Supplemental Information

Rapalog Pharmacology (RAP PAC) Study Design and Methods

Experimental Design and Allocation

RAP PAC will follow a Bayesian Optimal Interval Design (BOIN)[1] to perform a phase I, dose finding clinical trial in healthy older men and women (n=72, 55-80yrs) to identify a recommended phase 2 dose (RP2D) for the mTOR inhibitors rapamycin and everolimus. For each mTOR inhibitor, we intend to test 3 dose levels (5, 10, 15 mg/week) across 6 weeks on treatment and 2-4 weeks follow-up after cessation of treatment. Participants will take 1 mg tablets to reach the desired dose. We will specifically focus on weekly dosing regimens due to data by our team in mice[2,3] and others in humans[4] that suggest intermittent dosing strategies may enable selective inhibition of mTORC1 and decrease off-target side effects largely mediated by mTORC2 inhibition. The dose limited toxicity (DLT) rate limit for RP2D is 0.3 per sex per drug, and the maximum sample size is 18 participants per sex per drug. **Figure S1** shows the schema of the design. The trial starts at the lowest dose level of 5 mg/week and we will enroll and treat 3 women and 3 men to identify different toxicity profiles by sex. DLTs are defined as ≥Grade 2 adverse event following the common terminology criteria for adverse events (CTCAE v6.0). RAP PAC uses Grade 2 adverse events in healthy older adults because these AEs are bothersome and may interfere with some activities of living but are not typically considered dangerous nor prevent daily activities. We view this as the appropriate risk to potential benefit ratio for this trial in healthy adults.

The dose can be escalated, maintained, or de-escalated from the current dose based on the cumulative DLT rate calculated by total number of participants who experienced DLT at the current dose divided by the total number of participants at the current dose. The subsequent cohort of 3 individuals will receive a dose according to the cumulative DLT rate of the preceding cohorts. If the cumulative DLT rate is ≤ 0.236 the dose is escalated, if the cumulative DLT rate is >0.236 to <0.359 the dose is maintained and if the cumulative DLT rate is ≥ 0.359 the dose is de-escalated. If the DLT rate of the 5 mg/week is ≥ 0.359 or the DLT estimate of the 15 mg/week is ≤ 0.236, the dose remains at the same dose level. To avoid assigning many patients to a toxic dose, we additionally impose the dose elimination rule for the dose level j or higher satisfying the posterior probability that DLT rate of dose level j is larger than 0.3 is larger than 0.95. Two hypothetical scenarios are shown in **Figure S1** that arrive at either a RP2D of 5 or 15 mg per week.

All clinical trial activities will take place at the University of Wisconsin-Madison. RAP PAC has received approval by the Health Sciences IRB at UW-Madison (2023-0275) and is registered on clinicaltrials.gov (NCT05949658). Administration of once weekly sirolimus and everolimus will be conducted under the approved FDA Investigational New Drug application 166577 according to 21 CFR 312. RAP PAC will be monitored by an internal Central Monitoring Services required for FDA regulated research at UW-Madison and by an external Data Safety Monitoring Board appointed by the National Institute on Aging.

Dose Selection

Sirolimus and everolimus share a central macrolide chemical structure. Everolimus is a hydroxyethyl ester derivative with a different functional group added at C40. Everolimus has greater absorption, bioavailability, and clearance compared to sirolimus. Therefore, everolimus has a shorter half-life and faster elimination after the last dose than sirolimus. The shared macrolide structure of both everolimus

and sirolimus permit binding to FKBP12 to inhibit mTORC1, however, everolimus has a shorter half life (Everolimus: 28±4 hrs vs. Sirolimus: 62±16 hrs) which could be why 3 weeks of daily everolimus may have more selective inhibition on mTORC1 versus mTORC2 inhibition in mice compared to daily sirolimus[2].

The product label of rapamycin (sirolimus) recommends a daily dose of 2-5 mg, while everolimus is recommended at a daily dose of 1.5-10 mg given in 1-2 doses. The target trough concentration of both rapamycin and everolimus ranges around 5-15 ng/mL, depending on the indication. The majority of the available rapamycin PK data are from patients with renal transplant while there are no published data in otherwise healthy older adults. Using previously published PK models in this population[5–7], study pharmacist (SYL) performed PK simulations to compare the drug exposure parameters of 2 mg daily dosing and various intermittent rapamycin dosing regimens (5-15 mg per week) (**Table S1**). The 2 mg daily and the 15 mg weekly dose have similar predicted average exposures as estimated in average concentration at steady-state (C_{avg_ss}), and 28-day cumulative area under the curve ($AUC_{0\text{-}28d}$). Because of the higher doses and the longer interval between doses, once weekly dosing produced significantly higher maximum concentration at steady-state (C_{max_ss}) and lower minimum concentration at steady-state (C_{min_ss}) compared with the 2 mg daily dose. Everolimus also has a smaller volume of distribution (which contributes to a shorter elimination half-life), a higher C_{max_ss} and a lower C_{min_ss} compared with rapamycin. While higher C_{max_ss} from *daily* rapamycin dosing has been associated with renal toxicity, such risk in *weekly* rapamycin and everolimus dosing is unknown[5,8,9]. Trough concentrations (C_{min_ss}) of rapalogs are typically used for therapeutic monitoring and linked with side effects. Therefore, a dosing scheme with lower trough concentrations may be favorable to decrease adverse events.

Based on PK data obtained from renal transplant patients, oral 5 mg weekly dosing is selected as the starting dose for both rapamycin and everolimus. The simulated C_{max_ss} values are expected to be relatively low and C_{min_ss} (trough concentration) are <1.5 ng/mL. Therefore, minimal side effects are expected, which is consistent with everolimus treatment in healthy older adults[4]. Although 5, 10, and 15 mg doses have overlapping C_{max_ss} due to variability in the PK of mTOR inhibitors, the C_{max_ss} ranges, using sirolimus as an example, are estimated to provide incremental increases (~24 ng/ml per dose escalation). To assess the impact of C_{max_ss} on tolerability, 10 mg weekly dose is selected as next escalated dose. 15 mg weekly dose is the maximal dose because its exposure is equivalent to the 2 mg daily dose, which provides therapeutic effects in patient populations. 20 mg weekly dose was not considered since everolimus (20 mg/week) increased the rate of mild to moderate adverse effects and did not improve flu vaccine efficacy in older adults[4]. Although the adverse events were not serious with 20 mg/week of everolimus, the risk to benefit ratio does not warrant placing relatively healthy subjects on a dosing scheme of mTOR inhibitors that doubles the risk for adverse events.

Health and Medical Screening

Volunteers will be solicited from a recruitment list and databases, email, postal mail, and flyers for the study. We aim to complete the 6-week intervention and 2-4 week follow up in 36 participants per study drug (n=72 total; 55-80 yrs old) who are free from overt-chronic diseases. After documentation of informed consent, participants will be queried about their health and physical activity history, including any medical conditions, recent illnesses, hospitalizations, and medications and review of electronic medical records. Height and weight, resting blood pressure, and heart rate are also collected. Participants will complete medical screening, which consists of a pulmonary function test, a resting 12-lead ECG, and a fasting blood draw for HbA1c, complete metabolic panel (CMP), complete blood count with differential,

lipid panel, and insulin. Supervising study physician (NG) will review all information and determine subject eligibility, as defined in **Table S2**. As previously described[10], we will use the CDC and WHO guidelines as the objective distinction of chronic disease versus risk factors for chronic disease. We will screen subjects to eliminate those with a chronic disease (e.g. cardiovascular disease, type 2 diabetes), but will include people with the following risk factors for cardiometabolic disease: family history, physical inactivity, obesity, hyperlipidemia, hypertension, and impaired fasting glucose (<126 mg/dL). Table 2 also includes a list of drugs or drug classes that have been reported to be contraindicated with everolimus, have safety concerns with study procedures, or impact the primary outcomes. We are including subjects that use commonly consumed medications to control cholesterol and some medications to control blood pressure.

Body Composition, Continuous Glucose Monitoring, and Diet

Eligible subjects will complete body composition assessments via dual x-ray absorptiometry (DXA; Lunar iDXA, GE Electric, Boston, MA). After the DXA scan, subjects will wear a continuous glucose monitor (CGM; Dexcom G6 Pro) to evaluate ambulant glucose behavior and variability during 7-10 days to complement the highly controlled standardized oral glucose tolerance test. The CGM provides a high-resolution assessment of glucose behavior by measuring interstitial glucose values every 5 minutes (288 data points per 24 hours). CGM will be set in blinded mode to avoid subjects altering their lifestyle based on glucose results. We will analyze several indices of glucose variability including the range, total standard deviation, postprandial rate of rise, mean daily differences (MODD) and continuous overall net glycemic action over a 4hr and 8hr period ($CONGA_{4h}$ and $CONGA_{8h}$) as measures of inter-day and intra-day variability[12], respectively. Participants will track their diet using a 3-day dietary log and analyzed by a registered dietitian (MT) using ESHA Food Processor Nutrition Analysis Software[13]. Participants will repeat the DEXA, CGM and dietary log during the last week of the intervention. The CGM wear period will occur during the last 7-10 days leading up to the post-intervention skeletal muscle biopsy and oral glucose tolerance test.

Skeletal Muscle Biopsy and Oral Glucose Tolerance Test (OGTT)

All muscle biopsies and OGTTs are completed in the Clinical Research Unit (CRU) within the Institute of Clinical and Translational Research (ICTR) at University of Wisconsin Hospital. A skeletal muscle biopsy and 75-gram OGTT are performed once before and repeated once after the 6-week intervention. The post muscle biopsy and OGTT are completed approximately 2 days after the last study drug administration.

Subjects are asked to refrain from exercise, alcohol, and aspirin for 24 hours prior to the muscle biopsy and OGTT. The night before, participants consume a standardized study meal (~750 kcal) before and after the intervention matched for macronutrient composition (40% carbohydrates) to minimize inter and intra-subject variability. Subjects will arrive to the CRU the next morning (~0700) after an overnight fast where body weight and vitals (temperature, heart rate, and blood pressure) are recorded. After vitals, subjects will provide a urine and saliva sample. After laying supine for ~30 min, a retrograde intravenous catheter is placed in a heated hand vein for repeated arterialized-venous blood sampling. The initial blood samples will be collected for RNA sequencing, metabolomics, lipidomics, mTOR signaling, senescence associated secreted phenotype (SASP) and proposed biomarkers of aging.

Next, a skeletal muscle biopsy sample (100-300mg) will be obtained from the *vastus lateralis* after administration of local anesthetic (1-2% lidocaine without epinephrine) using a 5mm UCH needle

(Millennium Surgical) with manual suction[14,15]. Muscle samples are placed on a culture dish on ice and cleared of visible adipose and connective tissue. Samples are subsequently snap frozen in liquid nitrogen and stored at -80°C for subsequent immunoblotting and multi-omics.

Approximately 1 hour after the muscle biopsy, serial blood samples are obtained -20, -10, and 0 min before and 10, 20, 30, 40, 50, 60, 75, 90, and 120 minutes after the first sip of the glucose drink to measure blood glucose and glucoregulatory hormones. The participants will have 5 minutes to orally consume a 75-gram glucose drink. We will estimate insulin sensitivity using several indices, including the Matsuda Index and the Oral Glucose Sensitivity Index.

Pharmacokinetics and Pharmacodynamics (PK/PD)

Blood for PK/PD measurements will be obtained before, 0.5, 1.5, 4, 48 and 168 hours after the first dose (Day 1) and 6th dose (Day 36). PMBCs will be isolated from blood samples for circulating PD measures. Samples for PK will be sent to the San Antonio Nathan Shock Center to evaluate whole blood rapamycin and everolimus concentrations as previously performed[16]. Weekly therapeutic drug monitoring (TDM) of trough concentrations of sirolimus and everolimus will be completed at the CILA certified UW-Health Clinical Laboratory via Quantitative Liquid Chromatography-Tandem Mass Spectrometry

Pharmacokinetics/Pharmacodynamics (PK/PD) Modeling Overview: Led by study pharmacist (SYL), we will employ multiple PK/PD modeling approaches to quantify the PK and mTORC1 and mTORC2 inhibition following a weekly dose of rapamycin or everolimus. We will first perform a noncompartmental analysis to determine the descriptive PK parameters such as AUC, C_{max}, clearance, and elimination half-life. Then, we will use compartmental population PK modeling to further obtain the PK parameters that describe the time-course of drug blood concentration (**Fig. S2.1**). The inhibition of mTORC1 and mTORC2 depends on the drug concentration, therefore the time-course of the observed mTORC1 and mTORC2 activity depends on the drug PK (**Fig. S2.2**). We will use a *population PK/PD modeling* approach to connect the blood concentration with the observed mTORC1 and mTORC2 activity in the peripheral blood mononuclear cells (PBMC), to determine the pharmacological effects of rapamycin and everolimus (**Fig. S2.3**). Additionally, we will also use a *physiologically-based PK/PD (PBPK/PD) modeling* approach to predict the distribution of rapamycin and everolimus to various organs/tissues. Then, we will assess the mTORC1 and mTORC2 inhibition in various tissues based on the PK/PD relationship determined in PBMC. The predicted drug concentration and mTORC1 and mTORC2 inhibition in muscle tissues will be verified with observed data from muscle biopsies. Using these PK/PD models, we will simulate and select dosing schemes that achieve optimal mTORC1 inhibition with minimal disturbance to mTORC2 signaling for future trials.

Population PK/PD Modeling: The PK/PD data from all subjects receiving either rapamycin or everolimus will be analyzed simultaneously using a nonlinear mixed-effects modeling approach. We will determine the mean PK (e.g., clearance, volume of distribution) and PD (e.g., drug inhibitory capacity and sensitivity) parameters and their associated variability. We will use a two-compartment PK model with first-order absorption[5–7] to describe rapamycin and everolimus drug disposition kinetics (**Fig. S3**). Different PK models such as one- or three-compartment model will be tested to ensure adequate model performance. The mean PK parameters and the PK-related variability (between-subject, between-occasion, and analytical errors) will be determined using the maximum likelihood estimation method[17]. We will also explore the relationship of PK parameters and subject factor covariates (e.g., age and sex). Subsequently, the PK parameters for each participant will be estimated using the empirical Bayesian

estimation[18]. The participant-specific PK parameters and concentration-time profiles will then be connected with their observed mTORC1 and mTORC2 inhibition.

PD markers for mTORC1 (p-S6) and mTORC2 (p-AKT S473) activity in PBMC are collected at the same time points as PK samples. These PD markers are used as the surrogates of short-term mTOR inhibitor efficacy and safety. A turnover PD model has been used to describe the effects of mTOR inhibitors on mTORC1 and mTORC2 and will be used in our analysis (**Fig. S3**)[19]. In the absence of drugs, the baseline levels of mTORC1 and mTORC2 are a balance of their production and elimination (i.e., turnover). This balance is perturbed by mTOR inhibitors. For mTORC1, the concentration-dependent inhibition occurs when rapalogs form a complex with FK506-binding protein 12 (FKBP12) which then binds to the FKBP12-rapamycin binding domain of mTOR located on the surface of mTORC1 (31-33). As such, the drug concentration in the blood is linked to the drug-induced mTORC1 attenuation. For mTORC2, the inhibition appears to be indirect where rapamycin sequesters free mTOR and attenuates the formation of new mTORC2[20]. Therefore, the drug concentration in the blood is linked to the production of mTORC2. By linking the drug concentration and mTORC1 and mTORC2 activity, we will determine the PD parameters such as the maximum drug effect (E_{max}, I_{max}), drug potency (EC_{50}, IC_{50}), rates of mTORC1 and mTORC2 inhibition, and their rates of returning to baseline. These PD parameters will be compared between rapamycin and everolimus. Furthermore, we will explore the association between patient factors (e.g., age, sex) and the PD parameters. Model development and validation will be performed in accordance with FDA Guidance for Industry for population pharmacokinetics[21]. Both the noncompartmental analysis and PK/PD modeling will be performed using Phoenix® WinNonlin® version 8.3 (Certara USA, Inc., Princeton, NJ).

We will then determine whether the magnitude or duration of mTORC1 and/or mTORC2 inhibition are critical factors in mediating the positive effects on aging versus the detrimental side effects. The magnitude or duration of mTORC1 and/or mTORC2 inhibition will be determined by calculating the AUC of such inhibition, and calculating the percent of time the inhibition is over a prespecified threshold (e.g., 10%, 50%, and/or 90%). We will perform exploratory analyses to identify the association between: 1) mTORC1 inhibition and changes in aging-related pathways (e.g., metabolomics, lipidomics), 2) mTORC2 inhibition and observed adverse effects (e.g., elevated triglycerides, glucose). We will also explore the relationship between different drug exposure parameters (eg., AUC, Cmax, Cmin) and mTORC1 and mTORC2 inhibition. Based on these results, we will identify target exposures for safety and efficacy outcomes and perform PK/PD simulations to determine the doses for future trials.

Physiologically-Based PK/PD Modeling: We will further develop a PBPK/PD model to assess drug tissue distribution and the inhibition of mTORC1 and mTORC2 in various tissues (**Fig S4**). PBPK modeling integrates the knowledge of drug characteristics and physiology to predict the drug disposition and is commonly used to support decision-making in drug development and product labeling[22,23]. Rapamycin PBPK models in adults and children have been previously developed and published[24–26]. We will build on these models and extrapolate the rapamycin model to an older adult population by accounting for aging-related physiological changes[27]. Using the PBPK framework developed for rapamycin, we will build a PBPK model for everolimus by adjusting the physicochemical properties and metabolism data. The PBPK models for older adults will be qualified using both PK data from the literature and the current proposal[28,29]. Then, drug tissue distribution will be predicted using a perfusion-limited kinetic model, which uses blood flow, tissue composition, and drug tissue-to-plasma partition coefficient (Kp) to describe drug tissue distribution[23]. This model has been used with rapamycin[26]. We will use the PK/PD relationship

developed using the PBMC data to predict the organ/tissue mTORC1 and mTORC2 inhibition. The drug concentration and mTORC1 and mTORC2 inhibition in muscle tissues from model predictions will be verified with muscle biopsies. As described previously, the relationship between tissue mTORC1 and mTORC2 inhibition (e.g., inhibition AUC) and observed toxicity and efficacy will be examined. PBPK/PD modeling and simulation will be performed using Simcyp Simulator (version 21, SimCYP Ltd, Sheffield, UK).

Adverse Event Monitoring

Numerous side effects, with some being serious, have been reported in patients taking rapamycin and rapamycin analogs at doses consistent with the FDA label. Rapamycin and everolimus have immunosuppressive properties which increases the risk of infection from bacteria, viruses and fungi; decreases the immune response to vaccinations while actively on treatment; and increases risk of cancer, particularly skin cancer. Additionally, the most common side effects may include mouth, tongue, gum blisters, sores or ulcers; weakness or fatigue; fever, cough, headache; nausea, loss of appetite, diarrhea, indigestion; edema; increase in cholesterol and triglycerides; or increase in blood sugar or HbA1c and less likely new-onset diabetes. These side effects are largely based off patient populations being treated for cancer or organ transplant who receive 1.5 to 10 mg of rapamycin or everolimus per day. This study will use weekly administration of mTOR inhibitors in relatively healthy people to help minimize these risks.

A minimum of every 2 weeks, participants will receive a fasted blood draw to determine any changes to blood chemistries, blood cell counts, lipids, and insulin. Additionally, a weekly blood sample will be taken ~24hrs after the last study drug dose to perform therapeutic drug monitoring for everolimus and sirolimus. We will use a 20-item questionnaire to query for potential adverse events and also review diaries with participants to record any self-reported adverse events using Common Terminology Criteria for Adverse Events (CTCAE) V6.0. If needed, the supervising physician will perform a physical exam. All values and AEs will be published upon study completion.

Multi-Omics analysis

We will perform a broad and unbiased multi-omics approach to identify metabolic pathways and biological mechanisms engaged by mTORC1 inhibition by everolimus and sirolimus treatment (Led by CLG). We will collect 3mL of whole blood in a RNA-stabilizing tube, and isolate whole blood RNA. RNA will also be isolated from a portion of the skeletal muscle biopsy samples. RNA samples will be sequenced via Illumina sequencing at the UW-Biotechnology Center, which we have previously utilized for transcriptional profiling studies[30–32]. Analysis of significantly differentially expressed genes (DEGs) will be completed in R using *edge* [33] and *limma*[34]*.* To reduce the impact of external factors not of biological interest that may affect expression, data will be normalized to ensure the expression distributions of each sample are within a similar range, using the trimmed mean of M-values (TMM), which scales to library size. Heteroscedasticity will be accounted for using the voom function, DEGs were identified using an empirical Bayes moderated linear model, and log coefficients and Benjamini-Hochberg (BH) adjusted p-values will be generated for each comparison of interest[35]. DEGs will be used to identify enriched pathways, with both Gene Ontology (for Biological Processes) and KEGG enriched pathways determined for each contrast.

Blood plasma will be collected in a separate tube, and along with a portion of the skeletal muscle biopsy will be processed using a methyl-tert-butyl ether extraction. The organic phase will be used for untargeted lipidomics by quadrupole time of flight liquid chromatography mass spectrometry (QTOF-

LC/MS) while the aqueous phase will be processed for untargeted metabolomics using GCMS. Annotation of the lipids and metabolites will be performed with LipidAnnotator and with a curated personal compound database library, respectively[32,36,37]. Metabolomics and lipidomics data will be normalized and analyzed using the *metabolomics* package in R. Pathway enrichment of significantly altered metabolites and lipids will be conducted using Metaboanalyst[38] and Lipid Ontology (LION) respectively[39].

To identify clusters of physiological and molecular changes that respond similarly to everolimus and/or sirolimus treatment, significantly differentially expressed molecules found between mTOR inhibitors vs control groups will be identified and integrated. Metabolomics, transcriptomics, and lipidomics data will be log2 transformed, z-scale normalized across molecules and samples for each data type individually. Phenotypic data will be similarly z-scale normalized across phenotypes. We will concatenate all four data types for comparison. Correlations will be performed using Spearman's rank, hierarchical clustering will be used and number of clusters will be determined using silhouette scores. For each cluster, the over representation of KEGG pathways from genes will be determined using kegga and the gene ontology terms will be determined using goana from limma.

Surveys on health and geriatric conditions

Participants will also complete a series of validated questionnaires and surveys to understand whether mTOR inhibitors can improve participants perception of their overall health and wellness and select conditions. All questionnaires will be completed during the 2 hour OGTT. Participants will complete the short form 36 (SF36) which asks about eight general health concepts: physical function, general body pain, limitations due to physical health/function, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. SF36 also includes a single item that provides an indication of perceived change in health. We will also ask participants to complete a visual analog scale for bodily pain and questionnaires modeled after the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) to evaluate the impact of mTOR inhibitors on knee and hip pain, stiffness, and physical functioning of the joints. The WOMAC based questionnaire is intended for those with Osteoarthritis or joint pain. Therefore, by expanding to a general population, a limitation of this approach is that we anticipate only observing any potential changes in those with pre-existing joint pain or discomfort. The same is true for when participants complete sex-specific lower urinary tract symptoms (LUTS) questionnaires as well as questions that examine the risk of obstructive sleep apnea (STOP BANG), and sleep duration and quality (PROMIS).

Statistical approach

The primary endpoint is the occurrence of DLT for rapamycin and everolimus. With a sample size of 18 patients per group per sex, DLT is estimated and the 90% confidence interval has a half-width of no longer than 0.194. For example, if 1 of 18 subjects experience DLT, the observed DLT rate is 0.056 with the corresponding 90% confidence interval [0, 0.145); and if 5 DLTs are observed among 18 subjects, then the observed rate is 0.278 and the 90% confidence interval would be (0.104, 0.452). An exact binomial test will be performed based on an alternative DLT rate of 0.056 compared to a null hypothesized rate of 0.3 which is the maximum acceptable DLT rate. A sample size of 18 subjects provides 89.5% power to detect the difference based on a 1-sided test with α=0.05.

Data will be summarized both graphically and numerically using descriptive statistics. The summary statistics (e.g., AUC, mean, SD) will be generated and summarized by dose. The percentage of inhibitions will be modeled using linear regression. Residual plots (e.g., qq-plots and histograms) will

be examined to investigate approximate normality of the measurements and to identify appropriate transformation (e.g., logarithm) as needed. Associations will be summarized using fitted regression coefficients and corresponding 95% confidence intervals. The binary indicator of inhibition will be analyzed with the proportion and frequency. The data will be modeled using logistic regression. All analyses will be repeated stratifying on the sex of the patient to evaluate sex as a biological variable.

Table S1. Simulated Rapamycin exposure of various dosing regimens (n=200 per dose) over 4 weeks				
Dose Regimen	**Cave_ss (ng/mL)**	**Cmax_ss (ng/mL)**	**Cmin_ss (ng/mL)**	**$AUC_{0\text{-}28d}$ (h*ng/mL)**
	Median (5-95th Percentile)			
2 mg/day	8.4 (5.0-13.3)	15.6 (11.2-20.7)	5.96 (2.90-10.7)	5403 (3324-8001)
5 mg/week	3.0 (1.7-4.8)	24.0 (15.6-33.7)	0.45 (0.08-1.52)	2032 (1223-3063)
10 mg/week	6.0 (3.4-9.7)	47.9 (31.3-67.4)	0.90 (0.15-3.04)	4065 (2447-6126)
15 mg/week	9.0 (5.1-14.6)	71.9 (46.9-101.1)	1.36 (0.23-4.55)	6097 (3670-9189)

C_{avg_ss}: Average steady state concentration; C_{max_ss}: maximum concentration at steady-state; C_{min_ss} minimum concentration at steady-state; $AUC_{0\text{-}28d}$: 28-day cumulative area under the curve

TABLE S2
Inclusion Criteria
1. 55-80 years old; free of overt chronic disease
2. Willing to provide informed consent.
3. Willing to comply with all study procedures and be available for the duration of the study.
4. Able to use and be contacted by the telephone
5. Ability to take oral medication.
6. Not planning to change diet or physical activity status
7. Adequate organ function as indicated by standard laboratory tests: hematology (complete blood count), and clinical chemistry.
Exclusion Criteria
1. Heart disease (history, abnormal ECG)
2. Cerebrovascular disease (history)
3. Cancer or less than 5 years in remission (history)
4. Chronic respiratory disease (history, if both FEV1/FVC < 70 and FEV1 < 80% predicted)
5. Chronic liver disease (history, abnormal blood liver panel, ALT >104 IU/L, AST >80 IU/L)
6. Diabetes (history, HbA1C ≥ 6.5%, fasting blood glucose≥126 mg/dl, OGTT ≥ 200 mg/dl at 2 hrs)
7. Alzheimer's (history)
8. Chronic kidney disease (history, abnormal blood kidney panel including serum creatinine > 1.4, eGFR <60 ml/min/1.73m^2, urine protein to creatine ratio of >0.3 mg/mg)
9. Problems with bleeding, on medication that prolongs bleeding time (if subject cannot safely stop prior to biopsy)
10. Taking azathioprine (Imuran), cyclosporine (Gengraf, neoral, Sandimmune), dexamethasone (Decadron, Dexpak), methotrexate (Rhumatrex, Trexall), prednisolone (Orapred, Pediapred, Prelone), prednisone (Sterapred, sirolimus (Rapamune) and tacrolimus (prograf) or other medications proposed to lower immune system. Daily use of high potency topical corticosteroids used on greater than or equal to 10% of body surface area will not be eligible. Nasal sprays or inhaled corticosteroids will be reviewed on a case-by-case basis.
11. Taking strong or moderate CYP3A4 and/or P-glycoprotein (PgP) inhibitors such as ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole, amprenavir, fosamprenavir, aprepitant, erythromycin, fluconazole, verapamil, diltiazem
12. Taking strong CYP3A4 activators such as phenytoin, carbamazepine, rifampin, rifabutin, rifapentine, phenobarbital.
13. Subjects who are not willing to restrict the use of grapefruit, grapefruit juice, cannabidiol, and other foods/substances that are known to inhibit cytochrome P450 and PgP activity and may increase everolimus exposures and should be avoided during treatment
14. Subjects who are not willing to restrict the use of St. John's Wort (Hypericum perforatum) because it may decrease everolimus exposure unpredictably.
15. Subjects who use daily NSAIDs with exception of baby aspirin (81 mg)

16. Subjects who are not willing to avoid blood donations 8 weeks prior to the first visit and 8 weeks after the last visit.
17. Low white-blood cell count (<4,000 cell/μL)
18. History of stomatitis or ulcers in the mouth
19. Those on glucose lowering drugs
20. Participating in intensive exercise training program (high to moderate intensity exercise greater than 150 minutes per week) or planning to start new exercise program during study period.
21. Tobacco use
22. Allergies to lidocaine, everolimus, or sirolimus
23. Subjects currently enrolled in other clinical trials. Subjects may be eligible after a washout period that will be reviewed on a case-by-case basis.
24. Individuals with limited English proficiency
25. Subjects who are planning to have elective surgery 12 weeks prior to or during the intervention

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Figure Legends.

Figure S1 A. Schema for Bayesian Optimal Interval Design (BOIN) to perform a phase I, dose finding trial in healthy older men and women (n=72, 55-80yrs) to identify a recommended phase 2 dose (RP2D) for the mTOR inhibitors rapamycin and everolimus. **B and C** Two different hypothetical scenarios that arrive at different recommended phase 2 dose (RP2D). **B.** In the first scenario,1 participant in each of the first two cohorts both experience a DLT for a DLT rate of 0.33 (2 out of 6) which fits the criteria to maintain the dose level. In the third cohort, there are no DLTs to make the cumulative dose rate 0.22 (2 out of 9) which fits the criteria for dose escalation. The fourth cohort receives a 10 mg/week dose and 2 participants experience DLT for a rate 0.66 (2 out of 3) and meets the criteria for de-escalation. The fifth cohort experiences no DLTs at 5 mg/week for the cumulative DLT rate of 0.16 (2 out of 12) and meets criteria to again escalate to 10 mg/week. The last cohort is treated with 10 mg/week with 1 participant experienced a DLT making the cumulative DLT rate 0.50 (3 of 6). Therefore the 10 mg/week dose exceeds the pre-specified DLT rate while the 5 mg/week is below the prespecified dose rate. Therefore, the 5 mg/week dose is selected as the RP2D. **C.** The first cohort at 5 mg/week experienced no DLTs (0 out of 3) which meets criteria for dose escalation. The second cohort at 10 mg/week experienced no DLTs (0 out of 3) which meets criteria for dose escalation. The 3rd, 4th and 5th cohort at 15 mg/week each experienced 1 DLT (3 of 9) which each meets criteria for dose maintenance. The 6th cohort experienced no DLTs for a cumulative DLT rate of 3 of 12. Therefore, 15 mg/week is below our pre-specified DLT rate of 0.30 and is selected as the RP2D. DLT = dose limiting toxicity. Open circle (O) represents each participant without a DLT and filled circle (●) represents a participant with a DLT.

Figure S2 Population PK/PD modeling is used to connect (1) blood concentration-time data with (2) mTORC1 and mTORC2 activity data in PBMC that are collected at multiple time points, to determine (3) the pharmacological effects of mTOR inhibitors.

Figure S3 Left, a two-compartment model describes drug distribution and elimination after oral administration with first-order absorption rate constant (k_a). Right, blood concentration (C_1) drives the inhibition of mTORC1 and mTORC2 in PBMC. The mTOR inhibitor acutely acts on mTORC1, accelerating the mTORC1 elimination process ("Out" arrow). The drug effect on mTORC1 is described by a function of C_1, maximum effect (E_{max}), and potency of EC_{50} (drug concentration producing 50% of E_{max}). The mTOR inhibitor also binds to free mTOR (a precursor of mTORC1 and mTORC2). Over time, depletion of free mTOR results in reduced mTORC2 production ("In" arrow). Such effect is described by a function of C_1, I_{max} (the maximum inhibitory drug effect) and IC_{50} (drug concentration producing 50% of I_{max}). mTORC1 and mTORC2 activities are linked to geroprotective and adverse effects, respectively.

Figure S4 PBPK/PD modeling leverages physiological and drug data to predict drug tissue distribution and the inhibition of mTORC1 and mTORC2 in various tissues.

FIGURE S1

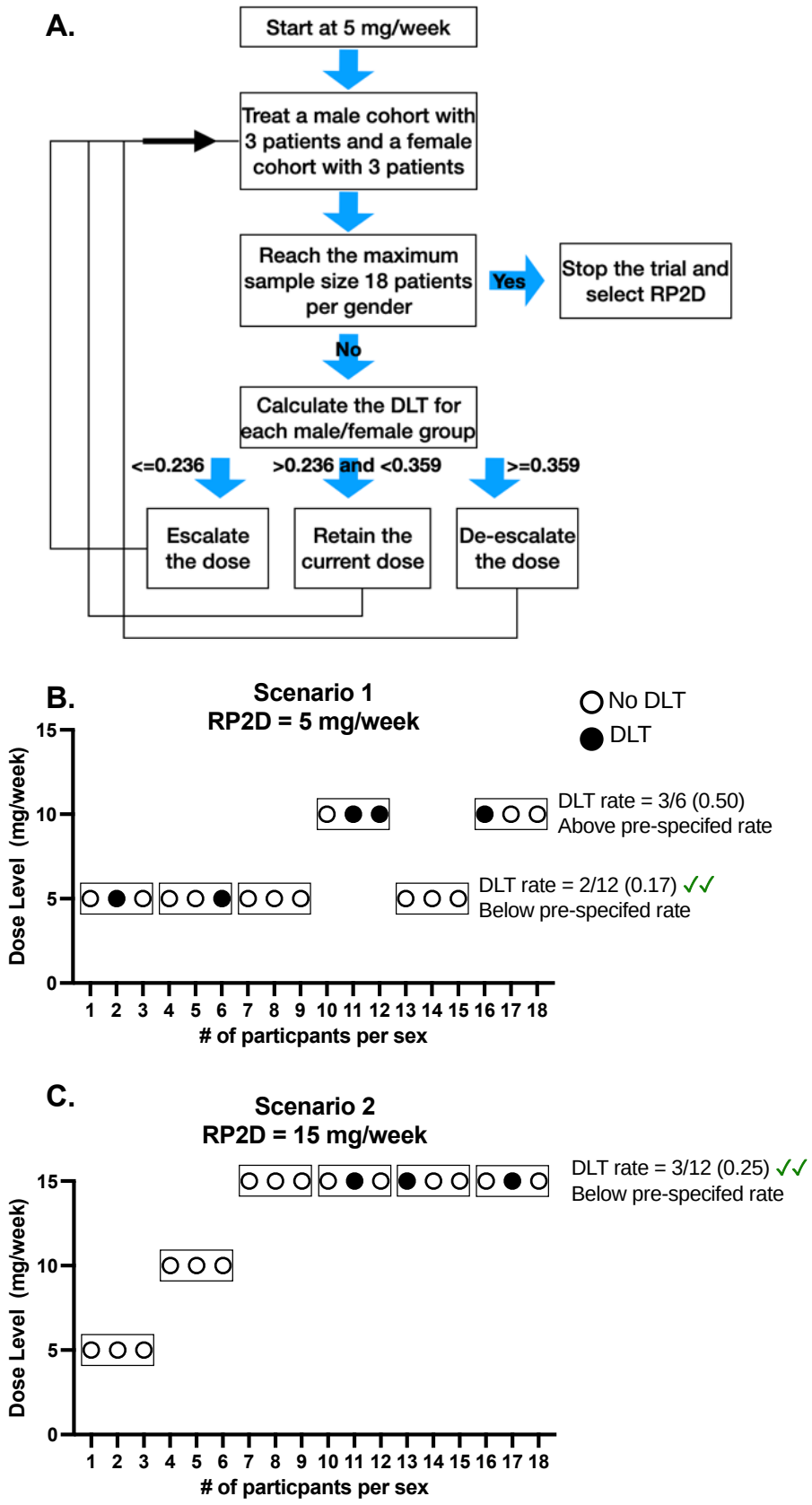

A.
Start at 5 mg/week
Treat a male cohort with 3 patients and a female cohort with 3 patients
Reach the maximum sample size 18 patients per gender
Yes
Stop the trial and select RP2D
No
Calculate the DLT for each male/female group
<=0.236
>0.236 and <0.359
>=0.359
Escalate the dose
Retain the current dose
De-escalate the dose
B.
Scenario 1
RP2D = 5 mg/week
No DLT
DLT
DLT rate = 3/6 (0.50)
Above pre-specifed rate
DLT rate = 2/12 (0.17) ✓✓
Below pre-specifed rate
Dose Level (mg/week)
# of particpants per sex
C.
Scenario 2
RP2D = 15 mg/week
DLT rate = 3/12 (0.25) ✓✓
Below pre-specifed rate
Dose Level (mg/week)
# of particpants per sex

Figure S2

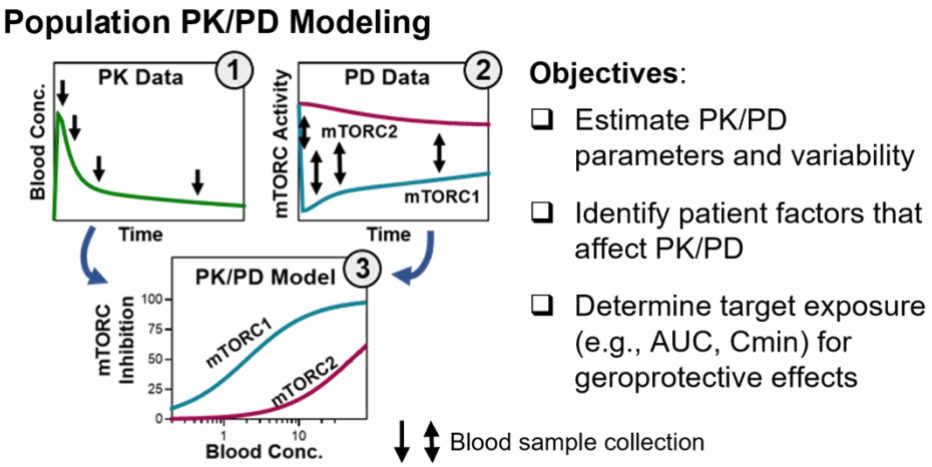

Population PK/PD Modeling
PK Data
1
Blood Conc.
Time
PD Data
2
mTORC Activity
mTORC2
mTORC1
Time
PK/PD Model
3
mTORC Inhibition
100
75
50
25
0
mTORC1
mTORC2
1
10
Blood Conc.
Objectives:
❑ Estimate PK/PD parameters and variability
❑ Identify patient factors that affect PK/PD
❑ Determine target exposure (e.g., AUC, Cmin) for geroprotective effects
Blood sample collection

Figure S3

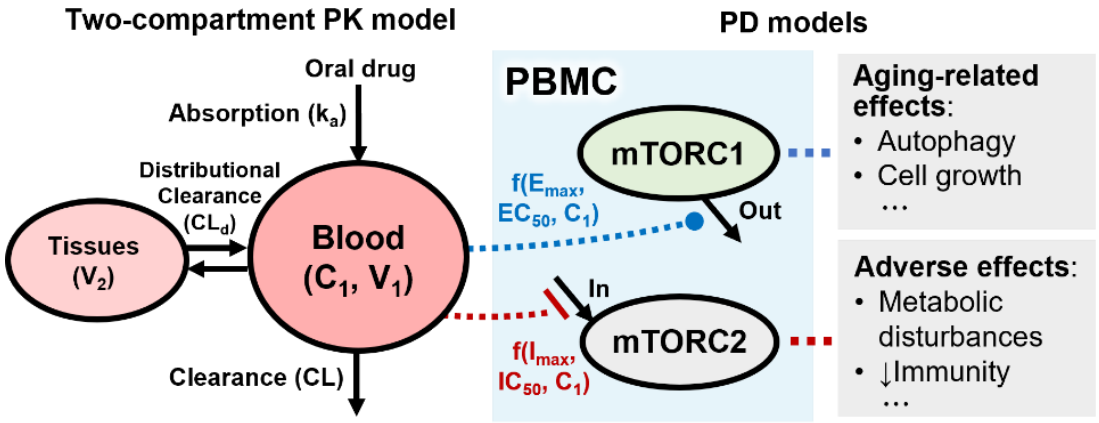
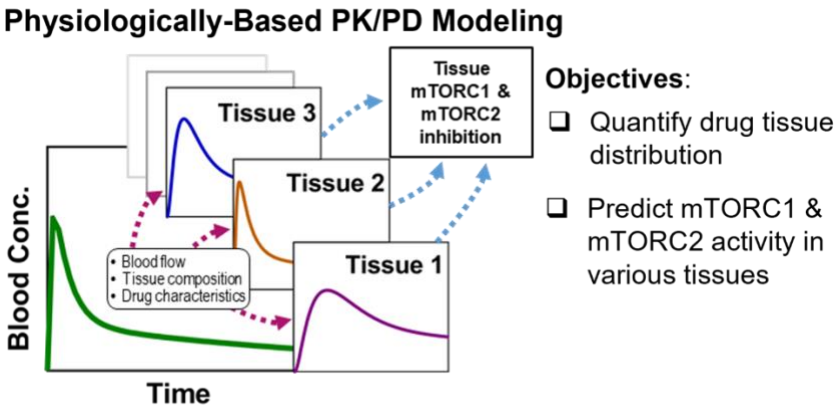

Two-compartment PK model
PD models
Oral drug
Absorption (k_a)
Distributional Clearance (CL_d)
Tissues (V_2)
Blood (C_1, V_1)
Clearance (CL)
PBMC
mTORC1
$f(E_{max}, EC_{50}, C_1)$
Out
In
mTORC2
$f(I_{max}, IC_{50}, C_1)$
Aging-related effects:
• Autophagy
• Cell growth
…
Adverse effects:
• Metabolic disturbances
• ↓Immunity
…

Figure S4


Physiologically-Based PK/PD Modeling
Tissue 3
Tissue 2
Tissue 1
Tissue mTORC1 & mTORC2 inhibition
Blood Conc.
Time
• Blood flow
• Tissue composition
• Drug characteristics
Objectives:
❑ Quantify drug tissue distribution
❑ Predict mTORC1 & mTORC2 activity in various tissues