and display several features resembling accelerated aging within a lifespan of 6 months[61], demonstrated that F significantly reduced senescence biomarkers, while late-life intervention with F in old WT mice significantly extended lifespan[40]. Similar findings were observed in human adipose tissue explants treated with F vs vehicle[40]. F also extended healthspan measured by a composite frailty score in the *Ercc1*$^{-/\Delta}$ mouse model of accelerated aging[40], thus establishing beneficial effects of F on physical function and aspects of frailty. Finally, our more recent unpublished data in young adult (4-month) WT mice transplanted with either relatively small numbers (*i.e.,* 10^6) of quiescent, non-senescent control cells or senescent cells (**Fig. 4**), demonstrate that senescent cell transplantation causes significant bone loss that is rescued by intermittent F therapy (**Fig. 4 A-B**).

Collectively, these data in mice establish that reducing the senescent cell burden with senolytics has broad potential implications for osteoporosis and other chronic diseases of aging.

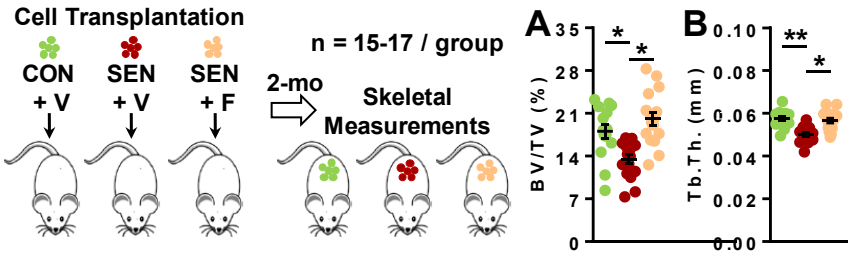

Fig. 4. After two months (2-mo), senescent (SEN) cell transplantation in young adult (4-mo-old) WT mice causes significant bone loss [(**A**) lower spine BV/TV and (**B**) trabecular thickness (Tb.Th.)] vs control (CON) cell transplantation that is rescued by intermittent (bi-weekly) fisetin (F) therapy vs vehicle (V). Mean ± SEM. *p<0.05; **p<0.01.

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 taken orally on an intermittent schedule (starting every 28 days) with no-therapy periods in between dosing regimens, repeated every 28 days over 20 weeks. More specifically, the oral treatment administration for each dosing regimen will be Dasatinib (D; 100 mg daily for two consecutive days) plus Quercetin (Q; 1000 mg total daily for three consecutive days) taken orally on an intermittent schedule (starting every 28 days) with no-therapy periods in between dosing regimens, repeated every 28 days over 20 weeks, resulting in five total dosing regimens throughout the entire intervention.

To our knowledge, there are no published studies utilizing Dasatinib or Quercetin or the combination of both drugs in the population of normal elderly postmenopausal women, independent of a diagnosis of CML. Dasatinib is a TKI used as an oral daily treatment for CML with fewer side effects and improved survival compared to older treatment agents for this disease[62]. Dasatinib is generally well-tolerated with the more common adverse effects consisting of rash, abdominal pain, diarrhea, nausea, myelosuppression, fluid retention, and headache.

There has, however, been a recent publication by Yilmaz et al.[63] in which a historical cohort review was conducted on 468 newly diagnosed patients with chronic-phase chronic myeloid leukemia at MD Anderson who were treated long-term with TKIs (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 four years. Among the six Dasatinib-treated patients with CKD (eGFR <60 mL/min) at baseline, there were no significant changes in eGFR over time within this relatively 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 (D) 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 therapy[64-66]. These events generally resolve with interruption of drug therapy and conservative management. Based on a case report and review by Wallace et al.[66], 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 (Q) is a natural-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 application[67], 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 improve kidney function[68-71].

1.4 Dose Rationale

Reducing the senescent cell burden with senolytics extends health- and lifespan in old mice[7,26,27,31,34-41], and prevents age-related bone loss[7,27]. The senolytics proposed in this application were discovered using a hypothesis-driven approach whereby senolytics selectively induce senescent cell apoptosis by transiently disabling the senescent cell anti-apoptotic pathways (SCAPs)[32,77] that defend senescent cells against their own pro-apoptotic environment[31]. However, not all senescent cells are the same: they can originate from different cell types, express unique SASPs[78-80], and use different mechanisms to resist apoptosis[32,77]. Intermittent D+Q or F therapy in old mice or young mice “aged” by senescent cell

transplantation alleviates a range of age-related chronic conditions, including osteoporosis[27]. Because senescent cells do not divide[1] and take at least weeks to re-accumulate[81] and because senolytics do not need to be continuously present to occupy a receptor or affect an enzyme[32,77], senolytic combinations, such as D+Q[31], and newer senolytics such as F[40], can be repurposed for immediate translation to humans. This premise is supported by a first-in-human pilot study of intermittent D+Q therapy[82], showing that senolytics alleviated aspects of physical dysfunction in patients with IPF, a progressive, fatal cellular senescence-associated disease[36,83,84]. This study provides "proof-of-concept" feasibility for targeting senescent cells in humans with senolytics to alleviate aspects of age-related diseases, and supports additional studies evaluating senolytics for other chronic diseases of aging, such as osteoporosis.

Dasatinib plus Quercetin (D+Q): D is an FDA-approved TKI used clinically to treat hematologic disorders with a long-standing, acceptable safety profile[28,29]. The oral dose of D (100 mg/d) selected is based on the FDA-approved dose for chronic therapy as effective for inducing apoptosis in human cancer cells. Follow-up analysis (5-years) from the phase III Dasatinib versus Imatinib Study in Treatment-Naïve Chronic Myeloid Leukemia (CML) Patients trial[85], demonstrated long-term efficacy and safety of oral D (100 mg/d). In addition, in the TKI era (*e.g.,* with daily D therapy) patients with CML have lifespans nearly as long as the general population[86]. In contrast to the daily D regimen these patients receive, D therapy in our study will be administered intermittently (see below). The oral dose of Q (a natural product present in many fruits and vegetables[30]) selected is 1000 mg/d (250 mg capsules x 4/d). Importantly, in the first-in-human pilot study of D+Q in patients with IPF[82], these doses of D+Q for 3 consecutive days over 3 consecutive weeks (9 total participant administered dosing days) at two separate U.S. clinical sites were well tolerated without notable changes in clinical chemistries or renal/hepatic function. Also, in a more recent study, we found that these doses of D+Q for 3 consecutive days demonstrated clearance of senescent cells in the skin and adipose tissue [87]. Based on pre-clinical models, we believe that 2 doses of D along with 3 doses of Q will be equally effective, but a single dose of D may not be; as such, we propose to use daily doses of D x2d and daily doses of Q x3d in our study. In our study, the D and Q doses will be the same, but the interval between dosing regimens will be longer (26 [Dasatinib] or 25 [Quercetin] days off senolytic therapy between dosing regimens) vs the first-in-human study in IPF patients (4 days off senolytic therapy between dosing regimens)[82]. Further, the study duration we propose is 20 total weeks to include a total of 5 participant dosing days administered intermittently (every 4 weeks, see **Section 3, Study Design**). More specifically, the two-consecutive day dosing days for Dasatinib will be at Baseline (Day 1, 2), start of Week 5 (Day 29, 30), start of Week 9 (Day 57, 58), start of Week 13 (Day 85, 86), and start of Week 17 (Day 113, 114) for a total of ten days of Dasatinib over the course of the 20-week study. More specifically, the three-consecutive day dosing days for Quercetin will be at Baseline (Days 1-3), start of Week 5 (Days 29-31), start of Week 9 (Days 57-59), start of Week 13 (Days 85-87), and start of Week 17 (Days 113-115) for a total of 15 days of Quercetin over the course of the 20-week study.

1.5 Risks and Benefits

Based on the above (see **Section 1.4, Dose Rationale**), our anticipation of any toxicity risk in subjects receiving these compounds (D+Q) for only two or three consecutive days followed by at least 25 days of no therapy, every four weeks over the course of 20 weeks, is <1%. Therefore, the potential benefits of intermittent senolytic administration to target senescent cells and thereby alleviate multiple functional consequences of age-related diseases in humans, as in

mice[7,26,27,31,34-41], is considerable. Further, although not yet established, the senolytic proposed here and next generation senolytics may have a better safety profile than existing pharmacologic options for osteoporosis prevention and treatment[88]. The studies proposed herein will be pivotal to this end. In addition, the risks of drawing blood include pain, bruising, lightheadedness, and/or fainting, or rarely, infection at the site of the needle stick. Study subjects will be exposed to low doses X-ray radiation during the dual-energy X-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (HR-pQCT) scans. The amount of radiation has a low risk of harmful effects. Based upon the review of the published literature and our pre-clinical as well as clinical data, we do not anticipate the occurrence of serious adverse events with any of the procedures or prescribed drug dosing regimen in this study. We have identified a variety of drug-drug interactions for which we established exclusion criteria or modification plans to minimize associated risks. Therefore, the overall procedure risks and any risks of using either drug regimen are likely minimal compared to the anticipated benefits and the knowledge that may be gained from this clinical investigation.

2 Study Objectives

Primary Objective

In a cohort of 60 older postmenopausal women, aged ≥60years, with a high senescent cell burden (as these individuals are most likely to benefit from pharmacological interventions that eliminate senescent cells – *i.e.,* senolytics), we will test the efficacy of intermittent senolytic therapy (D+Q) as compared to non-interventional therapy on improving serum bone resorption markers over the course of 20 weeks.

Secondary Objective

In the same cohort of 60 older postmenopausal women, aged ≥60years, with a high senescent cell burden, we will further assess the impact of senolytics (D+Q) as compared to non-interventional therapy on reducing systemic surrogate markers of senescent cell abundance and circulating senescence-associated secretory phenotype (SASP) factors.

3 Study Design

3.1 General Description

We will conduct a 20-week, open label, RCT comparing D+Q to the control group for improving bone turnover markers and skeletal parameters and in reducing systemic surrogate markers of senescent cell abundance and the SASP in 80 older postmenopausal women with an underlying senescent cell burden based on *p16*^{INK4a} mRNA expression in PBTL (>95th percentile of same sex young controls; cut-off is shown in **Fig. 6**). All studies will be performed at the Mayo Clinic outpatient Clinic Research Unit (CRU) following IRB approval. At screening, the nurse study coordinator will explain the study, including potential benefits and risks to volunteer subjects. After obtaining informed written consent and following comprehensive screening studies (including blood draw after 12-hr overnight fast for the *p16*^{INK4a} PBTL assay and clinical chemistries), subjects who meet eligibility criteria (as detailed below) and agree to take the assigned therapy for the entire study duration, will be randomized to one of two groups: i) untreated control group; ii) D (100 mg/d, for two days, Sprycel, Bristol Myers Squibb) plus Q (1000 mg/d; 250 mg capsules x 4/d Quercetin Phytosome, Thorne Research). As detailed above, D therapy will be taken orally on an intermittent schedule (every 27 days) with no-therapy periods in between doses, repeated every 28 days over 20 weeks, resulting in ten total dosing days throughout the entire intervention, whereas Q therapy

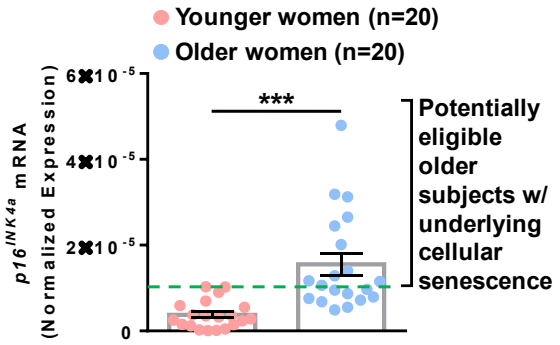

Fig. 6. *p16*^{INK4a} mRNA in PBTL as a biomarker to permit detection of *in vivo* human cellular senescence. Dashed line = 95th percentile of younger women. Mean ± SEM. ***p<0.001.

will be taken orally on an intermittent schedule (every 25 days) with no-therapy periods in between doses, repeated every 28 days over 20 weeks, resulting in 15 total dosing days throughout the entire intervention. Our proposed intermittent dosing regimen is based on the following: i) effects of D+Q on various senescent cell types in culture[31,34,40]; ii) our preclinical studies in which senolytics were administered monthly to naturally aged old WT mice (**Fig. 3**); iii) senescent cell clearance from freshly isolated human tissue explants treated with D+Q[39] or F[40]; iv) peak concentrations and the short elimination half-lives (<12 hrs.) of these senolytics in humans[89,90]; and v) dose escalation studies using these senolytics in old mice and old Rhesus monkeys. Block randomization will be performed by our statistician, Ms. Elizabeth Atkinson, M.S., to ensure balanced group assignment as the study proceeds. The first dose of each dosing regimen will be administered onsite by the nurse study coordinator who will provide eligible, randomized subjects with a screw cap bottle(s) from the Mayo Clinic Research Pharmacy containing their group allocated therapy (D+Q). Throughout the study, all subjects will be instructed to take 1000 IU of Vitamin D (supplied to them) and will be counseled to obtain total calcium intake of ~1200 mg/d. At each visit, a fasting morning blood draw will be obtained. Subjects will be given a blank notebook for recording AEs that the study team will review and document at each visit. The primary study endpoints will be percent changes in serum bone turnover markers (amino-terminal propeptide of type I collagen [P1NP] and C-terminal telopeptide of type I collagen [CTX]) from baseline over 20 weeks; changes in these markers over intermediate time points (weeks 4, 8, 12, 16) and changes in the P1NP/CTX ratio will be considered secondary endpoints. Additional secondary endpoints will be patient reported quality of life, safety and tolerability and changes in the following from baseline to study endpoint: i) bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) at the lumbar spine, hip (total and femoral neck [FN]), and radius (total and ultra-distal); ii) high-resolution peripheral quantitative computed tomography (HRpQCT)-derived trabecular/cortical bone parameters and micro-finite element analysis (μFEA)-derived bone strength at the radius and tibia; iii) *p16^{INK4a}* mRNA expression in PBTL; and iv) plasma SASP factors.

3.2 Number of Subjects

60 elderly postmenopausal women, aged ≥60 years, with increased senescent cell abundance in blood will proceed to randomization. We will screen 240 elderly postmenopausal women, aged ≥60years, as we anticipate ~50% will have eligible PBTL *p16^{INK4a}* mRNA expression assays. Note that 14 subjects have already completed the F group, but no additional subjects will be enrolled into that group per approval from NIH and our NIH DSMB (see documentation). Data from these 14 subjects will be used as pilot data to design future studies using F, depending on the outcome from D+Q.

3.3 Duration of Participation

Participants will remain in the study for 20 weeks post accrual. In addition, a follow-up phone call will be performed at 1 and 2-year post-study to inquire about any new malignancies that may have occurred since completing the study.

3.4 Primary Study Endpoints

The primary study endpoints will be percent changes in the serum bone resorption marker (C-terminal telopeptide of type I collagen [CTX]) from baseline over 20 weeks.

3.5 Secondary Study Endpoints

Changes in the bone formation marker, amino-terminal propeptid of type I collagen [P1NP] and CTX markers over intermediate time points (weeks 4, 8, 12, 16) and changes in the P1NP/CTX ratio will be considered secondary endpoints. Additional secondary endpoints will be patient reported quality of life, safety and tolerability and changes in the following from baseline to study endpoint: i) bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) at the lumbar spine, hip (total and femoral neck [FN]), and radius (total and ultra-distal); Due to the recent availability of trabecular bone score (TBS) software on the CRTU iDXA as an alternative method for assessment of bone quality from DXA images, we will explore how vertebral TBS compares to the cortical measures (BMSi and cortical porosity) in secondary analyses. Note that TBS requires no additional DXA imaging; it is performed on L_1-L_4 AP spine scans which have already been performed. ii) high-resolution peripheral quantitative computed tomography (HRpQCT)-derived trabecular/cortical bone parameters and micro-finite element analysis (μFEA)-derived bone strength at the proximal and distal radius and tibia; iii) $p16^{INK4a}$ mRNA expression in PBTL; and iv) plasma SASP factors.

3.6 Primary Safety Endpoints

Safety assessments are undertaken with the measurement of safety laboratory tests and procedures, vital signs, and recording of adverse events. In addition, follow-up phone calls will be performed at 1 and 2-year post-study completion to inquire about new malignancies. The following safety labs will be monitored and limits on these are as noted, which would result in withdrawal of the subject from the study: ECG (QTc > 470 msec); CBC (PI's assessment of clinically meaningful changes in blood counts; if ambiguous, this will be reviewed with an independent clinician [Dr. Matthew Drake]); AST (>2x above the normal limit); cystatin C (> 50% increase or reduction of eGFR to below 30 ml/min/1.73 m^2).

3.7 Identification of Source Data

Source data will be obtained from the electronic medical record and documented in study specific case report forms (paper and/or electronic data capture). No information in source documents about the identity of the subjects will be disclosed.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

We will randomize 60 eligible older postmenopausal women aged ≥60years to one of two groups: untreated control group or to D+Q; Inclusion criteria are as follows:

- Able and willing to provide informed consent.
- Normal postmenopausal women.
- Aged ≥60years.

4.2 Exclusion Criteria

- Subjects who are Type II diabetic and on insulin due to a risk of hypoglycemia.
- Hemoglobin A1c ≥8.0% at screening
- Abnormal screening labs (see below): Calcium >10.1 mg/dL, Phosphorus >4.7 mg/dL, Thyroid stimulating hormone (TSH) level <0.3mU/L, Fasting blood glucose >200 mg/dL.

- Presence of significant liver (with total bilirubin >2X upper normal limit, AST >2xupper normal limit, alkaline phosphatase >2x upper normal limit) or kidney disease (see eGFR below). If any elevations were to be noted (>2x the normal level), the study participant would stop treatment and have levels re-drawn in a month, per the clinical judgement of the investigator.
- If any of the laboratory blood work drawn at the study visits return with lab values outside of the "normal limits" or show a significant change from a previous value, a repeat blood draw would be done before the subject is excluded.
- Presence of a clinical diagnosis of heart failure
- Known active malignancy (including myeloma)
- Current diagnosis of Malabsorption or currently undergoing treatment for malabsorption disease
- gastric bypass / reduction
- Hyperthyroidism
- Acromegaly
- Cushing's syndrome
- Hypopituitarism
- Subjects with a fracture within the past six months
- Undergoing treatment with any medications that affect bone turnover, including the following:
    - adrenocorticosteroids (> 3 months at any time or > 10 days within the previous yr., except for use of topical steroid creams or gels or inhaled steroids), anticonvulsant therapy (within the previous year, include only those taking Carbamazepine, Phenobarbital and Phenytoin),
    - bisphosphonates (within the past 3 yrs., including injections or infusions); oral use permitted if taken for less than 1 month,
    - denosumab,
    - estrogen (E) therapy or treatment with a selective E receptor modulator, except for vaginal estrogen cream use, or teriparatide, abaloparatide, romosozumab (>1 month within the past yr.)

- QTc >450 msec
- Current diagnosis of hypo- or hyperparathyroidism or currently undergoing treatment for the disease
- Inability to provide consent
- Inability to tolerate oral medication
- eGFR<30 ml/min/1.73 m^2 (using the cystatin C blood levels for analysis)
- Subjects on therapeutic doses of anti-coagulants (*e.g.* warfarin, heparin, low molecular weight heparin, factor Xa inhibitors, *etc*)

- Subjects with hypovitaminosis D (25-hydroxyvitamin D [25(OH)D] <20 ng/ml, whose level does not improve above 20 ng/ml after two courses of 4-week treatment of 50,000 IU/d of Vitamin D. They will be referred to their primary provider should this occur.
- Subjects taking anti-arrhythmic medications known to cause QTc prolongation
- Subjects taking potentially senolytic agents within the last 6 months: Fisetin, Quercetin, Luteolin, Dasatinib, Piperlongumine, or Navitoclax
- Subjects currently taking drugs that induce cellular senescence: alkylating agents, anthracyclines, platins, other chemotherapy
- Subjects taking H2 antagonists, unless randomized to the control group
- Tyrosine kinase inhibitor therapy
- Subjects not having a PBTL $p16^{INK4a}$ mRNA expression level >95 percentile of young female controls (this cut-off is depicted by the dotted line in **Fig. 6**)
- Known hypersensitivity or allergy to Dasatinib or Quercetin
- Subjects taking the following antimicrobial agents: Aminoglycosides, Azole antifungals (fluconazole, miconazole, voriconazole, itraconazole), Macrolides (clarithromycin, erythromycin), Antivirals (nelfinavir, indinavir, saquinavir, ritonavir, elbasvir/grazoprevir), Rifampin
- 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 necessary from an infectious disease perspective, then they will be allowed only if the levels are therapeutic.
- Subjects taking strong inhibitors of CYP3A4
- 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 study drug dosing periods. Subjects may continue their previous regimen between study drug dosing periods.
- Subjects on quinolone antibiotic therapy for treatment or for prevention of infections within ten days.
- Subjects taking proton pump inhibitors and unwilling to discontinue therapy for one week before and two weeks following dosing.
- Subjects with clinically evident fluid retention
- Subjects with evidence of right heart strain on ECG
- Subjects with a history of pulmonary hypertension
- Subjects with an abnormal Complete Blood Count (clinically insignificant changes would be acceptable based on the judgement of the investigators)
- Presence of any condition the Investigator believes would place the subject at risk or would preclude the subject from successfully completing all aspects of the trial.
- If the DXA assessment reveals a spine or femur neck T-score < -2.5, the participant will be advised of this. She would then be given the option of withdrawing from the study to immediately start an osteoporosis drug through her primary care physician or continue in

the study and defer osteoporosis drug treatment for the duration of the study (20 weeks). Given that osteoporosis is a chronic, long-term disease, the 20-week deferral would pose a minimal risk to the participant and she would be free to make this choice.

Behavioral Modification: Participants will be educated about the risk of excessive caffeine usage. Participants will be encouraged to reduce use by 50% prior to and during the drug dosing periods. Due to drug-drug interaction, subjects may not clear the caffeine from their system properly/as usual. Because of possible effects on the metabolism of dasatinib, subjects will also be advised not to consume grapefruits or grapefruit juice during their participation in the study. Patients will be asked to contact the study coordinator by phone if prescribed any new medication during the trial.

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

Recruitment sources will include: 1) Previous research study participants, 2) flyers, 3) classified advertising, 4) radio advertising, 5) newspaper advertisement, 6) appropriate internet/social media platforms [redacted] 7) elderly population-focused newsletters [redacted]; Family Service Rochester Senior Independence; [redacted]; Rochester Family Service-Neighbors Helping Neighbors; [redacted]; Elder Network; [redacted]), 8) Mayo clinic patients (EMR search)and 9) at off-site campuses (e.g., retirement communities). Permission will be obtained from each location before recruitment begins. Trained clinical coordinators will contact subjects and assist in recruitment and carry-through of the protocol. Description of the studies and review of the informed consent forms with each patient will be conducted in person directly with the clinical coordinators 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. Please see Inclusion and Exclusion criteria above. Documentation of recruitment and enrollment efforts will be maintained in a secure database. We anticipate enrolling 60 subjects (30 per group). All studies will be performed and samples collected in the Clinical Research and Trials Unit (CRTU) at Mayo, with study subjects maintaining their usual diet (including usual calcium intake) and Vitamin D use. Screening will include a brief history, physical exam, ECG, and the following fasting blood screening tests: CBC, HbA1c, serum calcium, creatinine, aspartate aminotransferase, 25(OH)D, fasting blood glucose, bilirubin, serum phosphorus, alkaline phosphatase, thyroid-stimulating hormone and cystatin C.

At the initial screen (Visit 1), subjects will undergo a blood test for peripheral blood $CD3^+$ T cell assay (in PBTL) for $p16^{INK4a}$ mRNA expression and at Baseline (Visit 2) they will undergo a test using an AGES reader. These tests will be analyzed in our laboratory as we have established criteria for determining the presence of significant cellular senescence. If there is absence of cellular senescence in the T cell assay, the subject will not undergo randomization. However, if the T cell assay does reveal cellular senescence (as depicted in **Fig. 6**), then they will proceed to

randomization. The rationale for this selection is that senolytic drugs are unlikely to be effective in the absence of underlying cellular senescence.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

All subjects will be assessed during the three days of medication administration, and subsequent days of medication administration and all study visits. If a severe adverse event (SAE) occurs at any time during administration of the three-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 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 study- related procedures. Some reasons may include:

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

Withdrawn subjects may not re-enter 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 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 appropriate 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, sex, race, and T cell assay senescent cell positivity).

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 receiving intervention (Dasatinib plus Quercetin), for safety monitoring telephone contact will be attempted and subjects will be encouraged to return to complete the laboratory evaluations at the Week 20 (Day 140) visit. No follow up phone call needed beyond four months, except to screen for malignancies at year 1 and 2 post-study completion. It will be highly recommended that withdrawn subjects return for clinical blood (CBC, creatinine, etc.) 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.
- For those subjects in the intervention groups who are not tolerating the medication and considering withdraw, they will be given the option to continue in the study with intent to treat.

5 Study Drug

5.1 Description

Dasatinib (commercially available) 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 (TKI) used to treat cancer, particularly Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) or Ph+ acute lymphoblastic leukemia (ALL). 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. Unlike standard treatment regimens for CML (lasting several months), this medication will only be administered intermittently. Elimination half-life is <12 hours in adults. Similarly, in animal models, drug clearance is observed within 72 hours. Yet, based on animal studies, senescent cell clearance persists for at least ~2-4 weeks.
    a. Dosing: Dasatinib in the form of Sprycel® (Bristol Myers Squibb) 100 mg orally daily for two days, for each of the five dosing courses; 10 total dosing days. One (100 mg) tablet will be taken in the morning, for each dosing period. Thus, Dasatinib will be taken orally on an intermittent, one-day schedule (starting every 27 days) with 26-day no-therapy periods in between dosing regimens, repeated every 28 days over 20 weeks, resulting in ten total dosing days throughout the entire intervention.
2) Quercetin is a natural-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 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.
    a. Dosing: Quercetin dehydrate capsules (250 mg each) equating to 1000 mg total daily dosage will be administered orally for three consecutive days for each of the five dosing periods; 15 total dosing 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 three days, within each dosing period. Thus, Quercetin will be taken orally on an intermittent, three consecutive day schedule (starting every 28 days) with 25-day no-therapy periods in between dosing regimens, repeated every 28 days over 20 weeks, resulting in five total three-day dosing regimens (15 total dosing days) throughout the entire intervention.

5.3 Method for Assigning Subjects to Treatment Groups

Subjects who meet eligibility criteria and agree to take the assigned therapy for the entire study duration, will be randomized (using block randomization, as detailed below) to one of two groups: i) Untreated control group; ii) Dasatinib (D; 100 mg/d, for two days, Sprycel, Bristol Myers Squibb) plus Quercetin (Q; 1000 mg/d; 250 mg capsules x 4/d Quercetin Phytosome, Thorne Research). As detailed above, therapy (D+Q) will be taken orally on an intermittent schedule (every 28 days) with no-therapy periods in between doses, repeated every 28 days over 20 weeks. Block randomization (using blocks of four) will be performed by our statistician, [redacted] M.S., to ensure balanced group assignment as the study proceeds. Subjects

will be randomized in a 1:1 ratio to the untreated control group and D+Q. Randomization data will be provided to the Research Pharmacy by the statisticians.

5.4 Preparation and Administration of Study Drug

The request for the study drugs (investigational products) will be sent to the Research Pharmacy and individually prepared for each subject. This will be managed by the Research Pharmacy according to their established stand procedures. Subjects randomized to the Dasatinib plus Quercetin (D+Q) intervention arm will be provided with one bottle containing two tablets of Dasatinib, and one bottle containing 12 capsules of Quercetin at outpatient CRU study visits at Weeks 1 (Day 1), 5 (Day 29), 9 (Day 57), 13 (Day 85), and 17 (Day 113). Study subjects will be instructed to take the Quercitin within 5 minutes of the Dasatinib and to ingest all the pills/capsules from each bottle each day. They will record their administration in the provided drug diary.

5.5 Subject Compliance Monitoring

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

5.6 Prior and Concomitant Therapy

Drugs listed as part of the exclusion criteria are not permitted during each of the study drug periods. If patients are required to initiate these medications within the study drug period, then they will be removed from the study primarily due to risk of drug-drug interactions.

5.7 Packaging

The investigational products for this study will be delivered to, managed, and packaged by the Research Pharmacy according to their established standard procedures. At outpatient CRU study visits at Weeks 1 (Day 1), 5 (Day 29), 9 (Day 57), 13 (Day 85), and 17 (Day 113), subjects randomized to the Dasatinib plus Quercetin intervention arm will be provided with one bottle containing two tablets of Dasatinib, and one bottle containing 12 capsules of Quercetin. As noted above, 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.

5.8 Masking/Blinding of Study

In order to minimize the study bias, this study will use a process for randomized group assignment to either the untreated control group or to D+Q. Furthermore, the laboratories analyzing the collected samples and initial data will not have access as to which group (Control, D+Q) the samples came from. Since this is an open label study, the subject and study staff will be aware of which group they are in.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 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.

5.9.2 Storage

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

5.9.3 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.

5.9.4 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. Return or Destruction of Study Drug

Table 1. Overview of study visits.

Time (Days) Visits	**Screen** **-90 to 1** 1	**Baseline (Day 1)** **1** 2	**Week 3 (Day15)** **±5** 3	**Week 5 (Day 29) ±5** 4	**Week 9 (Day 57) ±5** 5	**Week 13 (Day 85) ±5** 6	**Week 17 (Day 113) ±5** **7**	**End of Week 20 (Day 140) ±5** 8	**Early Termination**
Informed Consent	X								
Verify Eligibility	X	X							
Patient History	X								
Medication History	X	X	X	X	X	X	X	X	X
ECG	X	X		X	X	X	X	X	X
Physical Exam[a]	X							X	X
Screening Labs[b]	X								
Vital Signs[c]	X	X	X	X	X	X	X	X	X
p16Ink4a in T Cells	X								
Randomization		X							
Skin AGES Reader		x						x	X
Urine tests		x	X	X				x	x
Buccal Swab		x	x	X				x	x
Bone Markers[d]		X	X	X	X	X	X	X	X
Plasma SASP Factors		X	X	X				X	X
Safety Labs[e]			X	X	X	X	X	X	X
DXA: full body, spine, hip, radius		X						X	X[>10 weeks]
HR-pQCT[f]		X						X	X[>10 weeks]
Study Drugs		X		X	X	X	X		
Record book		X		X	X	X	X	X	X
New fractures		X	X	X	X	X	X	X	X
Questionnaires[g,h]		X[g,h]		X[g]	X[g]	X[g]	X[g]	X[g,h]	X[g,h]
Adverse Events	X	X	X	X	X	X	X	X	X

a. Blood pressure, temperature, respiration, height, weight, and heart rate
b. Fasting blood glucose, CBC w/differential, creatinine, 25-hydroxyvitam D, AST, hemoglobin A1c, TSH, bilirubin, phosphorus, calcium, cystatin C, and alkaline phosphatase
c. Temperature, blood pressure, pulse, weight, and respiration
d. Fasting blood for plasma and cells for future research/bone markers (P1NP & CTx)
e. CBC, AST, cystatin C
f. High-resolution peripheral quantitative computed tomography, radius & tibia
g. SF 36 QOL
h. Health interview (baseline only). Geriatric depression scale and Pittsburg Fatigability questionnaires

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 Visit

A tabulated summary of all visits and assessments described in the following sections is provided in **Table 1**. To the extent possible, subjects will be expected to adhere to the established visit schedule. After discussing the study with the investigator/appropriate study staff and after agreeing to study participation by signing the consent, subjects will be assigned a subject number. For any subject, it is the responsibility of the investigator or study team member to obtain written informed consent (subject's signature) prior to performing any protocol-mandated assessment. However, assessments performed as part of the routine care of the subject may be used to assess eligibility. The subject number will identify the subject throughout the study. In case of re-screening, the subject number assigned during the first screening procedure will be retained.

Patients meeting the entry criteria and consenting to participate in the protocol will undergo the following:

- Eligibility confirmation and informed consent discussion and documentation.
- Review of medications.
- Medical History.
- EKG/ ECG – assessment of QTc interval.
- Fasting Blood work – screening labs, peripheral blood CD3+ T cell assay for $p16^{INK4a}$.
- Vital signs (blood pressure, temperature, height, weight, respiration, and heart rate).
- Query subject concerning adverse events.

6.2 Visit 2 – Baseline/Enrollment (Start of Week 1, Day 1)

Patients who meet eligibility criteria during Visit 1 will attend Visit 2. This visit will be recorded as "Day 1" and will require a "fasting" visit to the outpatient CRTU research center at Mayo Clinic where the following tests will be performed:

- Eligibility confirmation.
- Health interview, Geriatric depression scale and Pittsburg Fatigability scale questionnaires
- Vital signs (blood pressure, temperature, respiration, weight, and heart rate).
- Review of medications.
- SF 36 (QOL) Health Survey.
- Query subject concerning adverse events: performed by a "blinded" staff member.
- Blood work (P1NP, CTx, SASP markers).
- Skin AGES – Skin AGEs will be assessed using the AGE Reader®.
- DXA scans (Total Body, Spine [AP], Hip, & Forearm).
- New fracture assessment.
- Xtreme CT high-resolution peripheral quantitative computed tomography (HR-pQCT) scans (distal and proximal radius & tibia).
- ECG to monitor QTc prolongation related to dasatinib
- Urine tests (senescent markers, urine stored for future research)
- Buccal swab (senescent markers and biological clock)

- Plasma & Cells for future research.
    - This is a routine procedure and will be performed by an experienced phlebotomist using aseptic technique. About 100 mL of blood will be withdrawn in total, which poses no significant risk. Blood will be collected and stored until analysis.
- Investigation product administration – subjects will be provided with their randomized study drug (D+Q) in a sealed bottle(s) from the Mayo Clinic Research Pharmacy on Day 1 in the presence of a study coordinator. Subjects must consume all medications within the allotted visit time, taking Quercitin within 5 minutes of Dasatinib. Administration verification will be documented on the drug diary form. Subjects will be sent home with the remaining two days of study drugs (Day 2, 3), the drug diary form and the record book (for adverse event recording). Drug education will be provided by the study coordinator to those subjects receiving D+Q.
- ECG to monitor QTc prolongation related to dasatinib.

6.3 Visit 3 – Start of Week #3 (Day15 ± 5 Days)

This visit will be recorded as "Day 15". A summary of the events during this day are below:

- Vital signs (blood pressure, respiration, temperature, weight, and heart rate).
- Review of any additional medications taken since last visit.
- New fracture assessment.
- Query subject concerning adverse events: performed by a "blinded" staff member.
- SF 36 (QOL) Health Survey
- Blood work (P1NP & CTx; safety labs for possible dasatinib toxicities – CBC, AST, and cystatin C.
- Plasma & Cells for future research: 50 ml blood drawn to be stored until analysis
- Urine tests (senescent markers, urine stored for future research)
- Buccal swab (senescent markers and biological clock)

6.4 Visit 4 – Start of Week #5 (Day 29 ± 5 Days)

This visit will be recorded as "Day 29". A summary of the events during this day are below:

- Vital signs (blood pressure, respiration, temperature, weight, and heart rate).
- Review of any additional medications taken since last visit.
- New fracture assessment.
- Query subject concerning adverse events: performed by a "blinded" staff member.
- SF 36 (QOL) Health Survey.
- Blood work (P1NP & CTx; safety labs for possible dasatinib toxicities – CBC, AST, and cystatin C.
- Urine tests (senescent markers, urine stored for future research)
- Buccal swab (senescent markers and biological clock)
- ECG to monitor QTc prolongation related to dasatinib
- Investigation product administration – subjects will be provided with their randomized study drug (D+Q) in a sealed bottle(s) from the Mayo Clinic Research Pharmacy on Day 29 in the presence of a study coordinator. Subjects must consume all medications within the allotted visit time, taking Quercitin within 5 minutes of Dasatinib. Administration verification will be documented on the drug diary form. Subjects will be sent home with the remaining two days of study drugs (Day 30, 31), the drug diary and the record book.

Drug education will be provided by the study coordinator to those subjects receiving D+Q.

- Plasma & Cells for future research: 50 ml blood drawn to be stored until analysis

6.5 Visit 5 – Start of Week #9 (Day 57 ± 5 Days)

This visit will be recorded as "Day 57". A summary of the events during this day are below:

- Vital signs (blood pressure, respiration, temperature, weight, and heart rate).
- Review of any additional medications taken since last visit.
- New fracture assessment.
- Query subject concerning adverse events: performed by a "blinded" staff member.
- SF 36 (QOL) Health Survey.
- Blood work (P1NP & CTx; safety labs for possible dasatinib toxicities – CBC, AST, cystatin C).
- ECG to monitor QTc prolongation related to dasatinib.
- Investigation product administration – subjects will be provided with their randomized study drug (D+Q) in a sealed bottle(s) from the Mayo Clinic Research Pharmacy on Day 57 in the presence of a study coordinator. Subjects must consume all medications within the allotted visit time, taking Quercitin within 5 minutes of Dasatinib. Administration verification will be documented on the drug diary form. Subjects will be sent home with the remaining two days of study drugs (Day 58, 59), the drug diary form and the record book. Drug education will be provided by the study coordinator to those subjects receiving D+Q.
- Plasma & Cells for future research: 50 ml blood drawn to be stored until analysis

6.6 Visit 6 – Start of Week #13 (Day 85 ± 5 Days)

This visit will be recorded as "Day 85". A summary of the events during this day are below:

- Vital signs (blood pressure, respiration, temperature, weight, and heart rate).
- Review of any additional medications taken since last visit.
- New fracture assessment.
- Query subject concerning adverse events: performed by a "blinded" staff member.
- SF 36 (QOL) Health Survey.
- Blood work (P1NP & CTx; safety labs for possible dasatinib toxicities – CBC, AST, creatinine).
- ECG to monitor QTc prolongation related to dasatinib.
- Investigation product administration – subjects will be provided with their randomized study drug (D+Q) in a sealed bottle(s) from the Mayo Clinic Research Pharmacy on Day 85 in the presence of a study coordinator. Subjects must consume all medications within the allotted visit time, taking Quercitin within 5 minutes of Dasatinib. Administration verification will be documented on the drug diary form. Subjects will be sent home with the remaining two days of study drugs (Day 86, 87), the drug diary form and the record book. Drug education will be provided by the study coordinator to those subjects receiving D+Q.
- Plasma & Cells for future research: 50 ml blood drawn to be stored until analysis

6.7 Visit 7 – Start of Week #17 (Day 113 ± 5 Days)

This visit will be recorded as "Day 113". A summary of the events during this day are below:

- Vital signs (blood pressure, respiration, temperature, weight, and heart rate).
- Review of any additional medications taken since last visit.
- New fracture assessment.
- Query subject concerning adverse events: performed by a "blinded" staff member.
- SF 36 (QOL) Health Survey.
- Blood work (P1NP & CTx; safety labs for possible dasatinib toxicities – CBC, AST, cystatin C).
- ECG to monitor QTc prolongation related to dasatinib.
- Investigation product administration – subjects will be provided with their randomized study drug (D+Q) in a sealed bottle(s) from the Mayo Clinic Research Pharmacy on Day 113 in the presence of a study coordinator. Subjects must consume all medications within the allotted visit time, taking Quercitin within 5 minutes of Dasatinib. Administration verification will be documented on the drug diary form. Subjects will be sent home with the remaining two days of study drugs (Day 114, 115), the drug diary form and the record book. Drug education will be provided by the study coordinator to those subjects receiving D+Q.
- Plasma & Cells for future research: 50 ml blood drawn to be stored until analysis

6.8 Visit 8 – End of Week #20 (Day 140 ± 5 Days) or Early Termination

This visit will be recorded as "Day 140". A summary of the events during this day are below:

- Vital signs (blood pressure, respiration, temperature, weight, and heart rate).
- Review of medications.
- New fracture assessment.
- Geriatric depression scale and Pittsburg Fatigability Scale questionnaires
- SF 36 (QOL) Health Survey.
- Query subject concerning adverse events: performed by a "blinded" staff member.
- Blood work (P1NP, CTx, SASP markers; safety labs for possible dasatinib toxicities – CBC, AST, cystatin C).
- ECG to monitor QTc prolongation related to dasatinib.
- Skin AGES – Skin AGEs will be assessed using the AGE Reader®.
- DXA scans (Total Body, Spine [AP], Hip, & Forearm).
- Xtreme CT high-resolution peripheral quantitative computed tomography (HR-pQCT) scans (distal and proximal radius & tibia).
- Plasma & Cells for future research: 100 ml blood drawn to be stored until analysis.
- Urine tests (senescent markers, urine stored for future research)
- Buccal swab (senescent markers and biological clock)

6.9 Assessments

An electrocardiogram (ECG) will be conducted at screening/enrollment on all subjects and during visits 3-8 for the Dasatinib-randomized group members only. A timer will be set to ensure this is

done 2 hours after dosing. A QTc interval greater than 450 msec will require exclusion from the study at screening and greater than 470 msec during visits 3-8, will require withdrawal for the Dasatinib-randomized group members.

6.9.1 Examinations and Procedure

Vital signs, including pulse, blood pressure, heart rate, respiratory rate will be measured. Height and weight will be obtained at the pre-screening visit; weight will be obtained at each additional visit. Body mass index (BMI) will be recorded at screen only.

6.9.2 Laboratory Assessments

The following will be collected at the time points specified in the schedule of events (**Section 6, Table 1**)

Screening Tests:

- CBC w/diff
- Creatinine
- Vitamin D2 & D3
- AST
- Alkaline Phosphatase
- Bilirubin
- TSH
- Cystatin C
- HbA1c
- Calcium
- Phosphorus
- Fasting blood glucose

Blood Tests:

- Kidney Function: creatinine, cystatin C
- Bone Mineral Metabolism: calcium
- Anemia: complete blood count
- Senescence: Senescence (*p16^{INK4a}* in T cells) and SASP markers (including IL-6, IL-8, MCP-1, Activin A)

Urine Tests:

- Senescent markers: urinary exosomes
- Extracellular vesicles

Buccal swab: Testing will occur for determination of biological clock marker. This is an epigenetic test measuring molecules and is associated with predicting epigenetic age.

Peripheral blood CD3^{+} T cell assay for *p16^{INK4a}*:

10ml blood will be collected using EDTA tubes to measure *p16^{INK4a}* positive lymphocyte population in the peripheral blood- a biomarker of senescence and chronological aging. CD3 +lymphocyte will be collected using Whole Blood CD3, Human Microbeads (Miltenyi Biotec,

Cat# 130-090-874) and magnetic-activated cell sorting (MACS) machine. CD3 positive cells will be lysed and RNA will be isolated. *P16^{INK4a}* expression will be measured by rt-qPCR using Taq-man primer-probes, as done in **Fig. 6**.

Future Research Studies – Plasma & Cells:

- Collection of plasma, serum, and cells 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 100 mL plasma/serum will be obtained from each subject at Baseline and End of Week 20 (Day 140). The specimens will be kept on ice and immediately transported to Dr. Khosla's laboratory for processing and storage.

6.9.3 Questionnaires:

The Short Form (36) Health Survey will be administered to subjects at each visit. This survey consists of 36 questions and eight scaled scores (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health). The Geriatric Depression Scale is a 15-item screening tool which can be used to identify depression in older adults. This will be given at baseline and study end visits. If the GDS questionnaire is indicative of depression (score ≥ 10), the study team will make the subject aware of this and contact the subject's primary care provider. If the subject does not have a primary care provider, then consultation with psychiatry will be offered. If there are concerns regarding acute suicidal risk, the study team will contact one of the PIs who will then make appropriate arrangements for acute management.), diarrhea and sleep disorders.
The health interview includes questions about general health, gynecological history, social habits, and fall or fracture history and will be given at the baseline visit. The Pittsburg Fatigability Scale is a 10-item questionnaire that assesses self-report whole-body physical and mental tiredness related to activities of fixed intensity and duration in adults age ≥60.

6.9.4 Skin AGEs Assessment:

Skin AGEs will be assessed using the AGE Reader®. This non-invasive method has a light source which illuminates a skin surface of approximately 4 cm^2 on the volar side of the forearm. The device uses an excitation light source with peak intensity of approximately 370 nm to excite fluorescent moieties in the tissue (skin auto-fluorescence), which then will emit light of a different wavelength. In the used wavelength band, the major contribution in fluorescence comes from fluorescent AGEs linked mostly to collagen. Emission light and reflected excitation light from the skin are measured with a spectrometer in the 300–600 nm range.

7 Statistical Plan

In the case of an adverse event, information related to that particular patient will be revealed to the investigators.

7.1 Sample Size Determination

Based on our recent 20-week RCT in postmenopausal women treated with β-blockers[93], we calculated the sample size to compare the percent change from baseline to 20 weeks in CTX between the senolytic (D+Q) and the untreated control group, while allowing for an estimated

withdrawal rate of 10% (which is realistic given the relatively short trial duration) across the two groups. This calculation demonstrated that 30 subjects per group will provide the study with 90% power to detect a 19.8% difference in the bone resorption marker, CTX (17.1% with 80% power), based on a two-sample *t*-test.

7.2 Statistical Methods

Descriptive Statistics

For the analyses, categorical data will be described by counts and percentiles and quantitative data by means and standard errors or standard deviations. Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated for the different groups. Adherence to medication regimes will be described in terms of percent of medication taken (actual or reported) for each group.

Handling of Missing Data

All participants who are randomized will be included in the primary efficacy analysis dataset. The analysis will follow the intent-to-treat (ITT) principle (*i.e.,* according to the treatment arm they are assigned to). For patients whose 20-week CTX and P1NP measurements are missing, data will be imputed. For subjects missing the 20-week measurements, individual regression models will be fit regressing each specific bone turnover marker (CTX or P1NP) on time (weeks); 20-week values will then be extrapolated based on these regression lines.

Primary Hypothesis: In a cohort of 60 older postmenopausal women, aged ≥60years, with a high senescent cell burden (as these individuals are most likely to benefit from pharmacological interventions that eliminate senescent cells – *i.e.,* senolytics), we hypothesize that intermittent senolytic therapy (D+Q) as compared to non-therapy will improve bone resorption markers over the course of 20 weeks.

The Primary Hypothesis will be tested using an ANCOVA model with treatment as a fixed effect and the baseline measurement as a covariate, followed by an F-test to determine the overall difference between the groups. The 20-week percent changes in secondary endpoints will also be examined using this ANCOVA model. Although randomization should ideally obviate the need to correct for baseline factors, given two groups and 30 subjects per group, there is the possibility that randomization may not perfectly balance the subjects regarding potential covariates, such as the baseline values of the respective response variables. A detailed analysis of ANOVA versus ANCOVA by Van Breukelen[95], concluded that for RCTs with pre- and post-intervention measurements, both methods are appropriate and unbiased, but the ANCOVA has more power and is therefore recommended. All randomized subjects will be included in the primary efficacy analysis, which will follow the intention-to-treat (ITT) principle; *i.e.,* according to the treatment arm to which they were randomized. To optimize the sensitivity of the ITT analysis, every effort will be made to obtain follow-up data at 20 weeks on any subject who discontinues the study at an earlier time point.

Secondary Hypothesis 1: In the same cohort of 60 older postmenopausal women, aged >60years, with a high senescent cell burden, we hypothesize that intermittent senolytic therapy (D+Q) as compared to non-therapy will reduce systemic surrogate markers of senescent cell abundance ($p16^{INK4a}$ in PBTLs) and circulating senescence-associated secretory phenotype (SASP) factors.

The Secondary Hypotheses will be tested using an ANCOVA model with treatment as a fixed effect and the baseline measurement as a covariate. For secondary exploratory analyses, a mixed-effects model will be used to allow the treatment effect to vary across time and to include all subjects (*i.e.,* even those who did not contribute data at all time points). All subjects who receive any drug (D+Q) will be included in the safety and tolerability evaluations. Frequency tables will summarize AE occurrences by treatment arm, though group differences are likely since the treatment arm is known to the subjects.

7.3 Subject Population(s) for Analysis

Analysis of the primary hypothesis will include subjects who have completed the final visit (at Week 20; Day 140), regardless of study drug adherence. Analysis of the secondary hypotheses will follow an intent-to-treat (ITT) model and will include all subjects who have completed at least one follow-up visit following initial randomization, again regardless of drug adherence. Secondary analysis will include only those subjects who completed the entire study drug course and were seen at all visits.

8 Safety and Adverse Events

Safety evaluations will include adverse event (AE) and serious AE (SAE) reporting. Adverse event reporting will be conducted throughout the study for all subjects. The reporting period begins at the time of informed consent and continues through study completion. Adverse events will be assessed at every visit (except the screen visit) by a "blinded" staff member.

Definitions, documentation and reporting of AE's are described in Section 8.1-8.4.

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 disability or incapacity
- 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 30 days following the last administration of study treatment.

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.

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

A clinical laboratory abnormality should be documented as an adverse event if the abnormality changes from a value within the normal range before treatment to one outside or worse than the normal range after treatments. A clinical laboratory abnormality will also be documented as an adverse event if the abnormality meets the definition of an SAE or requires the subject to have the investigational product discontinued or interrupted or requires the subject to receive specific corrective therapy. Such changes will prompt a repeat test, telephone call to check on the subject's status, a repeat visit and/or referral to the subject's primary care physician. For example, the subject will receive specific corrective therapy if their Vitamin D level is <20ng/mL, as described in **Section 4.2, Exclusion Criteria**. In the event that a subject experiences an elevated liver function test (AST, ALT, ALP, and/or bilirubin) greater than 2-fold over their screening/baseline measurement, dosing with Dasatinib should be halted and the subject will continue in the trial and complete the study assessments including laboratory assessments. Additional treatment and continued participation will be at the discretion of study investigators.

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.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

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 will be obtained 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, 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 during the Adverse Event Reporting Period is 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 appropriate 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 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-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's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention*):
- If the adverse event was expected:
- 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 sponsor-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 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 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.

The sponsor-investigator must also notify the FDA (and sponsors must notify all participating investigators) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under § 312.32(c)(1)(i)-(iv).

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 20 weeks of study drug administration (D+Q) and during each study visit following administration of each cycle of drugs. If a severe adverse event occurs at any time during administration of the 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 severe adverse events accrue, the 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 Mayo IRB. If any elevations were to be noted (>2x the normal level), the study participant would stop treatment and have levels re-drawn in a month, per the clinical judgement of the investigator.

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 Internal Data and Safety Monitoring Board

None.

8.5.2 Independent Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be formed to monitor the study. Members will be determined and a charter developed once the NIH grant has been funded.

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 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 ensuring inclusion/exclusion criteria and review of adverse events/toxicity. All data requested on the CRF will 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 will be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. Errors will not be erased and "white-out" will not be used to correct errors. For clarification of illegible or uncertain entries, the clarification will be printed above the item, initialed, and dated. If the reason for the correction is not clear or needs additional explanation, details will be added related to the justification for 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 Processing
All data will be housed and analyzed by statistician, as detailed in **Section 7**.

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 will be 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. 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.

Data Clarification Process
To be determined.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents. The sponsor-investigator will retain the specified records and reports for:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual "Retention of and Access to Research Data Policy" [redacted]

Whichever is longer.

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.

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 will be funded by the NIH (National Institutes of Health).

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

Remuneration will consist of up to $400.00 if subjects complete the entire study and will be explained in the informed consent form. Subjects will receive remuneration for each visit as follows:

1. Screen $25.00

2.	Baseline (Day 1)	$50.00
3.	Start of Week 3 (Day 15)	$25.00
4.	Start of Week 5 (Day 29)	$50.00
5.	Start of Week 9 (Day 57)	$50.00
6.	Start of Week 13 (Day 85)	$50.00
7.	Start of Week 17 (Day 113)	$50.00
8.	Week 20 (Day 140) or early termination	$50.00

Completion of the study period means that the subject followed all study-related procedures for each day as described in the consent form. In a token of appreciation, subjects will receive an additional $50.00 for completing all visits in the 20-week period. In addition, subjects will receive parking passes or taxi reimbursement for the time involved with completing the study visits or travels to the research center for study visits. Subjects will be directed where to park in order to receive the parking passes.

13 Publication Plan

The principal investigators, Drs. Khosla and Farr, hold 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 outcome.

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