GeroScience (2023) 45:2769–2783
https://doi.org/10.1007/s11357-023-00935-x

ORIGINAL ARTICLE

Blazing a trail for the clinical use of rapamycin as a geroprotecTOR

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Received: 23 June 2023 / Accepted: 4 September 2023 / Published online: 6 October 2023
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Abstract Treatment with rapamycin, an inhibitor of the mechanistic Target Of Rapamycin Complex One (mTORC1) protein kinase, has been repeatedly demonstrated to extend lifespan and prevent or delay age-related diseases in diverse model systems. Concerns over the risk of potentially serious side effects in humans, including immunosuppression and metabolic disruptions, have cautiously limited the translation of rapamycin and its analogs as a treatment for aging associated conditions. During the last decade, we and others have developed a working model that suggests that while inhibition of mTORC1 promotes healthy aging, many of the negative side effects of rapamycin are associated with "off-target" inhibition of a second mTOR complex, mTORC2. Differences in the kinetics and molecular mechanisms by which rapamycin inhibits mTORC1 and mTORC2 suggest that a therapeutic window for rapamycin could be exploited using intermittent dosing schedules or alternative rapalogs that may enable more selective inhibition of mTORC1. However, the optimal dosing schedules and the long-term efficacy of such interventions in humans are unknown. Here, we highlight ongoing or upcoming clinical trials that will address outstanding questions regarding the safety, pharmacokinetics, pharmacodynamics, and efficacy of rapamycin and rapalogs on several clinically oriented outcomes. Results from these early phase studies will help guide the design of phase 3 clinical trials to determine whether rapamycin can be used safely to inhibit mTORC1 for the treatment and prevention of age-related diseases in humans.

Keywords Aging · mTOR · Sirolimus · Everolimus · Metabolism · Muscle

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11357-023-00935-x.

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Introduction

The world is experiencing an unprecedented increase in the number of aged individuals [1]. The number of people over the age of 65 is expected to reach 2 billion by the year 2050 [2]. Age is a significant risk factor for most major causes of morbidity and mortality in the USA, including type 2 diabetes (T2D), cardiovascular disease (CVD), frailty, and dementia (Fig. 1). The financial impact of caring for elderly individuals is daunting with Medicare expenditures expected to top $1 trillion this year [4]. The high comorbidity of age-related diseases in the elderly individuals limits the benefit that can be obtained by targeting any one chronic condition individually [5]. Therefore, it is important to test geroprotective interventions that have shown efficacy in preclinical models to determine if these agents can simultaneously delay or even prevent multiple age-related conditions in humans.

The evolutionarily conserved mTOR protein kinase is a key regulator of growth, metabolism, and aging. Genetic inhibition of mTOR complex 1 (mTORC1) signaling extends the lifespan in yeast, worms, flies, and mice [6–9]. mTORC1 inhibition by rapamycin is one of the most repeatedly and rigorously tested pharmacological approaches to extend lifespan in diverse model systems and across multiple independent laboratories. In addition to mTORC1 inhibition, rapamycin has significant impact on several proposed biomarkers and fundamental mechanisms of the biology of aging, such as increased markers of autophagy, suppression of the senescent-associated secretory phenotype (SASP), regulation of nuclear transcription factors, selective protein translation, and altering epigenetic patterns in rodents [10–14]. The goal of this review is to (1) briefly showcase how mTORC1 inhibition by rapamycin and rapamycin analogs (rapalogs) can extend healthspan by maintaining, improving, or restoring select indices of physiological function in pre-clinical and some human studies (Table 1); (2) discuss potential risks and key knowledge gaps regarding the clinical use of rapamycin for geroprotection; and (3) highlight several ongoing or upcoming clinical trials that will begin to test if the benefits of rapamycin in pre-clinical models can be safely translated to humans. It is important to note that this is not an endorsement for or against the use of mTOR inhibitors, but rather a compendium of upcoming clinical trials leveraging the last two decades of preclinical data to help advance our understanding of the impact of rapamycin on human healthspan.

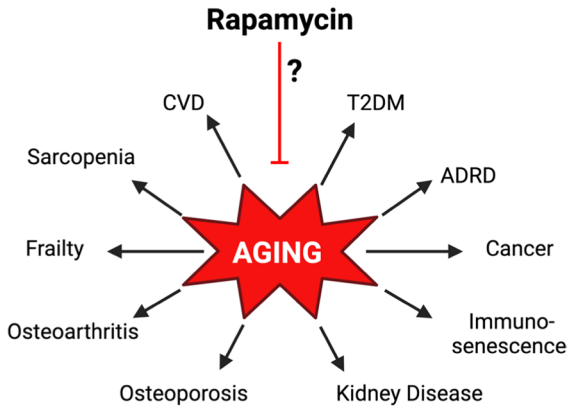

Fig. 1 Age is one of the greatest risk factors for nearly every chronic condition. Rapamycin is the most repeatable and effective pharmacological approaches to extend lifespan and delay or treat many age-related pathologies in diverse model systems. A critical translational gap in knowledge is whether rapamycin or rapamycin analogs (rapalogs) can safely maintain or improve healthy aging in humans. CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; ADRD, Alzheimer's disease and related dementias. Figure adapted from Kaeberlein et al. [3]

Impact of rapamycin on select physiological outcomes in pre-clinical and clinical studies

Metabolic function The incidence of metabolic dysfunction, such as insulin resistance, prediabetes, and overt T2D, increases with age and further increases the risk of nearly every age-related condition including heart disease, frailty, cerebrovascular disease, and dementia [23–27]. The nutrient overload model of insulin resistance posits that insulin resistance results, in part, from constitutive activation of mTORC1 leading to S6K1 and Grb10 mediated feedback inhibition on insulin receptor substrate 1/2 (IRS-1/2) [28–30]. Consistent with this model, (1) mice lacking *S6K1*, a downstream target of mTOR, are protected from age and diet-induced insulin resistance [29, 31] and (2) rapamycin given intermittently or at a low dose improves metabolic health and insulin sensitivity in mouse models of obesity [32–34]. In healthy young men, a single dose of rapamycin (6 mg) attenuated mTORC1 during an amino acid infusion and

Table 1 Summary of completed rapamycin clinical trials*

Population	mTOR inhibitor dosing regimen	Duration	Study design	Primary endpoint	Primary endpoint met?	Reference
11 men (28 ± 1 years)	Rapamycin, 6 mg single dose	Once	Randomized, double-blinded, placebo controlled, cross-over design	Increase in peripheral insulin sensitivity during insulin clamp plus AA infusion	Yes	Krebs et al. 2007 [15]
15 men (29 ± 2 years)	Rapamycin 12 mg (12 × 1 mg tablets) single dose 2h before resistance exercise (RE)	Once	Acute bout of RE ± rapamycin	Difference in post-RE mixed-muscle protein synthesis rates	Yes	Drummond et al. 2009 [16]
16 men (26 ± 1 years)	Rapamycin 16 mg (16 × 1 mg tablets) single dose 1h before blood flow restricted resistance exercise (RE)	Once	Acute bout of blood flow restriction RE ± rapamycin	Difference in post-RE mixed-muscle protein synthesis rates	Yes	Gunderman 2014 [17]
218 adults age ≥ 65 years	Everolimus 0.5 mg daily Everolimus 5 mg weekly Everolimus 20 mg weekly Placebo	6 weeks	Phase 2, randomized, single observer blinded, placebo-controlled	Improvement in influenza vaccination response	Yes for 0.5 mg daily and 5 mg weekly	Mannick et al. 2014 [18]
264 adults age ≥ 65 years	Everolimus 0.1 mg daily Everolimus 0.5 mg daily BEZ235 10 mg daily BEZ235 10 mg+0.1 mg everolimus daily Placebo	6 weeks	Phase 2a, randomized, double-blinded, placebo-controlled	Improvement in influenza vaccination response	Yes for BEZ235 10 mg + 0.1 mg everolimus daily	Mannick et al. 2018 [19]
25 healthy adults 70–95 years	Rapamycin 1 mg Placebo	8 weeks	Phase 2, Randomized, double-blinded, placebo-controlled	Immunological response	No	Kraig et al., 2018 [20]
36 adults with aging skin > 40 years	Topical rapamycin (10 μM) Topical placebo	8 months	Phase 2, randomized, placebo-controlled	Decreased p16-positive senescent cells in skin	Yes	Chung et al. 2019 [21]
1024 adults age ≥ 65 years	RTB101 10 mg daily Placebo	16 weeks	Phase 3, randomized, placebo-controlled	Decrease in clinically symptomatic respiratory illness	No	Mannick et al. 2021 [22]

*Studies are presented in chronological order from the publication date. AA, amino acid

increased peripheral insulin sensitivity by nearly 20% [15]. Conversely, long-term treatment with rapamycin doses ≥2 mg/kg/day in mice disrupts a second mTOR complex (mTORC2) and leads to insulin resistance [7, 35, 36] as evident by increased fasting insulin (104 vs. 86 pmol/L), elevated basal endogenous glucose production (EGP: 30.4 vs. 16.1 mg/kg/min), impaired suppression of EGP during a hyperinsulinemic-euglycemic clamp (39% vs. 67%), and glucose intolerance (39711 vs. 23075 AUC) in rapamycin treated versus vehicle controls [7]. We have also shown that hyperglycemia induced by 12 weeks of dietary rapamycin (14ppm) was correlated with greater osteoarthritis severity in guinea pigs prone to idiopathic osteoarthritis [37] suggesting that metabolic disruptions by long-term daily rapamycin may exacerbate some geriatric conditions in animal models.

Cognitive function Decreased cerebral blood flow (CBF) occurs during normal aging and deficits in CBF are worsened by vascular risk factors like impaired glucose homeostasis and insulin resistance [24, 38, 39]. The decline in CBF that accompanies age and insulin resistance is associated with memory dysfunction before the development of overt diseases like T2D and Alzheimer's [24, 25]. Therefore, therapies that can slow the biological rate of aging and age-related conditions may provide an effective strategy to mitigate the age-related risk of dementia. Rapamycin can cross the blood-brain barrier and mTOR attenuation by rapamycin prevents and reverses cognitive and cerebrovascular dysfunction in multiple models of Alzheimer's disease as well as high-fat diet–induced vascular cognitive impairment [40–42]. In C57BL/6 mice, dietary rapamycin (14ppm) enhances cognitive function by ~25–100%, depending on the outcome, in young adult mice and blocks age-associated cognitive decline in older mice (12 and 25 months old) [40]. Additionally, 15 months of dietary rapamycin (14ppm) improves spatial learning and memory in 34-month-old F344BNF1 rats by ~50% [43]. In aging animals, improvements in cognitive performance by mTOR inhibition are linked to restoring cerebrovascular blood flow to adult animals [43].

Cardiac function With age, there is an increase in cardiac stiffness and a decrease in ventricular volume that are associated with increased risk for heart failure and CVD [44]. Rapamycin reverses pre-existing age-dependent cardiac hypertrophy and diastolic dysfunction in mice [45, 46] and the effects of rapamycin on diastolic function, hypertrophy, and myocardial stiffness persist even following cessation of treatment [47]. Similarly, 10 weeks of rapamycin treatment (0.05–0.1 mg/kg 3×/week) increased diastolic and systolic cardiac function compared to placebo in a small cohort of dogs, with the greatest improvements in those dogs with lowest baseline scores [48].

Physical function Skeletal muscle health and cardiorespiratory fitness (CRF) play a critical role in maintaining mobility, whole-body metabolism, and survival [49–53]. Age-related loss of skeletal muscle mass and CRF increase the risk of disability, loss of independence, and mortality [53–56]. Higher skeletal muscle function and CRF are associated with protection from multi-morbidity and mortality [57–60]. In animal models and humans, there is evidence of an age-related increase in skeletal muscle mTOR signaling [61–67] and an impaired muscle protein synthetic response to acute anabolic stimuli [68]. Genetic activation of mTORC1 by knockout of the upstream inhibitor TSC1 contributes to muscle loss [69, 70]. Correcting hyperactive skeletal muscle mTORC1 signaling in aged rats by low-dose treatment with the rapalog everolimus (equivalent to 0.5 mg/day in humans) and dietary rapamycin in old mice partially or completely prevents age-related muscle atrophy [61, 65]. Furthermore, 3 months of high-dose dietary rapamycin (126ppm) slowed the age-related decline in physical function in older mice as evident by greater forelimb grip strength and longer rotarod run time in rapamycin treated versus control mice even 3 months after stopping treatment [71]. These findings, like that of cardiac function, suggest that the benefits on physical function in older mice may persist even after ending rapamycin treatment. However, a single dose of rapamycin (12 or 16 mg) attenuated the acute increase in muscle protein synthesis rates after resistance exercise in young men [16, 17], which is one process involved in muscle growth. Therefore, it remains unclear if attenuation of mTOR signaling by rapamycin with or without exercise may positively or negatively impact skeletal muscle and physical function in older adults.

Immune function Older adults are characterized by a decline in immune function that contributes to lower vaccine efficacy and increased vulnerability to infection. Age-related loss of immune function contributes to mortality linked to influenza and COVID-19 which disproportionately affects older persons. Just 6 weeks of treating older mice with rapamycin restored immune cell function and improved response to the influenza vaccine [72]. In older humans, a 6-week treatment with daily (0.5 mg/day) or intermittent (5 mg/week) everolimus rejuvenated aspects of the immune system and boosted the subsequent response to the influenza vaccination by >1.25-fold against two of three strains tested without metabolic side effects [18]. However, a higher weekly dose (20 mg/week) did not meet primary endpoints of increasing the serologic response to the influenza vaccine and led to more than double the number of adverse events compared to placebo and lower everolimus doses. While phase 2b and 3 clinical trials also showed that mTOR inhibitors increased antiviral gene expression in older patients, primary endpoints of decreasing laboratory confirmed or clinically symptomatic respiratory illness were not met [22]. The discrepancies between studies of meeting or not meeting primary endpoints are likely related to the pre-specified primary outcome. Although mTOR inhibitors appear to generally have a positive impact on immune function in older adults, additional work is needed to clarify the role of mTORC1 inhibition on immune function and vaccine efficacy in older adults.

Potential risks and unknowns

Despite the positive effects on lifespan and many indices of healthspan, mTOR inhibitor–based therapies for diseases of aging have not yet translated to clinical practice, largely due to a small number of human trials and potential safety concerns. Consistent with many animals studies, chronic treatment with rapamycin or rapamycin analogs (rapalogs) at the FDA-approved immunosuppressive doses (i.e., rapamycin 2–5 mg per day; everolimus up to 10 mg/day) to prevent organ transplant rejection or to treat some cancers are associated with deleterious metabolic consequences, including glucose intolerance (22% of patients), dyslipidemia (30–72% of patients), and an increased risk of developing new-onset diabetes (hazard ratio: 1.36 to 1.9) [73–78]. Furthermore, in a small study of healthy older adults, 8 weeks of daily rapamycin (1mg/day) tended to increase HbA1c, triglycerides, and VLDL [20] which challenges whether this represents an appropriate long-term dosing regimen. While these adverse side effects may be acceptable to patient populations, alternative dosing schedules are likely needed to minimize these adverse effects in older adults to achieve an acceptable risk to benefit ratio for geroprotection.

Even with the potential risks, unknown impact, and limited testing in humans, recent estimates suggest over 2000 people across the USA are currently taking rapamycin off label. Additionally, telemedicine services are beginning to launch that will increase accessibility to rapamycin. A recent observational study compared rapamycin users (n=333) to non-rapamycin users (n=172) [79]. Most users followed a weekly rapamycin dosing schedule, across a range of doses, for the purpose of "healthy longevity/anti-aging." Mouth ulceration was the only self-reported adverse event (AE) that was greater in rapamycin vs. non-rapamycin users [79]. Interestingly, 50% of rapamycin users agreed that rapamycin improved their health while ~25–38% of rapamycin users felt younger, more confident, more energetic, and/or helped with other perceived health benefits. However, the remaining ~62–75% of rapamycin users did not perceive that rapamycin improved these domains [79]. Although difficult to assess without a double-blinded, placebo-controlled study design, rapamycin users did report less abdominal cramps and pain, signs of depression, muscle tightness, anxiety, and eye pain. No clinical laboratory results were evaluated to determine any potential differences in health between rapamycin versus non-rapamycin users.

Working model

The mTOR kinase is the catalytic core of mTORC1 and mTORC2, each of which is composed of shared as well as unique protein subunits (Fig. 2), and which phosphorylate different substrates to regulate different metabolic processes. One of the major differences between mTORC1 and mTORC2 is that rapamycin acutely inhibits mTORC1 while chronic treatment with rapamycin also inhibits mTORC2 signaling in cultured cells and in mice [7, 80, 81].

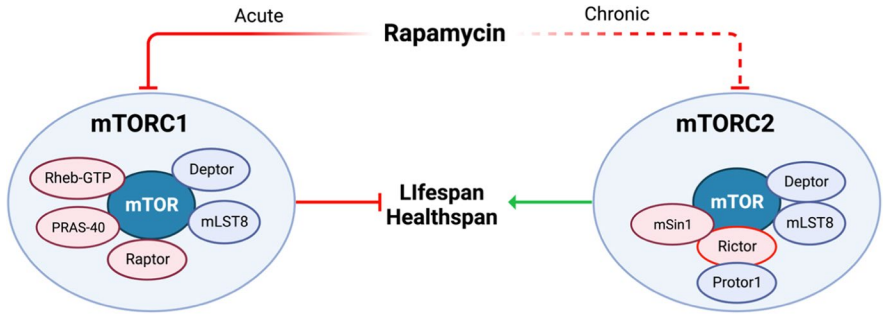

Fig. 2 Rapamycin acutely and potently inhibits mTORC1 while prolonged exposure to high, daily doses of rapamycin can lead to off-target inhibition of mTORC2. We propose to test the model that inhibition of mTORC1 is geroprotective, while inhibition of mTORC2 mediates metabolic side effects of rapamycin**.** Rapalog strategies that safely exploit the potent geroprotective effects of mTORC1 inhibition may warrant further testing for the treatment and prevention of age-related diseases in larger phase 3 clinical trials

Rapamycin inhibits mTORC1 by first forming 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 [82–84]. mTORC2 is not acutely sensitive to rapamycin because components of mTORC2, specifically Rictor and mSin1, hinder the binding of FKBP12-rapamycin to mTOR [85–87]. When high doses of rapamycin treatment are continued for a prolonged period of time, mTORC2 activity is decreased in most mouse tissues [7, 80, 81, 88] and this effect of rapamycin on mTORC2 is believed to be indirect, with rapamycin sequestering free mTOR and hindering the formation of new mTORC2 [80].

Using genetic models, inhibition of mTORC1 signaling alone extends lifespan and healthspan [7, 8] while tissue-specific and whole body genetic depletion of mTORC2 has negative effects on metabolic health, frailty, and survival in mice [89–93]. Similarly, genetic inhibition of mTORC2 activity in the heart impairs cardiac function in flies, while genetically increasing mTORC2 activity preserves cardiac function with aging and extends the lifespan of flies [94, 95]. These data support a model shown in Fig. 2 in which rapalog-mediated inhibition of mTORC1 is geroprotective, while the "off-target" inhibition of mTORC2 may be responsible for many negative effects of rapamycin. Therefore, to enhance translation of mTOR inhibitor-based therapies from pre-clinical models to human clinical trials and clinical practice, rapalog dosing strategies that preferentially inhibit mTORC1 rather than mTORC2 should be tested to potentially capitalize on healthspan extension while minimizing adverse side effects.

Collectively, these data indicate an urgent need to build on the exciting pre-clinical and clinical work that has already been completed to determine if rapamycin and rapalogs can be used safely and efficaciously for geroprotection in humans. There is currently a lack of pharmacokinetic and pharmacodynamic (PK/PD) data in healthy older adults. Therefore, it remains unknown what dose or dosing schedule of rapamycin or rapalogs minimizes undesirable side effects in older adults and whether rapamycin can have a beneficial impact on proposed biomarkers of aging and human healthspan as it does in pre-clinical models. We also do not understand if the dose of rapamycin for geroprotection will differ between sex, the age-related condition(s), or impacted tissue(s). Furthermore, we do not know how rapamycin will interact with healthy lifestyle practices such as exercise and diet. To address this need for additional information, a number of new clinical trials at the University of Wisconsin-Madison and around the world have begun or will begin in the near future to better study the effects of rapamycin and its analogs on age-related conditions (Table 2). The studies that are summarized within this review were identified by using search criteria *rapamycin* and *mTOR* on clinicaltrials.gov and through recent funding announcements. Studies were not included that focused on transplant or cancer patients. These relatively small trials will be signal-generating and will be used to inform on future, well-powered phase 3 clinical trials that will be needed for more definitive assessments of

Table 2 Summary of ongoing or upcoming rapamycin clinical trials*

Population	mTOR Inhibitor Dosing Regimen	Duration	Study Design	Primary Endpoint	Status	Study Name and/or NCT#
100 adults (30−90y)	Off label use of rapamycin or everolimus, varied doses	Single time point	Cross-sectional	Lower HOMA-IR in rapamycin treated vs. non-treatment control	Ongoing	RAP PROTECT, Communication with Dudley Lamming
10 young men (22−35y)	Single dose of Rapamycin 16 mg or Placebo	Once	Non-randomized, double-blinded, placebo controlled, cross-over design	Post-exercise, insulin stimulated muscle glucose uptake	Ongoing	NCT05233722, Communication with Jørgen Wojtaszewski
35 residents ≥ 65y in nursing homes with COVID	RTB101 10 mg daily Placebo	4 weeks	Randomized, double-blinded, placebo-controlled	Percentage of people who develop laboratory confirmed COVID-19	Pending	NCT04409327, Communication with Joan Mannick
72 adults (55−80y)	Rapamycin 5, 10, or 15 mg weekly Everolimus 5, 10, or 15 mg weekly	6 weeks	Allocated, open label, dose finding trial	Dose limited toxicities to determine RP2D	Active, Not yet recruiting	RAP PAC, NCT05949658 Adam Konopka
50 patients with periodontitis	Rapamycin 5 mg weekly Placebo	8 weeks	Randomized, placebo-controlled	Clinical attachment loss	Not active	Communication with Jonathan An
50 Women with premature ovarian failure	Rapamycin 5 mg weekly Placebo	12 weeks	Randomized, placebo-controlled	Ovarian reserve	Ongoing	VIBRANT, NCT05836025 Communication with Samuel Williams and Yousin Suh
16 males age ≥ 65y	Rapamycin 1 mg daily Placebo	16 weeks	Randomized, single-blinded, placebo-controlled, unilateral resistance exercise training vs. sedentary contralateral leg	Change in muscle mass	Ongoing	NCT05414292
72 adults with insulin resistance (55−80y)	Everolimus 0.5 mg daily Everolimus 5 mg weekly Placebo	24 weeks	Randomized, double-blinded, placebo-controlled	Change in peripheral insulin sensitivity	Ongoing	EVERLAST, NCT05835999 Adam Konopka
150 adults (≥ 50y)	Rapamycin 5 mg weekly Rapamycin 10 mg weekly Placebo	12 months	Prospective, randomized, placebo-controlled	Change in visceral fat	Ongoing	PEARL, NCT04488601 Communication with Sajad Zalzala
40 patients with MCI/early AD	Rapamycin 1 mg daily Placebo	12 months	Randomized, double-blinded, placebo-controlled	Number of adverse events	Ongoing	REACH, NCT04629495 Communication with Mitzi Gonzales

*Studies are presented as observational study first, followed by studies with increasing rapamycin treatment duration

the therapeutic potential of rapalogs for human aging-related conditions.

Observational and clinical trials testing mTOR inhibitors for geroprotection at the University of Wisconsin-Madison

The Evaluate the safety and efficacy of RAPamycin as a geroPROTECTor (RAP-PROTECT) study, led by Dr. Dudley Lamming and colleagues, is an ongoing study examining some of the growing number of individuals who are taking rapamycin off-label under the belief that it will extend their lifespan. This study is actively recruiting approximately 100 subjects taking or planning to take rapamycin or rapamycin analogs from across the contiguous United States to examine how clinical labs, the blood/plasma transcriptome, metabolome, and lipidome, and the blood methylation clock compare in rapamycin users vs. matched control subjects. The primary outcome of this cross-sectional trial is that rapamycin users will have a lower HOMA-IR than controls. While these data will expand on the insight from a recent observational study [79], there may be some difficulties in interpreting the data in the absence of longitudinal data from each subject, blood samples prior to rapamycin initiation, and double-blinded placebo-control as well as the diverse dosing regimens. Despite these limitations, this cross-sectional comparison will begin to reveal if there are differences in rapamycin users versus non-users in clinical laboratory results as well as molecular and metabolic signatures associated with aging. Therefore, this study may be informative to the geroscience community and those interested in potentially enrolling in one of the physician-supervised clinical trials described within this review.

The Rapalog Pharmacology (RAP PAC) Trial led by Dr. Adam Konopka and colleagues at the University of Wisconsin-Madison will identify a recommended phase 2 weekly dose (RP2D) for the mTOR inhibitors rapamycin and everolimus by performing a phase I, dose finding trial in healthy older men and women (n=18 per drug per sex, 55–80years). For each mTOR inhibitor, we propose to test up to 3 weekly dose levels (5, 10, 15 mg/week) for 6 weeks. RAP PAC will evaluate safety, pharmacokinetics (PK), pharmacodynamics (PD), and mTORC1/2 inhibition. The occurrence of dose limited toxicities (DLTs), defined as ≥grade 2 AE using the Common Terminology Criteria for Adverse Events (CTCAE), will serve as the primary endpoint. We will determine AEs by using a 20-point questionnaire to query about common mTOR inhibitor AEs, review participant diaries, perform clinical bloodwork, and if needed, complete a physical exam. Secondary and exploratory outcomes include investigating the impact of mTOR inhibitors on whole-body glucose metabolism and insulin sensitivity via a 75-g oral glucose tolerance test (OGTT) and 7–10 days of continuous glucose monitoring before and at the end of the 6-week treatment. mTOR signaling will be determined by conventional immunoblotting and immunoprecipitation as well as novel approaches to identify a molecular signature that distinguishes mTORC1 versus mTORC1/2 signaling by integrating transcriptomics, metabolomics, and lipidomics. Therefore, this study will combine comprehensive molecular, pharmacologic, and metabolic approaches to evaluate PK/PD in humans and identify dosing regimens that safely inhibit mTORC1 and intervene on the biology and metabolism of aging. A detailed review of the clinical trial protocol and experimental design can be found in the Supplement.

The Everolimus Aging Study (EVERLAST) led by Dr. Adam Konopka and colleagues at the University of Wisconsin-Madison is an ongoing phase 2 trial of 72 insulin-resistant, older adults (55–80 years old) who are at increased risk of multiple aged-related conditions, including type 2 diabetes, cardiovascular disease, frailty, and dementia [23, 24, 96]. In this randomized, double-blinded study, subjects will receive either 0.5 mg/day everolimus, 5 mg/week everolimus, or placebo-control for approximately 24 weeks. Everolimus tablets and placebo will be over-encapsulated to be indistinguishable from each other. To determine if everolimus can change molecular and physiological aging toward that of young healthy individuals, an additional group of 14 subjects between 18 and 35 years of age will serve as a young healthy reference group to complete baseline testing only (no intervention). The primary endpoint is the change in peripheral insulin sensitivity determined using a dual isotope oral glucose tolerance test (OGTT). Safety and incidence of treatment-associated adverse events will be determined from participant diaries, questionnaires, and changes in baseline blood chemistry, cell count, lipids, glucose, and insulin. Secondary and other pre-determined

exploratory end points include additional indices of metabolic function (glucose tolerance, glycemic variability, hepatic insulin sensitivity), cognitive function (micro- and macro-vessel cerebral blood flow, learning, memory), cardiac function (fractional shortening, E/A ratio, ejection fraction, etc.), and physical function (VO_2max, maximal knee extensor power and strength, body composition). To comprehensively examine the molecular target specificity and the impact on mechanisms of aging by everolimus, EVERLAST will evaluate mTORC1 and mTORC2 signaling, assess mitochondrial bioenergetics, and perform multi-omics (epigenomics, transcriptomics, proteomics, lipidomics, and metabolomics) in muscle biopsy and/or blood samples. EVERLAST will also explore the role of everolimus on senescent-associated secretory phenotype (SASP), DNA methylation clocks, and other proposed biomarkers of aging in saliva, urine, blood, and/or skeletal muscle. A detailed review of the clinical trial protocol and experimental design can be found in the online Supplement.

At this time, one EVERLAST ancillary study application is under review and another ancillary study led by Dr. Alexey Terskikh and colleagues at Sanford Burnham Prebys Medical Discovery Institute is funded. The funded study will perform microscopic imaging of epigenetic age (miEpiAge) to determine how the epigenetic landscape and heterogeneity in peripheral mononuclear blood cells (PMBCs) and skeletal muscle biopsy samples correlate to metabolic, cognitive, cardiac, and physical function with or without mTOR inhibition by everolimus. miEpiAge is a novel technique rooted in the analysis of epigenome topography at the single cell level to quantitate change in chromatin landscape. Therefore, this ancillary study will capture patterns of nuclear staining of epigenetic marks (e.g., acetylated and methylated histones) and employ automated microscopy and machine learning to determine a multiparametric signature of the cellular state.

Clinical trials of mTOR inhibitors for aging-related conditions in humans

Metabolism and body composition

The mTOR as Mediator of Exercise-induced Insulin Sensitivity Study led by Dr. Jørgen FP Wojtaszewski and colleagues in Denmark will evaluate the role of mTOR signaling on insulin sensitivity and muscle protein synthesis after a single exercise bout. This is a non-randomized, double-blinded, cross-over study where young men (22–35 years old) will perform two identical visits separated by at least 14 days that only differ by subjects taking either oral rapamycin (16 mg) or placebo 2 h before completing 1 h of one-legged kicking exercise (80–100% of maximal work). The combination of skeletal muscle biopsies, stable isotopes, femoral arterial and venous blood samples, and blood flow measurements will allow the measurement of post-exercise skeletal muscle glucose uptake and protein synthesis rates with and without insulin stimulation. The primary outcome is insulin stimulated skeletal muscle glucose uptake during recovery from exercise. Secondary outcomes include insulin stimulated skeletal muscle protein synthesis during recovery from exercise and phosphoproteomics to identify the intracellular signaling network regulating muscle glucose uptake and protein synthesis. Although this study is restricted to young men, these findings could provide the framework to understand how rapamycin may impact the acute benefits of exercise on substrate metabolism in both older men and women.

A relatively small trial in the UK, led by Drs. Philip Atherton and Lynne Cox, is aiming to determine how rapamycin affects skeletal muscle and immune function in men between the ages of 50 and 90 years. A total of 16 subjects will be randomized to either 1 mg/day rapamycin or placebo for 16 weeks. Subjects will complete a 2-week rapamycin lead-in before engaging in 14 weeks of unilateral, leg extension resistance exercise (3×/week at 75% of 1 repetition maximum) while the contralateral leg will remain sedentary. Although there may be some crossover effects between the trained and sedentary legs, this trial may be able to determine both the independent and combined effects of rapamycin and resistance exercise on study endpoints. The primary outcomes are the change from baseline in skeletal muscle mass assessed by MRI and ultrasound of the thigh muscles after 5, 8, and 16 weeks. Secondary outcomes include change in skeletal muscle strength (1-RM), power (counter jump movement), and physical function (short-performance physical battery). The combination of muscle biopsy samples and the use of the stable isotope deuterium oxide will be used to measure cumulative skeletal muscle protein synthesis rates. Blood samples taken will be used to assess immune cell senescence and inflammation.

The Participatory Evaluation of Aging with Rapamycin for Longevity Study (PEARL), led by Dr.

James Watson of the University of California, Los Angeles and Dr. Sajad Zalzala of AgelessRx, is now underway. A total of 150 subjects located throughout the USA aged 50–85 have been randomized to receive either 5 mg or 10 mg of rapamycin once per week or a placebo-control. The primary outcome of the 12-month-long trial will be changes in visceral fat as estimated by dual-energy X-ray absorptiometry (DXA). Secondary outcomes will include bone density, fat-free mass, changes in blood glucose, HbA1c, and electrolytes, and effects on liver and renal function, as well as adverse events. Outcomes relevant to molecular aging may also include microbiome analysis, DNA methylation clock, and PhenoAge score. A potential limitation is the standardization between DXA scanners located across the country; however, participants will have pre- and post-measurements conducted on the same DXA machine.

Specific geriatric conditions

The Rapamycin – Effects on Alzheimer's and Cognitive Health (REACH) trial led by Drs. Mitzi Gonzales, Sudha Seshardi, and collaborators at the University of Texas San Antonio Health Sciences Center is now recruiting subjects to explore the possibility that rapamycin could be used as an intervention in Alzheimer's disease (AD). This trial is based on the extensive pre-clinical evidence that orally administered rapamycin attenuates cerebral mTOR signaling, restores cerebral blood flow, reduces amyloid beta (Aβ) and tau accumulation, and ameliorates cognitive deficits in AD and aged rodent models [42, 97, 98]. In this trial, 40 subjects with amnestic mild cognitive impairment (aMCI) or early-stage AD will be randomized to receive 1 mg/day of rapamycin or placebo for 12 months, followed by a 6-month observational period. The safety, efficacy, and feasibility of rapamycin treatment for aMCI/AD will be determined by tracking compliance, adverse events, rapamycin levels within the CSF, and changes in cognition. Molecular effects of the treatment on CSF levels of Aβ and tau will also be determined, and neuroimaging will be performed to examine how the treatment impacts AD biomarkers, including blood flow, glucose metabolism, neurovascular coupling, and cerebral volumetry.

Based on the lessons learned from previous phase 2b and phase 3 trials (Table 1) [19, 22], a pilot trial was recently completed to test an endpoint that assessed *severity* rather than *incidence* of respiratory tract infections (RTIs) caused by a specific virus (COVID-19) in nursing home patients. Specifically, the trial assessed whether 10mg daily dose of the mTOR catalytic inhibitor BEZ235 decreased the incidence of severe COVID-19 in residents of nursing homes experiencing a COVID-19 outbreak. Results of the clinical trial are pending.

The Validating Benefits of Rapamycin for Reproductive Aging Treatment (VIBRANT) trial led by Drs. Samuel Williams and Yousin Suh at Columbia University will explore the benefits of rapamycin in women undergoing early ovarian failure. Fifty subjects are being actively recruited and will be randomized to either 5 mg of rapamycin once weekly or placebo for 3 months, and then followed for 9 months. This trial is based in part on studies in rodents showing that short-term treatment with rapamycin can increase ovarian lifespan [99, 100]. The primary endpoint of this study will be ovarian reserve determined by transvaginal ultrasound, with secondary endpoints related to concentration of reproductive hormones.

An upcoming clinical trial led by Dr. Jonathan An at the University of Washington is aiming to start recruitment later in 2023 to study the effects of rapamycin on periodontal disease. This trial is based on pre-clinical work in mice showing that rapamycin can partially restore oral health in aged mice [101, 102]. Approximately 50 subjects aged 50 and up will be recruited and randomized to receive either rapamycin (most likely 5–6 mg per week) or placebo for 8 weeks. The primary endpoint of the study will be clinical attachment loss, an established clinical readout for the progression of periodontal disease.

Conclusions

Overall, there is significant interest by the scientific community and greater public to determine if rapamycin can safely and effectively extend healthy longevity in humans like it does in multiple model systems. The a priori goal of this review was to highlight ongoing and upcoming clinical trials testing the safety and utility of rapamycin to intervene in fundamental mechanisms of aging with the goal of slowing, improving, or restoring the age-related decline in physiological function. At the completion of these trials, it is expected that the geroscience community will identify dosing strategies that minimize adverse events for older adults and improve our understanding

of whether rapamycin can improve key predictors of clinical outcomes in aging humans either at risk for or with overt, pre-existing geriatric conditions. Positive or null findings will be equally informative on the experimental design of future clinical trials in terms of dosing regimens, pre-specified primary endpoints, candidate subject populations, and statistical approaches. Furthermore, there is a need for the ongoing mTOR inhibitor trials to make data publicly available so that datasets can be combined for further exploratory analyses on dose, safety, aging biomarkers, and overlapping outcomes. Many of these studies have just recently started or will begin enrollment within the next 2 years. Therefore, study details were not always widely available for each protocol. For example, it was unclear whether some studies had a matching placebo and/or if studies were double or single-blinded. Therefore, the evaluation of rigor was not completely possible but, inherently, each of the studies mentioned within this review, including our own, will have strengths and limitations. Additionally, while there is growing infatuation for the potential of using rapamycin for geroprotection, there are currently no human data to suggest that long-term use of mTOR inhibitors can safely extend healthy longevity in humans and underscore the importance of the emerging clinical trials discussed within this review to close this translational knowledge gap.

Acknowledgements We appreciate Drs. Jonathan An, Philip Atherton, Mitzi Gonzales, Joan Mannick, Samuel Williams, Sajad Zalzala and Jørgen Wojtaszewski for sharing information about their studies. We thank Jacob Rome, JD for his assistance in navigating the submission process for FDA Investigational New Drug Applications (161611 and 166577). For EVERLAST, we thank Dr. Lindsay Clark for her input on cognitive assessments, Dr. Adrian Vella for assistance with the design and calculations to determine glucose kinetics during the dual isotope oral glucose tolerance test, Dr. Scott Bauer for the suggestion to include sex-specific lower urinary tract symptoms (LUTS) questionnaires, and Mihaela Teodorescu for sleep quality questionnaires. We also thank the faculty and staff within the Clinical Research Unit and the Pharmaceutical Research Center for initiating EVERLAST and RAP PAC which are supported by the Clinical and Translational Science Award (CTSA) program, through the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR002373. EVERLAST is supported by NIH/NIA U01-AG076941 (to ARK), EVERLAST Biomarker Study is supported by the Impetus Longevity Grant by the Norn Group (to ARK), miEpiAge is supported by R21-AG083782 (to AT), RAP PAC is supported by U01-AG081482 (to ARK and DWL), and RAP-PROTECT is supported by the Impetus Longevity Grant by the Norn Group (to DWL). The Konopka Laboratory is also supported in part by NIH/NIA R21-AG067464 and New Investigator Award from AFAR/Hevolution. The Lamming laboratory is also supported in part by the NIH/NIA (AG056771, AG062328, and AG061635) and the NIH/NIDDK (DK125859 and DK133479). Both Konopka and Lamming Laboratories are also supported by startup and other funds from the University of Wisconsin-Madison School of Medicine and Public Health and Department of Medicine. This work was supported using facilities and resources from the William S. Middleton Memorial Veterans Hospital.

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Declarations

Conflict of interest DWL has received funding from, and is a scientific advisory board member of, Aeovian Pharmaceuticals, which seeks to develop novel, selective mTOR inhibitors for the treatment of various diseases. Aeovian Pharmaceutical mTOR inhibitors will not be used in the clinical trials discussed within this manuscript.

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