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Targeting the biology of aging with mTOR inhibitors

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Inhibition of the protein kinase mechanistic target of rapamycin (mTOR) with the Food and Drug Administration (FDA)-approved therapeutic rapamycin promotes health and longevity in diverse model organisms. More recently, specific inhibition of mTORC1 to treat aging-related conditions has become the goal of basic and translational scientists, clinicians and biotechnology companies. Here, we review the effects of rapamycin on the longevity and survival of both wild-type mice and mouse models of human diseases. We discuss recent clinical trials that have explored whether existing mTOR inhibitors can safely prevent, delay or treat multiple diseases of aging. Finally, we discuss how new molecules may provide routes to the safer and more selective inhibition of mTOR complex 1 (mTORC1) in the decade ahead. We conclude by discussing what work remains to be done and the questions that will need to be addressed to make mTOR inhibitors part of the standard of care for diseases of aging.

mTOR is an evolutionarily conserved serine–threonine protein kinase found in diverse species including mice and humans. The mTOR kinase forms the catalytic core of two distinct protein complexes, mTORC1 and mTORC2, each of which are composed of shared as well as unique protein subunits and phosphorylate different substrates. mTORC1 is regulated by a wide range of nutrients and hormonal cues, most notably by the availability of amino acids, but also glucose, oxygen and cholesterol[1–3]. mTORC1 activity drives a wide variety of anabolic processes through phosphorylation of substrates including ribosomal protein S6 kinase (S6K) β1 (S6K1) and eukaryotic translation-initiation factor 4E-binding proteins (4E-BPs). mTORC1 activity also inhibits autophagy via phosphorylation of substrates including Unc-51-like autophagy-activating kinase (ULK1)[4]. In contrast to mTORC1, which is responsive to many different environmental cues, mTORC2 primarily acts as an effector of phosphoinositide 3-kinase (PI3K) signaling by tuning the activity of substrates including the kinase AKT, serum–glucocorticoid-regulated kinase (SGK) and protein kinase Cα (PKCα).

Beginning 20 years ago, researchers discovered a role for mTORC1 signaling in the aging process. Studies in yeast, worms and flies found that genetic inhibition of mTORC1 or signaling pathways downstream of mTORC1, including S6K and translation-initiation factors, extends lifespan[5–11]. mTORC1 signaling was also observed to be lower in long-lived Ames dwarf mice than in wild-type controls[12]. Studies in mice have likewise shown that genetic depletion of mTORC1 subunits, deletion of *S6K1* (also known as *Rps6kb1*) or downstream substrates or expression of dominant negative 4E-BP1 in specific tissues extends the lifespan and healthspan of mice[13–16]. Even partial inhibition of mTORC1 in genetic mouse models (for example, *S6K1*$^{-/-}$, *Mtor*$^{+/-}$, *Mlst8*$^{+/-}$, *Mtor*$^{\Delta/\Delta}$ and *Tsc1*^{tg}) can extend lifespan and healthspan in mice[13,17–19].

These results quite logically spurred substantial interest in the possibility that a potent chemical inhibitor of mTORC1, rapamycin, could extend lifespan. This was indeed the case, and there are now numerous studies showing that rapamycin can extend the lifespan not only of model organisms including yeast, worms and flies[20–22] but also, as we detail below, of both wild-type mice and in many disease models. In this Review, we will discuss the results of these studies, as well as the possible mechanism by which reduced mTORC1 signaling via both dietary and pharmacological means may improve healthspan[23–25].

Many of the side effects of rapamycin, which include immunosuppression, hyperlipidemia and hyperglycemia, have raised concerns about the feasibility of using rapamycin to promote healthy longevity in humans and have slowed clinical evaluation of mTOR inhibitors for diseases of aging[26]. In this Review, we will discuss data generated over the past decade suggesting that the beneficial effects of rapamycin on healthspan and lifespan are mediated by inhibition of mTORC1, whereas

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the negative effects of rapamycin on glucose and lipid metabolism are mediated by inhibition of mTORC2.

Importantly, these data predict that interventions that more selectively target mTORC1, by limiting the amount of mTOR inhibitor used and the time of the exposure, will have reduced side effects as compared to chronic treatment. Here we discuss preclinical animal data and clinical human data exploring the use of intermittent and low-dose mTOR-inhibitor-treatment regimens, the results of which suggest that rapamycin analogs (rapalogs) and other mTOR inhibitors may be able to be dosed in a way that is safer as well as geroprotective. Finally, we discuss future studies and the quest for mTORC1-selective molecules derived in part through recent discoveries about the molecular machinery through which mTORC1 senses nutrients and hormonal cues.

Extension of lifespan with the mTOR inhibitor rapamycin in mice

Rapamycin is a macrolide first discovered in soil from Easter Island almost 50 years ago[27]. Rapamycin is an acute inhibitor of mTORC1 but not of mTORC2 (ref. 28). The reason for this difference in rapamycin sensitivity is structural; the mTOR-interacting protein RICTOR, which is present in mTORC2 but not in mTORC1, masks the rapamycin-interacting domain of mTOR[29–31]. Although mTORC2 is not acutely inhibited by rapamycin, subsequent studies have shown that mTORC2 is inhibited in cell culture as well as in vivo in mice when exposed to high concentrations of rapamycin for a prolonged period of time, most probably due to the sequestration of free mTOR by rapamycin so that it is unavailable for incorporation into mTORC2 (refs. 17,32). Rapamycin and rapalogs inhibit T cell activation and are approved to prevent organ transplant rejection. In addition, rapalogs inhibit tumor cell growth and are approved for the treatment of a subset of tumors. Finally, rapalogs are approved for the treatment of specific genetic disorders that result in hyperactive mTOR signaling.

Multiple groups have studied the effect of rapamycin on the longevity of mice, starting with a landmark study by the National Institute on Aging Interventions Testing Program (NIA ITP) published in 2009, which demonstrated that rapamycin extended the lifespan of genetically heterogeneous mice[33]. Since that time, additional studies of rapamycin on mouse lifespan have been conducted not only by the NIA ITP but by at least ten other groups across the globe (Table 1). A number of lessons can be drawn from this set of studies, primarily that rapamycin is an extraordinarily robust geroprotective intervention, extending lifespan in multiple wild-type mouse strains handled by multiple teams and including inbred, outbred and genetically heterogeneous male and female mice. Every study observed an extension of lifespan in one or more dosing groups, with females typically benefiting more than males at equivalent doses of rapamycin. Importantly, unlike caloric restriction, another well-validated longevity intervention, which is less efficacious when begun later in life[34], rapamycin robustly extended lifespan when dosing was not initiated until late in life when the mice were 20 months of age (equivalent to about a 60-year-old human)[33,35]. Dosing regimens for rapamycin are very flexible, and beneficial effects of rapamycin on longevity are observed even when rapamycin is dosed intermittently, when it is given for only a short period of time immediately postnatally or when it is administered to older mice already past middle age[36–43]. Finally, rapamycin may be effective when combined with other geroprotectors, including metformin and acarbose[44,45].

Rapamycin has also been tested in a wide number of disease models, including multiple different cancers, mitochondrial disease and progeria (Table 2). Here, the beneficial effects of rapamycin have been more varied than in wild-type mice. As might be expected, rapamycin has shown significant benefits in mouse models of mTORopathies[46], diseases resulting from genetic activation of mTOR, including tuberous sclerosis complex (TSC) and some epilepsies. Rapamycin also has substantial positive effects on the survival of mouse cancer models. Finally, rapamycin extends the lifespan of several progeroid mouse models quite effectively, suggesting that progeria mouse models, while not perfect models of aging, may have utility for rapidly assessing the potential benefits of geroprotectors.

While by far the most work on rapamycin and aging has been done in mice, there is substantial interest in exploring the use of rapamycin in primates. A total of 66 middle-aged marmosets (*Callithrix jacchus*) have been fed either rapamycin- or vehicle-containing diets, with the lifespan and healthspan of the animals followed longitudinally. Both sexes are being examined in this study, which is still underway. A limitation of these studies is both the relatively small number of animals studied (less than 20 per sex and treatment) and the range of ages and the genetic heterogeneity of the population. Initial studies suggested that rapamycin, dosed at ~1 mg per kg of body weight via the diet, was well tolerated, with only minor effects of rapamycin on hematological parameters and no statistically significant changes in blood glucose, cholesterol or triglyceride levels[47]. In the subset of six animals treated with rapamycin or vehicle for 9 months, two rapamycin-treated animals showed an increase in fasting blood glucose and two rapamycin-treated animals showed an increase in triglycerides, suggesting the possibility of some inhibition of TORC2 at this dose level[47]. However, a definitive examination of the metabolic impact of rapamycin in marmosets will require studies in a larger number of animals, conducted over a longer period of time.

The second major study of rapamycin done outside of the rodent context is examining the effect of rapamycin on the aging of dogs. As companion animals, dogs share the human environment, receive regular medical care similar to humans and develop many of the same age-related diseases that humans do. Furthermore, many dog owners have shown interest in enrolling their dogs in studies to promote healthy aging. The short lifespan of dogs, particularly that of large dog breeds, allows for studies of shorter duration than for humans. A small pilot study found that treatment with 0.05 mg per kg or 0.1 mg per kg rapamycin three times per week did not cause statistically significant side effects but did improve measurements of cardiac function (fractional shortening and diastolic function)[48]. A larger study in which middle-aged, large-breed dogs will be treated with rapamycin or vehicle for 1 year will complete enrollment during 2023 (ref. 49).

Mechanisms underlying the effects of rapamycin on healthspan and lifespan

There are many possible mechanisms underlying the geroprotective effects of rapamycin. In this section, we will discuss the effects of mTORC1 inhibition on both specific disease processes and the molecular mechanisms that may contribute to the beneficial effects of rapamycin on lifespan and healthspan.

Protein translation

mTORC1, via S6K1 and 4E-BPs, has a central role in the regulation of translation, and defects in translation have long been theorized to contribute to aging. The error catastrophe theory suggests that errors in protein translation could lead to increasingly inaccurate protein synthesis and functional decline[50]. Conceptually, slowing protein translation by treatment with rapamycin might allow mRNA to be translated into protein with higher fidelity or to fold more accurately. In agreement with such a model, experiments in model organisms have shown that deletion or knockdown of genes encoding ribosomal subunits, S6K1 or translation-initiation factors results in increased lifespan[6,10].

However, modern measurement techniques have not found evidence that protein translation errors increase with age[51]. Furthermore, neither mTOR inhibition by rapamycin nor deletion of *S6K1* significantly slows protein translation in mice or cells[52–54]. Studies in *Caenorhabditis elegans* have shown that the lifespan of worms lacking eukaryotic translation-initiation factor 4E (eIF4E), which are long lived and have a global reduction in protein synthesis, can be still further increased by knockdown of the gene encoding TOR[10]. These results

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Table 1 | The effect of rapamycin on the lifespan of wild-type mice

Strain	Sex	Starting age	Rapamycin dose	Route	Control (d)	Δ lifespan (%)	Reference
Wild-type mice: rapamycin alone via diet							
UM-HET3	Male	20 months	14 ppm	Diet	–	9	Harrison et al.[33]
UM-HET3	Female	20 months	14 ppm	Diet	881–895	14	
UM-HET3	Female	9 months	14 ppm	Diet	843–891	18	Miller et al.[36]
UM-HET3	Male	9 months	14 ppm	Diet	780–851	10	
129/Sv	Female	2 months	1.5 mg per kg	s.c. 3×/week, 2 weeks per month	759	10	Anisimov et al.[41]
C57BL/6J.Rj	Male	4, 13, 20 months	14 ppm	Diet	~900	~10[a]	Neff et al.[87]
C57BL/6J.Nia	Male	19 months	14 ppm	Diet	954	−3.5[NS]	Zhang et al.[194]
C57BL/6J.Nia	Female	19 months	14 ppm	Diet	874	5	
UM-HET3	Male	9 months	4.7 ppm	Diet	807	3[NS]	Miller et al.[195]
UM-HET3	Male	9 months	14 ppm	Diet	807	13	
UM-HET3	Male	9 months	42 ppm	Diet	807	23	
UM-HET3	Female	9 months	4.7 ppm	Diet	896	16	
UM-HET3	Female	9 months	14 ppm	Diet	896	21	
UM-HET3	Female	9 months	14 ppm	Diet	896	26	
C57BL/6J.Nia	Male	4 months	14 ppm	Diet	806*	11*	Fok et al.[196]
C57BL/6J.Nia	Female	4 months	14 ppm	Diet	826*	16*	
C57BL/6J.Nia	Male	20–21 months	126 ppm for 90 d	Diet	914	14	Bitto et al.[38]
C57BL/6J.Nia	Female	20–21 months	126 ppm for 90 d	Diet	960	9	
UM-HET3	Male	20 months	42 ppm	Diet	772	11	Strong et al.[193]
UM-HET3	Male	20 months	42 ppm for 3 months	Diet	772	11	
UM-HET3	Male	20 months	42 ppm	Diet every other month	772	9	
UM-HET3	Female	20 months	42 ppm	Diet	905	15	
UM-HET3	Female	20 months	42 ppm for 3 months	Diet	905	4[NS]	
UM-HET3	Female	20 months	42 ppm	Diet every other month	905	8	
C57BL/6 *Terc*$^{+/+}$	Male	3 months	42 ppm	Diet	509	43	Ferrara-Romeo et al.[197]
C57BL/6 *Terc*$^{+/+}$	Female	3 months	42 ppm	Diet	711	23	
UM-HET3	Male	Birth (via dam)	42 ppm until 45 d	Diet	783	11.9	Shindyapina et al.[42]
UM-HET3	Female	Birth (via dam)	42 ppm until 45 d	Diet	822	9.1[NS]	
Wild-type mice: rapamycin alone via i.p.							
C57BL/6J.Nia	MF	22–24 months	4 mg per kg	i.p. every other day	~795	>14[a]	Chen et al.[39]
C57BL/6J.Nia	Female	20 months	2 mg per kg	i.p. 1×/5 d	897	7	Arriola Apelo et al.[37]
C57BL/6J.Nia	Male	20–21 months	8 mg per kg for 90 d	i.p. 1×/d	925	14	Bitto et al.[38]
C57BL/6J.Nia	Female	20–21 months	8 mg per kg for 90 d	i.p. 1×/d	847	0[NS]	
Mixed	Male	600–700 d	4 mg per kg	i.p. every other day	911	13	Fang et al.[198]
Mixed	Female	600–700 d	4 mg per kg	i.p. every other day	896	22	
CD1	Male	Day 4	10 mg per kg until 40 d	i.p. 1×/d	655	8.9	Aiello et al.[43]
CD1	Male	Day 30	10 mg per kg until 60 d	i.p. 1×/d	655	3.5[NS]	
CD1	Female	Day 4	10 mg per kg until 40 d	i.p. 1×/d	714	8.4	
CD1	Female	Day 30	10 mg per kg until 60 d	i.p. 1×/d	714	3.8[NS]	

Table 1 (continued) | The effect of rapamycin on the lifespan of wild-type mice

Strain	Sex	Starting age	Rapamycin dose	Route	Control (d)	Δ lifespan (%)	Reference
Wild-type mice: rapamycin via diet as part of a combination							
UM-HET3	Male and female	9 months	14 ppm with metformin	Diet	It is complicated; see text for details.		Strong et al.[44]
UM-HET3	Male and female	9 or 16 months	14 ppm with acarbose	Diet	It is complicated; see text for details.		Strong et al.[45]

The impact of rapamycin on median lifespan in mouse studies since 2009 in which longevity or mortality rate was determined is shown. Sex is listed separately for males and females when sex-specific data exist. The rapamycin dose listed for dietary administration indicates the drug concentration in the ad libitum fed diet; the dose listed for administration in water or administered intraperitoneally (i.p.) or subcutaneously (s.c.) indicates the dose in mg per kg of body weight. Control indicates median lifespan of the control group in days; Δ lifespan is the per cent change in median lifespan (* indicates that the mean is reported instead). MF indicates that the lifespan results were not broken down by sex or that sex was not reported. [a]Lifespan study per cent increase was not determined. NS, not statistically significant. Control lifespan and percentage change are estimated when precise information is not available from the authors or are not listed in the referenced study. The table was adapted with permission from ref. 199, Oxford University Press.

strongly suggest that rapamycin does not regulate longevity solely by downregulating protein translation.

There may be a more subtle effect of rapamycin on protein translation, namely, that rapamycin may alter the translation of specific mRNA species. Both rapamycin and complete mTOR inhibition preferentially inhibit translation of mRNA with 5′ terminal oligopyrimidine motifs, suggesting a potential role for these genes in longevity[55,56]. In yeast, the deletion of genes encoding ribosomal subunits can extend lifespan; this is partially dependent upon inducing translation of the mRNA encoding the transcription factor general control nondepressible 4 (Gcn4)[57]. Expression of the Gcn4 protein is limited by multiple upstream open reading frames that normally sequester ribosomes that bind to the mRNA. Under conditions of large ribosomal subunit abundance, the upstream open reading frames are more frequently bypassed to initiate translation of *GCN4*. It is not clear whether this system is conserved in mammals because, in mammals, mTORC1 inhibition decreases translation of the mammalian homolog of Gcn4, ATF4 (refs. 58,59). ATF4 instead may act as a brake on mTORC1 in response to mitochondrial distress, with ATF4 inhibiting mTORC1 activity by upregulating the mTORC1 inhibitors sestrin 2 and REDD1 (ref. 60). Despite these differences, it is possible that changes in translation of specific mRNA species may contribute to the beneficial effects of rapamycin on healthspan and lifespan.

One potential example of this is that mice expressing a constitutively active (dephosphorylated) form of 4E-BP1 in skeletal muscle are protected from age- and diet-induced declines in insulin sensitivity and metabolic rate[15]. These effects may be mediated in a non-cell-autonomous manner via increased production of fibroblast growth factor 21 (FGF21) by skeletal muscle, activating brown adipose tissue. Interestingly, whole-body overexpression of 4E-BP1 also has positive effects on healthspan, protecting male mice from diet-induced obesity[16]. These mice also have increased levels of FGF21, in this case due to upregulation of hepatic *Fgf21* expression. Notably, expression of *Fgf21* is regulated in part by ATF4 (refs. 61,62). A recently described downstream effector of mTORC1 and S6K1 is glutamyl-prolyl tRNA synthetase (EPRS), which is phosphorylated by S6K1 (ref. 14). When phosphorylated, EPRS functions to inhibit the translation of select mRNA species by forming an interferon γ (IFNγ)-activated inhibitor of translation (GAIT) complex, which selectively inhibits mRNA containing GAIT elements[63]. Mice expressing $EPRS^{S999A}$, which cannot be phosphorylated, have reduced body weight and adipose mass and increased lifespan, similar to mice lacking *S6K1* (ref. 14). Other aminoacyl-tRNA synthetases may similarly have non-canonical functions[64] that may play a part in the response to rapamycin.

Autophagy

Autophagy is a process by which cells recycle their proteins and organelles, which not only allows cells to survive nutrient-limited conditions but is also a central mechanism by which damaged protein and subcellular organelles are removed. While autophagy thus seems to be very important from this description alone, studies in yeast, worms and flies have shown that inactivation of autophagy shortens lifespan, while promotion of autophagy extends lifespan[65–67]. Importantly, autophagy has also been shown to be required for the extension of lifespan by reduced mTOR signaling in both yeast and worms[65,68]. In mammals, autophagy is reportedly upregulated in calorie-restricted mice and in the cells of long-lived Snell dwarf mice and is required for some of the beneficial effects of a calorie-restricted diet on the heart, kidney and liver[69–72].

When nutrients are abundant, mTORC1 activity acts as a brake on autophagy by phosphorylating the autophagy-initiating kinase ULK1 and autophagy-related protein 13 (ATG13) as well as other components downstream of autophagy initiation and is reviewed in detail elsewhere[73–75]. Conversely, when nutrients are low, autophagy is active as a result of AMP-activated protein kinase (AMPK) activation and reduced mTORC1 activity. Autophagy is normally impaired with age in multiple types of mouse cells, and genetic activation of autophagy in the aged mouse liver rejuvenates the liver histologically and improves function[76–78]. While rapamycin itself is not a strong inducer of autophagy compared to mTOR kinase inhibitors[53], the effects of rapamycin on the pathology of mouse models of Alzheimer's disease have been attributed in part to increased autophagy[79,80].

Improvement in immune function

Premature aging of the immune system has been shown to drive aging of multiple other organ systems in mice[81]. These findings suggest that therapies that improve immune aging may have more systemic healthspan and lifespan benefits. mTOR inhibition has been shown to improve the function of the aging immune system in both mice and humans. Specifically, a short 6-week course of rapamycin has been shown to rejuvenate the function of hematopoietic stem cells (HSCs), increase production of naive lymphocytes and improve the response to influenza vaccination in old mice[39]. Of interest, rapamycin treatment extended lifespan in this study even though it was only administered for 6 weeks when mice were already old (26 months). In older humans, 6 weeks of mTOR-inhibitor treatment (a rapalog alone or in combination with a catalytic site mTOR inhibitor) also improved the response to influenza vaccination and was associated with a decrease in the percentage of exhausted $PD\text{-}1^+$ T cells in peripheral blood[82,83]. In addition, mTOR inhibition has been shown to upregulate antiviral immunity in older adults[83,84]. These clinical studies will be discussed in more detail below.

However, it is worth noting that rapamycin is approved by the FDA as an immunosuppressant, and infections are common, particularly in humans taking high doses of rapalogs for a long period of time. In mice, rapamycin has been shown to be immunomodulatory, improving $CD8^+$ T cell immunological memory while at the same time impairing defenses against acute viral and bacterial infections[85]. A recent meta-analysis suggests that mice treated with rapamycin have increased survival following an acute pathogenic challenge[86]. Thus, there may be tradeoffs, with rapalogs improving some aspects of immune function while impairing others.

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Table 2 | The effect of rapamycin on the survival of mouse disease models

Strain	Sex	Starting age	Rapamycin dose	Route	Control (d)	Δ lifespan (%)	Reference
Disease models							
Autosomal dominant polycystic kidney disease							
Vil-Cre;Pkd2$^{f3/f3}$	Female	10 d	50 mg per kg until 60 d	i.p. 1×/d	~137	~53	Li et al.[200]
Vil-Cre;Pkd2$^{f3/f3}$	Male	10 d	50 mg per kg until 60 d	i.p. 1×/d	~137	~33	
Vil-Cre;Pkd2$^{f3/f3}$	MF	10 d	50 mg per kg until 110 d	i.p. 1×/d	~137	~75	
Vil-Cre;Pkd2$^{f3/f3}$	MF	10 d	50 mg per kg until 60 d	i.p. 1×/d	~137	~53	
Vil-Cre;Pkd2$^{f3/f3}$	MF	60 d	50 mg per kg until 110 d	i.p. 1×/d	~137	~53	
Amyotrophic lateral sclerosis							
SOD1^{G93A}	MF	64 d	2 mg per kg	i.p. 1×/d	126	−15	Zhang et al.[201]
SOD1$^{H46R/HR8Q}$	MF	1.5 months	14 ppm	Diet	232	NS	Bhattacharya et al.[202]
Cancer							
Pten$^{-/-}$	MF	1 month	10 mg per kg (everolimus)	Oral	66*	>292*[a]	Hernando et al.[203]
K14-Cre Pten$^{F/F}$	MF	5 d	1 mg per kg	i.p. every other day	94	240	Squarize et al.[204]
Apc$^{Min/+}$ C57BL/6	MF	5 weeks	40 mg per kg; not encapsulated, diet changed out frequently	Diet	156*	77	Koehl et al.[205]
129 *Atm*$^{-/-}$	MF	2–3 months	15 mg per kg	i.p. 1×/d	~100	~32	Alexander et al.[206]
FVB/N HER-2/neu	Female	2 months	1.5 mg per kg	s.c. 3×/week 2 weeks per 4	288	13.6	Anisimov et al.[40]
BALB/c nu/nu+A549	Male	7 weeks	10 mg per kg (temsirolimus)	i.v. 1×/5 weeks for 5 weeks	53.5	36	Ohara et al.[207]
Trp53$^{-/-}$	Male	2 months	0.5 mg per kg	Oral 1×/d 5 d on/9 d off	161	35	Comas et al.[208]
BALB/c+4T1	Female	6 weeks	5 mg per kg	i.p. every other day	16[d]	31[d]	Hussein et al.[209]
Trp53$^{+/-}$	Male	<5 months	1.5 mg per kg	Water	373*	28*	Komarova et al.[210]
Trp53$^{+/-}$	Male	>5 months	1.5 mg per kg	Water	373*	10*	
NOD.Cg-*Prkdc*^{SCID} ALL (*JAK2*$_m$/*CRLF2*$_R$)	MF	Sufficient disease burden	5 mg per kg	Oral 5×/week	23	174	Maude et al.[211]
NOD.Cg-*Prkdc*^{SCID} ALL (*JAK1*$_m$)	MF	Sufficient disease burden	5 mg per kg	Oral 5×/week	58	57	
Rb1$^{+/-}$	Male	2 months	14 ppm	Diet	369	13.8	Livi et al.[212]
Rb1$^{+/-}$	Female	2 months	14 ppm	Diet	378	8.9	
Apc$^{Min/+}$	Female	8 weeks	14 ppm	Diet	174	284	Hasty et al.[213]
Apc$^{Min/+}$	Female	8 weeks	42 ppm	Diet	174	439	
Pdx1-Cre;Kras$^{G12D/+}$;*Pten*$^{fl/+}$	MF	Clinically detectable pancreatic tumors	10 mg per kg	i.p. 1×/d	10	460	Morran et al.[214]
Pdx1-Cre;Kras$^{G12D/+}$;*Trp53*$^{R172H/+}$	MF	Clinically detectable pancreatic tumors	10 mg per kg	i.p. 1×/d	2	250	
HER-2/neu	Female	2, 4 or 5 months	0.45 mg per kg	s.c. 3×/week 2 weeks per 4	282, 278, 289	5.7^{NS}, 6.1, 5.5	Popovich et al.[215]
Trp53$^{+/+}$	MF	2 months	14 ppm	Diet	681	17.8	Christy et al.[216]
Trp53$^{+/-}$	MF	2 months	14 ppm	Diet	520	11.9^{NS}	
Trp53$^{-/-}$	MF	2 months	14 ppm	Diet	199	−3^{NS}	
C3H/HeJJcl+LM8	Male	9 weeks	50 mg per kg	i.v. every other day	~30	~28	Ando et al.[217]

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Table 2 (continued) | The effect of rapamycin on the survival of mouse disease models

Strain	Sex	Starting age	Rapamycin dose	Route	Control (d)	Δ lifespan (%)	Reference
Trp53$^{5KR/5KR}$	MF	Weaning	42 ppm	Diet	258	35	Kon et al.[218]
Trp53$^{-/-}$	MF	Weaning	42 ppm	Diet	164	55	
Apc$^{Min/+}$	Male	4 weeks	42 ppm	Diet	222	348	Parihar et al.[219]
Apc$^{Min/+}$	Female	4 weeks	42 ppm	Diet	260	156	
Apc$^{Min/+}$ DSS	Male	4 weeks	42 ppm	Diet	112	171	Parihar et al.[220]
Apc$^{Min/+}$ DSS	Female	4 weeks	42 ppm	Diet	113	220	
Pten$^{+/-}$	Male	6 weeks	14 ppm	Diet	394	65	Tibarewal et al.[221]
Pten$^{+/-}$	Female	6 weeks	14 ppm	Diet	239	54	
Clock							
Bmal1$^{-/-}$	MF	16 weeks	0.5 mg per kg	Water	~240	47	Khapre et al.[222]
Diabetes							
C57BL/6NCr HFD	Male	12 months	1.5 mg per kg	i.p. 1×/week	684	[b]	Leontieva et al.[223]
C57BLKS/J *Lepr*$^{db/db}$	Male	4 months	14 ppm	Diet	349	−16	Sataranatarajan et al.[224]
C57BLKS/J *Lepr*$^{db/db}$	Female	4 months	14 ppm	Diet	487	−18	
Epilepsy							
*Depdc5*cc+	MF	3 weeks	6 mg per kg	i.p. 3×/week	126	>62[c]	Yuskaitis et al.[225]
Depdc5-Emx1-Cre	MF	13–15 d	3 mg per kg	i.p. 5×/week	22	>70[c]	Klofas et al.[226]
Growth hormone							
Ghr$^{-/-}$	Male	600–700 d	4 mg per kg	i.p. every other day	558	−12.5	Fang et al.[198]
Ghr$^{-/-}$	Female	600–700 d	4 mg per kg	i.p. every other day	556	−28	
Immune deficiency							
Rag2$^{-/-}$	MF	3 months	14 ppm	Diet	310	121	Hurez et al.[227]
Ifng$^{-/-}$	MF	5 months	14 ppm	Diet	398	34	
CByB6F1 BM	Female	8–9 weeks	2 mg per kg Day 0–9 after LN	i.p. for 5–13 d	16.5[d]	36[d]	Feng et al.[228]
CByB6F1 BM	Female	8–9 weeks	2 mg per kg Day 0–12 and day 3–12 after LN	i.p. for 5–13 d	16.5[d]	>506[c]	
Inflammation							
Nfkb1$^{-/-}$	Male	4–5 months	14 ppm	Diet	598	−6[NS]	Correia-Melo et al.[229]
Lupus							
MRL/l	Female	8 weeks	12.5 mg per kg	Oral 3×/week	90	72	Warner et al.[230]
MRL/l	Female	8 weeks	25 mg per kg	Oral 3×/week	90	163	
Marfan syndrome							
mgR/mgR	Female	7–8 weeks	2 mg per kg	i.p. 1×/d for 2 weeks	199	36	Zaradzki et al.[231]
mgR/mgR	Male	7–8 weeks	2 mg per kg	i.p. 1×/d for 2 weeks	101	45	
Mitochondrial disease							
Ndufs4$^{-/-}$	Female	Weaning	8 mg per kg	i.p. every other day	~50	38	Johnson et al.[132]
Ndufs4$^{-/-}$	Male	Weaning	8 mg per kg	i.p. every other day	~50	25	
Ndufs4$^{-/-}$	Female	10 d	8 mg per kg	i.p. 1×/d	~50	~128	
Ndufs4$^{-/-}$	Male	10 d	8 mg per kg	i.p. 1×/d	~50	~128	
Ndufs4$^{-/-}$	MF	Weaning	8 mg per kg	i.p. 1×/day	52	119	Johnson et al.[232]
Ndufs4$^{-/-}$	MF	Weaning	42 ppm	Diet	52	29[NS]	
Ndufs4$^{-/-}$	MF	Weaning	378 ppm	Diet	52	92	
Tk2$^{KI/KI}$	MF	To dams from conception	0.8 mg per kg before birth, 4.0 mg per kg after birth	Water to dam	~15	~60	Siegmund et al.[233]
Prion							
Tg(PrP^{A116V})	MF	6 weeks	10 mg per kg	i.p. 3×/week	173*	1[NS]	Cortes et al.[234]
Tg(PrP^{A116V})	MF	6 weeks	20 mg per kg	i.p. 3×/week	173*	9	

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Table 2 (continued) | The effect of rapamycin on the survival of mouse disease models

Strain	Sex	Starting age	Rapamycin dose	Route	Control (d)	Δ lifespan (%)	Reference
Progeria							
Lmna$^{-/-}$	MF	1 month	14 ppm	Diet	46	35	Ramos et al.[235]
Lmna$^{-/-}$	MF	1 month	8 mg per kg	i.p. every other day	55	56	
Lmna$^{-/-}$	Female	4 weeks	8 mg per kg	i.p. every other day	59	70	Liao et al.[236]
Lmna$^{-/-}$	Male	4 weeks	8 mg per kg	i.p. every other day	52	86	
Lmna$^{H222P/H222P}$	MF	2 months	14 ppm	Diet	309	−2^{NS}	
G2 *Terc*$^{-/-}$	Male	3 months	42 ppm	Diet	274	−19	Ferrara-Romeo et al.[197]
G2 *Terc*$^{-/-}$	Female	3 months	42 ppm	Diet	274	−12.5^{NS}	
Ercc1$^{Δ/-}$	Female	4 weeks	14 ppm	Diet	148	−7.4^{NS}	Birkisdottir et al.[237]
Ercc1$^{Δ/-}$	Female	8 weeks	14 ppm	Diet	141	−6.4^{NS}	
Ercc1$^{Δ/-}$	Female	8 weeks	4.7 ppm	Diet	180	−2.2^{NS}	
Ercc1$^{Δ/-}$	Female	8 weeks	42 ppm	Diet	180	−1.6^{NS}	
Ercc1$^{Δ/-}$	Male	8 weeks	4.7 ppm	Diet	160	5^{NS}	
Ercc1$^{Δ/-}$	Male	8 weeks	42 ppm	Diet	160	−1.7^{NS}	
TSC							
GFAP-Cre Tsc1$^{L/L}$	MF	2 weeks	3 mg per kg	i.p. 5×/week	63	[e]	Zeng et al.[238]
GFAP-Cre Tsc1$^{L/L}$	MF	6 weeks	3 mg per kg	i.p. 5×/week	~74	[f]	
CD-1 nude with *Tsc2*$^{-/-}$, *Trp53*$^{-/-}$ MEF tumors	MF	Tumor ≥40–50 mm^3	8 mg per kg	i.p. 3×/week	24.5	88	Lee et al.[239]
LSL-Kras^{G12D} *Tsc1*$^{L/L}$	MF	13–16 weeks	6 mg per kg	i.p. every other day	20[d]	551[d]	Liang et al.[240]
LSL-Kras^{G12D} *Tsc1*$^{L/+}$	MF	13–16 weeks	6 mg per kg	i.p. every other day	48[d]	138[d]	
CD-1 nude with *Tsc2*$^{-/-}$, *Trp53*$^{-/-}$ MEF tumors	MF	Tumor ≥100 mm^3	8 mg per kg	i.p. 3×/week	31	173	Woodrum et al.[241]
Tsc1$^{L/L}$;*SM22-Cre*$^{+/-}$	MF	2 weeks	4 mg per kg	i.p. 5×/week	24	[g]	Malhowski et al.[242]
Tsc1$^{L/L}$;*SM22-Cre*$^{+/-}$	MF	2 weeks	4 mg per kg	i.p. 3×/week	24	[g]	
Tsc1$^{L/L}$;*SM22-Cre*$^{+/-}$	MF	3 weeks	4 mg per kg	3×/week	24	[g]	
Six2-Cre$^{tg/+}$;*Tsc1*$^{L/L}$	MF	To dams, E12.5, E14.5, E16.5	0.2 mg per kg	3×	2	500	Nechama et al.[243]
Tsc2$^{L/L}$, *Nphs2-Cre*$^{+/-}$ ICR	MF	4 weeks	2 mg per kg until 11 weeks	i.p. every other day	~53	~150	Iwata et al.[244]

The impact of rapamycin on median lifespan is shown in mouse studies since 2009 in which longevity or mortality rate was determined. Sex is listed separately for males and females when sex-specific data exist. The rapamycin dose listed for dietary administration indicates the drug concentration in the ad libitum fed diet; the dose listed for administration in water or administered intraperitoneally or subcutaneously indicates the dose in mg per kg of body weight. Control indicates median lifespan of the control group in days; Δ lifespan is the per cent change in median lifespan (* indicates that the mean is reported instead). MF indicates that the lifespan results were not broken down by sex or that sex was not reported. ALL, acute lymphoblastic leukemia xenograft model; BM, bone marrow failure model; DSS, mice were treated with dextran sodium sulfate to induce colon cancer; E, embryonic day; ICR, Institute of Cancer Research; HFD, high-fat diet; i.v., intravenous; LM8, murine subcutaneous allograft tumor model; MEF, mouse embryonic fibroblast; NS, not statistically significant; Prp, prion protein. *Vil-Cre* refers to the expression of Cre under the control of the *Vil1* promoter; *K14-Cre* refers to the expression of Cre under the control of the *KRT14* promoter; *LSL-Kras*^{G12D} refers to a the expression of a *Kras* mutant allele the expression of which is blocked by the a Lox-Stop-Lox cassette; *SM22-Cre* refers to Cre under the control of the *Sm22a* (also known as *Tagln*) promoter; mgR refers to a hypomorphic allele of *Fbn1*. Control lifespan and percentage change are estimated when precise information is not available from the authors or is not listed in the referenced study. This table is adapted with permission from ref. 199, Oxford University Press. [a]Lifespan study per cent increase was not determined. [b]One hundred per cent of rapamycin-treated mice survived to 2 years of age versus 40% of control mice. [c]Study was not continued long enough to determine median lifespan. [d]Survival following tumor induction, tumor implantation or lymph node cell infusion (LN). [e]Ninety-one per cent of rapamycin-treated mice survived to 6 months of age versus 0% of control mice. [f]One hundred per cent of rapamycin-treated mice survived to 18 months of age versus 0% of control mice. [g]There were no deaths in the treatment cohort at 8 weeks of age versus 100% of untreated mice.

Cancer

Cancer is the most common cause of death for laboratory mice, and rapamycin and its derivatives (rapalogs) inhibit the proliferation of cancer cell lines. Rapalogs are approved for use in certain oncology indications, and, as shown in Table 2, rapamycin is very effective at extending the lifespan of mouse models of cancer. A study by Neff and colleagues found that rapamycin significantly reduced the proportion of 16-month-old mice with cancer and/or precancerous lesions[87] and suggested that this indicated that the effects of rapamycin on lifespan were driven by its anti-cancer effects. This effect on cancerous and precancerous lesions was not observed in animals killed at 25 months of age, although the interpretation of these results was confounded by the fact that cancer was not assessed in animals that died of natural causes. By contrast, the NIA ITP has shown that the overall prevalence of cancer at death, as well as the spectrum of cancers observed, was very similar between rapamycin-treated mice and controls[36]. While cancer prevention clearly has a role in the benefits of rapamycin in mice, cancer is itself an age-related disease, and thus prevention of cancer is an expected consequence of geroprotective therapies.

Cancer cells often express pluripotency markers, and rapamycin reduces cell size and proliferation and enhances the differentiation of mouse and human embryonic stem cells[88–90]. Rapamycin may be particularly beneficial against cancer stem cells and has been shown to deplete leukemia-initiating cells and inhibit both self-renewal and differentiation of stem cells derived from infantile hemangioma[91,92]. Rapamycin inhibits cell proliferation, the epithelial–mesenchymal

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transition and cancer stem cell characteristics of lung cancer stem cells and colorectal cancer stem cells[93,94].

Stem cells

In contrast to its actions on cancer stems cells, rapamycin has been shown to have beneficial effects on self-renewal and function in normal stem cells, which may contribute to the benefits of rapamycin on tissue function. Several studies have been conducted on HSCs; aged mice have elevated mTORC1 activity in their HSCs as well as functional deficits similar to those observed in a mouse model of hyperactive mTORC1 activity[39]. Treatment of aged mice with rapamycin restored the functional capacity of their HSCs and boosted the immune response to influenza virus. In a separate study, rapamycin was shown to restore self-renewal capacity to a subpopulation of mouse HSCs with spontaneously high oxidative stress and reduced functional capacity[95]. Rapamycin also increases the self-renewal capacity of mouse intestinal stem cells via a non-cell-autonomous mechanism mediated by inhibition of mTORC1 in the adjacent Paneth cells[96].

Reduction of hyperactive mTOR in aging tissues

One reason that mTOR inhibition may have health benefits in older organisms is because mTOR activity may become inappropriately high with age. Higher mTORC1–S6K activity in muscle of older mice, rats and humans is associated with sarcopenia[97–101] and, in the brain, is associated with Alzheimer's disease[101–105]. Altered mTORC1 signaling with age has also been reported in other tissues of mice, with most studies reporting increased mTORC1 activity with age[39,97,106–108]. The ability of rapamycin to promote longevity is consistent with the idea that mTOR activity is an example of antagonistic pleiotropy, with high mTOR signaling being beneficial for development and reproduction but harmful during a post-reproductive old age[109]. Under such a model, the benefits of mTOR inhibition may arise less from specific benefits on processes such as translation and more from avoiding negative effects of hyperactive mTOR on processes such as cellular senescence. Indeed, rapamycin has been shown to inhibit the accumulation of senescent cells in mice as well as to suppress the senescence-associated secretory phenotype[110].

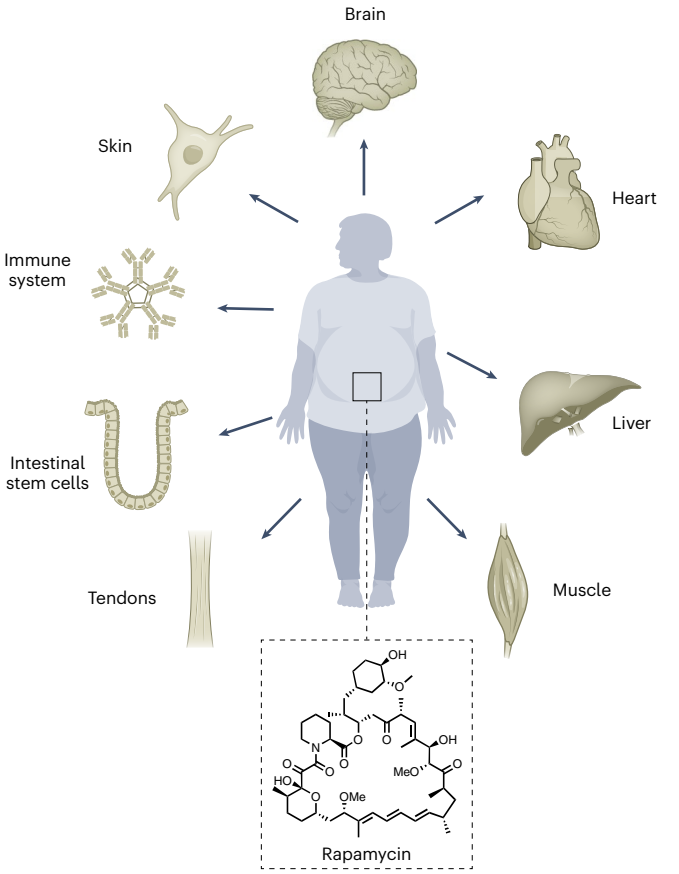

Fig. 1 | Potential healthspan targets of rapamycin. Based on studies in rodents and humans, rapamycin and rapalogs may have potential benefits for ameliorating or slowing age-related conditions associated with the brain[105,245–247], the heart[25,248,249], the liver[25], skeletal muscle[100], tendons[25,250], the immune system[39,82–84], the skin[135] and the intestine[96,251]. Me, methyl.

Inhibition of mTORC2

In sharp contrast to mTORC1, inhibition of mTORC2 has mostly negative effects on lifespan. In worms, mTORC2 inhibition has most often been associated with reduced lifespan, while, in flies, increasing mTORC2 activity through overexpression of *rictor* extends lifespan[21,111–113]. Whole-body deficiency of mTORC2 signaling or tissue-specific inhibition of mTORC2 signaling in the brain, liver or adipose tissue reduces lifespan in C57BL/6J mice[114–117]. Conversely, the lifespan of male mice is extended by acarbose and 17α estradiol, and these compounds increased hepatic mTORC2 activity[118]. mTORC2 activity is also elevated in long-lived Snell dwarf mice and *Ghr*$^{-/-}$ mice[119].

Inhibition of mTORC2 also results in negative effects on metabolism and immunity. Genetic inhibition of mTORC2 in one or more tissues of a mouse can result in frailty, hyperphagia, insulin resistance, hyperlipidemia, hypercholesterolemia, hyperglycemia, kyphosis and/or obesity depending upon the specific tissues in which mTORC2 is inhibited[68,114–117]. Specific inhibition of mTORC1 in mice using an mTORC1-specific inhibitor did not result in hyperglycemia, impaired glucose tolerance, hyperlipidemia or hypercholesterolemia, again demonstrating that these negative effects are mediated at least in part by inhibition of mTORC2 (ref. 120). Finally, mTORC2 has been shown in a number of studies to have a key role in immunity and wound repair[121–127], and, in accordance with this important role for mTORC2, compounds that selectively inhibit mTORC1 have a reduced effect on the immune system compared to rapamycin[120]. Thus, lower or intermittent doses of rapamycin or treatment with rapalogs that more specifically target mTORC1 are predicted to reduce mTORC2-associated side effects such as hyperlipidemia and hyperglycemia without impairing the lifespan-extending benefits of mTORC1 inhibition.

This is not to say that there may not be benefits of inhibiting mTORC2 in specific settings. Inhibition of mTORC2 signaling extends the lifespan of *C. elegans* under certain temperatures and dietary conditions[111,112], has anti-tumor effects[128–131], is beneficial in mouse models of mitochondrial disease[132,133] and has been shown to inhibit or reverse senescence in human cells[134,135]. Certain side effects, whether mediated by mTORC1 or mTORC2, may potentially even be a consequence of 'on-target' action of rapamycin and rapalogs, and avoiding these side effects may limit the benefits of such drugs for healthy aging. However, given the balance of the evidence, particularly the genetic studies showing that mTORC2 activity is associated with lifespan and that inhibition of mTORC1 alone can extend lifespan, we believe that inhibition of mTORC2 is 'off-target' with respect to the beneficial effects of rapamycin and rapalogs on healthspan and longevity.

Clinical trials of mTOR inhibitors for diseases of aging

Given the extensive preclinical data confirming that mTOR inhibition extends lifespan and healthspan, there is great interest in determining whether mTOR inhibitors will have benefits for human aging. As highlighted in Fig. 1, based on mouse studies, rapamycin and rapalogs may have benefits in many different systems, including the brain, the heart, the immune system, the intestine, the liver, senescent skin cells, skeletal muscle and tendons. Rapalogs are approved

Table 3 | Completed clinical trials of mTOR inhibitors for aging-related conditions

Population	mTOR-inhibitor-dosing regimen	Duration	Primary endpoint	Was the primary endpoint met?	Reference
218 adults aged ≥65 years	Everolimus, 0.5 mg daily Everolimus, 5 mg weekly Everolimus, 20 mg weekly Placebo	6 weeks	Improvement in influenza vaccination response	Yes	Mannick et al.[82]
264 adults aged ≥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	Improvement in influenza vaccination response	Yes	Mannick et al.[83]
652 adults aged ≥65 years at increased risk of RTIs	BEZ235, 5 mg daily BEZ235, 10 mg daily BEZ235, 10 mg twice daily BEZ235, 10 mg + everolimus, 0.1 mg daily Placebo	16 weeks	Decrease in incidence of laboratory-confirmed RTIs	Yes	Mannick et al.[84]
1,051 adults aged ≥65 years	BEZ235, 10 mg daily Placebo	16 weeks	Decrease incidence in symptoms consistent with an RTI	No	Mannick et al.[84]
25 healthy adults, 70–95 years old	Rapamycin, 1 mg Placebo	8 weeks	Safely achieving therapeutic blood level	Yes	Kraig et al.[149]
36 adults with aging skin	Topical rapamycin (10 μM) Topical placebo	8 months	Decrease in p16-positive senescent cells in skin	Yes	Chung et al.[135]

Information on completed clinical trials was compiled from a review of the scientific literature.

for use in patients with transplantation and patients with cancer at high doses that strongly suppress mTORC1 activity[136] and have substantial side effects including mouth ulcers similar to canker sores, gastrointestinal side effects, hyperlipidemia and hyperglycemia and impaired wound healing. The approved doses of rapalogs also inhibit immune function, and therefore the FDA approved prescribing information notes that taking rapalogs may increase the risk of infection and certain cancers associated with immunosuppression[137,138]. The risk of infectious diseases associated with higher doses of rapalogs in humans may not be adequately modeled in preclinical mouse experiments, as mice are usually housed in specific pathogen-free barrier facilities.

Chronic treatment of humans with high doses of rapalogs is also associated with deleterious metabolic consequences including hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, insulin resistance and glucose intolerance[139–143]. Hyperlipidemia and hyperglycemia have also been observed in rodents treated with rapamycin or rapalogs, although mice are less susceptible to developing hyperlipidemia-induced cardiovascular disease than humans. Trelinska and colleagues observed hyperlipidemia in 66% of patients with TSC taking high daily doses of everolimus for 15 months; hyperglycemia was observed in 22% of participants[139]. Another side effect of rapamycin at doses that extend lifespan in mice is testicular degeneration[25,144], and reduced male fertility has been observed in humans treated with rapalogs[145,146]. Finally, rapamycin at lifespan-extending doses in mice also promotes the formation of cataracts[25], although cataracts have not been associated with rapalog treatment in humans.

Recent studies have explored the safety and efficacy of lower or intermittent dosing regimens of mTOR inhibitors in older adults. Preliminary results from these studies suggest that lower or intermittent dosing regimens of mTOR inhibitors that turn down mTOR activity to 'younger' levels rather than turn off mTOR activity have the potential to safely improve the function of aging organ systems or ameliorate aging-related diseases in humans. Of interest, clinical trials to date suggest that low or intermittent doses of mTOR inhibitors enhance rather than suppress immune function and decrease infection risk in older adults. Table 3 summarizes the clinical trials published to date of mTOR inhibitors for aging-related conditions; we discuss each of these briefly below.

mTOR inhibition and the immune system

The largest clinical trials to date have investigated whether mTOR inhibition can improve the function of the aging immune system. The first clinical trial was done with 218 adults aged ≥65 years without unstable medical conditions to determine whether the rapalog everolimus improved the function of the aging immune system as assessed by response to influenza vaccination[82]. The rationale for the trial was a preclinical study demonstrating that 6 weeks of rapamycin treatment improved the immune response of old mice to influenza vaccination[39].

Safety was a key concern when designing this trial. Therefore, very low daily or intermittent doses of everolimus were used in the vaccination trial (1/6th–1/20th lower than the approved doses for patients with transplantation and oncological conditions) that were predicted to minimize adverse events and to lower rather than completely inhibit mTORC1 activity. The clinical trial demonstrated that 6 weeks of treatment with everolimus at either 0.5 mg once daily or 5 mg once weekly was well tolerated and significantly improved the response of older adults to influenza vaccination. Immunophenotyping revealed that everolimus at low or intermittent doses decreased the percentage of PD-1$^+$ exhausted T cells in the peripheral blood of older adults, and this may have been a mechanism underlying their improved immune function. This was the first study to demonstrate that mTOR inhibition may improve the function of an aging organ system in humans[82].

A subsequent clinical trial was done with 264 adults aged ≥65 years to extend these findings and determine whether low-dose mTOR-inhibitor treatment with either everolimus and/or or the catalytic site mTOR inhibitor BEZ235 improved the function of the aging immune system sufficiently to improve not only influenza vaccination response but also to decrease total infection rates in older adults[83]. The study confirmed that 6 weeks of treatment with mTOR inhibitors at a low dose was safe and was associated with a dose-dependent significant improvement in influenza vaccination response. In addition, low-dose mTOR-inhibitor treatment significantly decreased the rate of infections, the majority of which were respiratory tract infections (RTIs). Exploratory transcriptional profiling found that the older adults receiving mTOR inhibitors at low doses had significant upregulation of genes in type 1 interferon-induced antiviral pathways. This upregulation of antiviral gene expression may underlie the reduction in RTIs observed in older adults treated with mTOR inhibitors, as most RTIs are viral in origin[147,148].

Large follow-up phase 2b (n = 652) and phase 3 (n = 1,051) trials were done to confirm whether 16 weeks of treatment with mTOR inhibitor at a low dose upregulated antiviral immunity and decreased the incidence of viral RTIs in older adults[84]. Low doses of the mTOR inhibitor BEZ235 were observed to be well tolerated and to decrease the incidence of laboratory-confirmed RTIs (the phase 2b primary endpoint; odds ratio, 0.601 (90% confidence interval CI: 0.39, 0.92); P value, 0.025) but not the incidence of clinically symptomatic respiratory illness, which was defined as symptoms consistent with an RTI irrespective of whether an infection was laboratory confirmed (the phase 3 primary endpoint; odds ratio, 1.07 (90% confidence interval: 0.80, 1.42); P value, 0.65). In both trials, significantly more interferon-induced antiviral genes were upregulated in individuals treated with BEZ235 than in those treated with the placebo. Lessons learned from the combined phase 2b and 3 trial results suggest that upregulation of antiviral immunity by low-dose mTOR-inhibitor therapy may have a greater impact on (1) severity than incidence of viral RTIs, (2) RTIs caused by coronaviruses, rhinoviruses and influenza viruses as opposed to other respiratory viruses, and (3) the incidence and severity of viral RTIs in older adults ≥75 years of age as opposed to those 65–74 years of age. Based on these findings, it will be important to determine in future clinical trials whether mTOR inhibitors decrease the severity of specific viral RTIs in people ≥75 years of age.

Rapamycin in older adults

In contrast to the participants in the above trials, who tolerated everolimus or BEZ235 quite well, a small, 8-week long randomized clinical trial of 25 older adults between 70 and 95 years of age treated with rapamycin at 1 mg per day experienced more side effects than those treated with placebo, including a small increase in glycated hemoglobin (A1C) (within-group P = 0.03) and a 40% increase in triglyceride levels (within-group P = 0.05)[149]. While these results tend to align with our expectation that chronic rapamycin treatment may cause hyperglycemia and hyperlipidemia, a caution in interpreting these results is the small group size and the failure of the between-group comparisons to reach statistical significance (P = 0.07 and 0.12 for A1C and triglyceride changes, respectively). In addition, other parameters of glucose metabolism including insulin sensitivity and pancreatic beta cell function were not affected. It is possible that lower doses of rapamycin, intermittent treatment with rapamycin or treatment with everolimus would be better tolerated than the daily dose of 1 mg per day examined here, but this has not been directly tested.

Rapamycin in skin

Local administration of rapamycin with negligible systemic exposure poses fewer safety risks than systemic administration. In a randomized placebo-controlled study of participants aged ≥40 years with signs of skin aging, 36 participants topically applied cream containing rapamycin (10 μM) to one hand and a matching placebo cream to their other hand for 8 months[135]. A total of 17 participants completed the study, 13 participants had skin biopsies, and eight participants had sufficient biopsy material for analysis of p16 expression levels, the primary endpoint of the study. Rapamycin-treated as compared to placebo-treated participants were observed to have a significant decrease in senescent cells as assessed by p16 expression, increased collagen VII protein expression and clinical and histologically assessed improvements in skin appearance. Treated participants did not have detectable levels of rapamycin in their blood, and there were no treatment-related adverse events.

Forthcoming clinical trials

A number of small clinical trials focused on geroprotective outcomes have been proposed in the USA, the UK and New Zealand over the past 2 years, and many have obtained funding and/or began enrolling participants. While the details of each study differ, most are testing low or intermittent doses of rapamycin or everolimus in participants over the age of 50 years in the range of 0.5–1 mg per day to 5–6 mg per week. These studies are testing a range of outcomes for a range of age-related diseases, including Alzheimer's disease, diabetes and sarcopenia; primary outcomes include disease-specific outcomes, including effects on cognitive performance, changes in insulin sensitivity and changes in physical performance. Secondary outcomes for most planned studies include biomarkers of aging, including analysis of the DNA-methylation clock; most studies are also assessing safety-related endpoints, including infections and metabolic disruption. A weakness of these planned studies is that even the largest will be studying a maximum of only 150 participants, and the longest planned study is 18 months. Thus, while these studies are critical to gather data on the safety and effectiveness of these compounds as geroprotectors in humans and interesting new data will probably become available over the next few years, comprehensive follow-up with well-powered double-blind placebo-controlled randomized clinical trials will still be necessary.

New ways to inhibit mTORC1

Given the drawbacks of rapamycin and commercially available rapalogs, most notably, the side effects and the narrowness of any potential therapeutic window, there is intense interest in identifying new ways to selectively inhibit mTORC1. In the short term, some dietary interventions have shown potential promise. In particular, because mTORC1 activity is regulated by amino acids, while mTORC2 activity is not, reducing the content of dietary protein or specific dietary amino acids that normally stimulate mTORC1 activity has been explored as a way to promote healthy aging. Several studies have shown that restriction of protein or specific restriction of either methionine or one or more of the branched-chain amino acids (leucine, isoleucine and valine) reduces mTORC1 activity and extends lifespan in preclinical species[24,150–152]. Intriguingly, low blood levels of isoleucine are also associated with reduced mortality in humans[153]. Other potentially mTORC1-lowering diets include a ketogenic diet, which has been shown to extend the lifespan of mice while reducing mTORC1 activity[154].

Of course, any dietary intervention will have issues with adherence, and pharmaceutical interventions can take mTOR inhibition to a much broader swath of the population. High-resolution structures of mTORC1 have provided a previously unprecedented look at how mTORC1 is activated by nutrients and how it is inhibited by rapamycin[155–157]. High-resolution structures have also been generated of the complexes that regulate mTORC1 activity, including the TSC complex[158], Rag GTPases in complex with mTORC1 and Ragulator[159–161] and the binding of leucine by sestrin 2 (ref. 162). As discussed below, this has led to potential new approaches and the development of new molecules to target mTORC1 selectively.

mTORC1 is able to phosphorylate its substrates only after allosteric interaction with Ras homolog enriched in brain (RHEB)–GTP, which activates mTORC1 by promoting the alignment of kinase-site residues[163,164]. To bring mTORC1 and RHEB–GTP together, two signaling pathways converge at the surface of the lysosome. The first of these pathways brings mTORC1 to the lysosomal surface, while a second pathway controls the GTP–GDP binding status of RHEB. The recruitment of mTORC1 to the lysosomal surface has been studied for over a decade and has been reviewed in detail elsewhere[3]; we have outlined the major regulator mechanisms in Fig. 2. In brief, mTORC1 is recruited to the lysosome by interacting with heterodimeric pairs of the Rag family of small GTPases[165,166]. In the presence of amino acids, RagA or RagB binds GTP and RagC or RagD binds GDP (for example, $RagA^{GTP}$–$RagC^{GDP}$), permitting the Rag proteins to interact with mTORC1 and localize it to the lysosome. The nucleotide-binding status of Rag GTPases is controlled by several different protein complexes with guanine nucleotide exchange factor (GEF) or GTPase-activating protein (GAP) activity for Rag GTPases.

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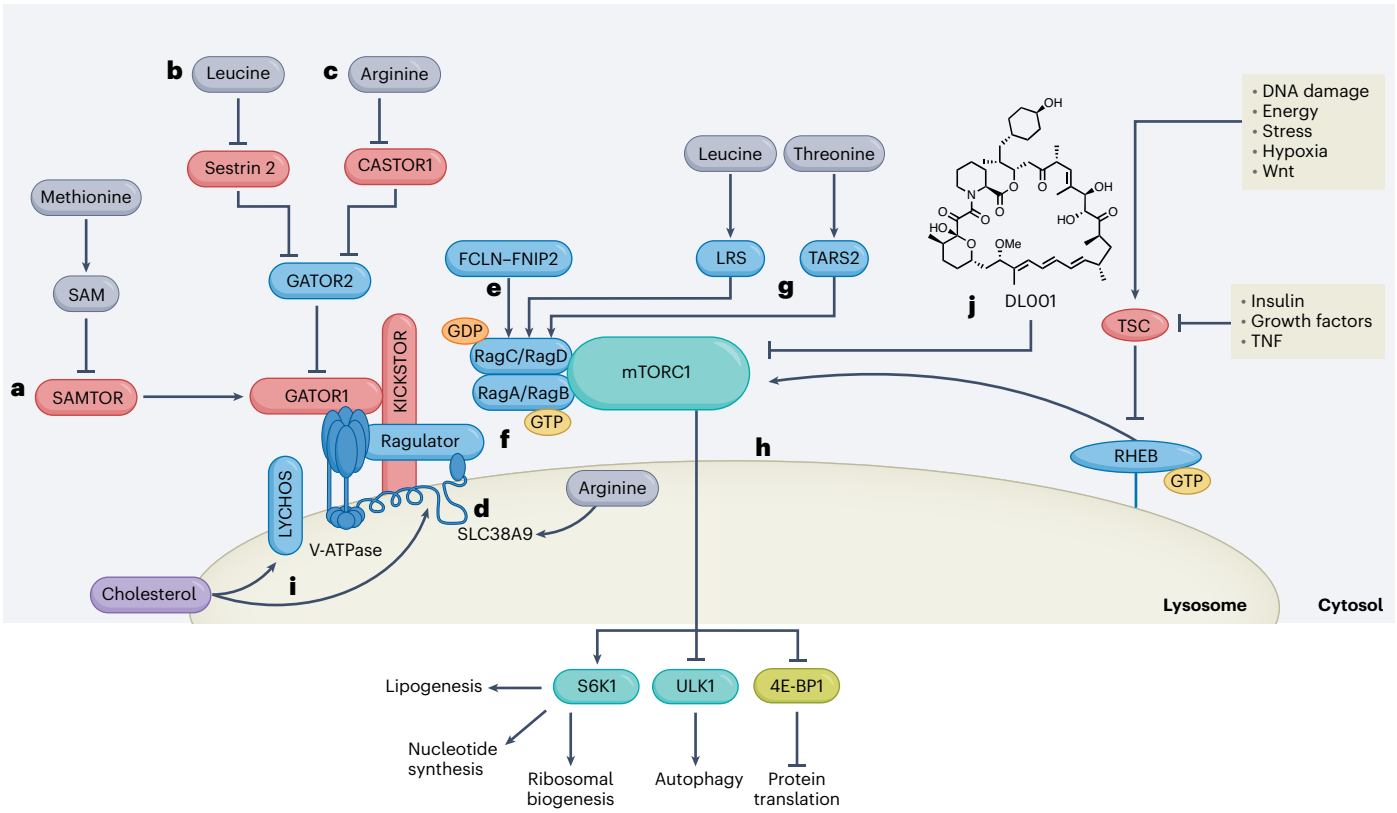

Fig. 2 | An overview of the mTORC1 signaling pathway with areas of potential pharmaceutical inhibition highlighted. Negative regulators (CASTOR1, GATOR1, SAMTOR, sestrin 2, TSC) and positive regulators (FLCN–FNIP2, GATOR2, KICKSTOR, LRS, Rag GTPases, Ragulator, RHEB, SLC38A9, vacuolar-type ATPase (V-ATPase)) are shown. Potential mechanisms for the development of mTORC1-specific inhibitors include: identifying small molecules that block the ability of amino acid sensors upstream of mTORC1 to sense the availability of leucine, arginine or SAM (**a**–**d**); developing compounds such as BC-LI-0186 that inhibit the GAP or GEF activities of FLCN–FNIP2, LRS, Ragulator or TARS2 (**e**–**g**); inhibiting the interaction of mTORC1 and RHEB, the mechanism of action of NR1 (**h**); identifying small molecules that block the ability of LYCHOS and SLC38A9 to sense the availability of cholesterol (**i**); and identifying rapamycin derivatives such as DL001 that specifically inhibit mTORC1 (**j**). TNF, tumor necrosis factor-α. Select downstream substrates of mTORC1 and processes mediated by them are also shown. The figure was adapted with permission from ref. 252, Oxford University Press.

One of the best described systems for regulating the nucleotide-binding status of Rag GTPases is the activity of the GATOR complexes. GATOR1 functions as a GAP for RagA and RagB, while GATOR2 acts to inhibit the activity of GATOR1 (refs. 167,168). The GATOR complexes are regulated by levels of amino acids, cholesterol and glycolytic intermediates, thus linking mTORC1 activity with nutrient availability. Three different amino acid sensors have been found that regulate mTORC1 activity by controlling GATOR1 or GATOR2 activity. The sestrin and CASTOR families of proteins link the availability of leucine and arginine, respectively, to the recruitment of mTORC1 to the lysosomal surface. Specifically, the sestrin family of proteins binds to and inhibits the action of GATOR2 when leucine levels are low, permitting GATOR1 to inhibit the recruitment of mTORC1 to the lysosome. Binding of leucine by the sestrins, particularly sestrin 2, relieves this inhibition of GATOR2, resulting in inhibition of GATOR1 GAP activity and allowing mTORC1 to be recruited to the lysosome[162,169].

The CASTOR proteins function similarly; when arginine levels are low, CASTOR proteins bind to and inhibit GATOR2. When arginine levels are high, CASTOR proteins bind to arginine and release GATOR2, which allows the recruitment of mTORC1 to the lysosome[170,171]. The SAMTOR protein acts as an indirect sensor of methionine levels, inhibiting GATOR1 when levels of the methionine metabolite *S*-adenosylmethionine (SAM) are low[128]. As SAM is extremely responsive to methionine levels, SAMTOR essentially functions as a sensor of methionine levels[172]. It was recently shown that cholesterol signals through a G protein-coupled receptor, LYCHOS, which links cholesterol levels to mTORC1 activity by sequestering GATOR1 in the presence of cholesterol[173]. Finally, while glucose is not directly sensed by mTORC1, the glycolytic intermediate dihydroxyacetone phosphate (DHAP) is sensed via a GATOR-dependent mechanism[2].

The regulation of RagC and RagD is somewhat less well understood, but the folliculin (FLCN) complex has been shown to act as a GAP for these GTPases[174–177]. Leucyl-tRNA synthetase (LRS) has been shown to function as a leucine sensor for mTORC1, acting as a GAP for RagD[178]. The mitochondrial threonyl-tRNA synthetase TARS2 functions as a threonine sensor, interacting with GTP–RagC to promote GTP loading of RagA, probably via recruitment of an unidentified RagA GEF[179].

At the lysosomal surface, mTORC1 activity depends upon the interaction of the mTOR protein kinase with GTP-bound RHEB. Cryo-electron microscopy has revealed that RHEB–GTP binds allosterically to mTOR, resulting in a global conformational change that allosterically realigns the active-site residues to enable substrate phosphorylation[157]. At the lysosomal surface, TSC inhibits mTORC1 activity by acting as a GAP for RHEB. A number of different factors have been reported to control the lysosomal localization of TSC, most notably, insulin–PI3K–AKT signaling, which acts to phosphorylates TSC on multiple residues, causing TSC to depart from the lysosome and permitting RHEB to be loaded by GTP[180]. The localization of TSC has also been reported to be sensitive to amino acids, including arginine, although the mechanism by which this takes place is unclear[181]. Rag GTPases have also been reported to recruit TSC to lysosomes in response to amino acid restriction, possibly via a GATOR2- or sestrin 2-dependent mechanism[182].

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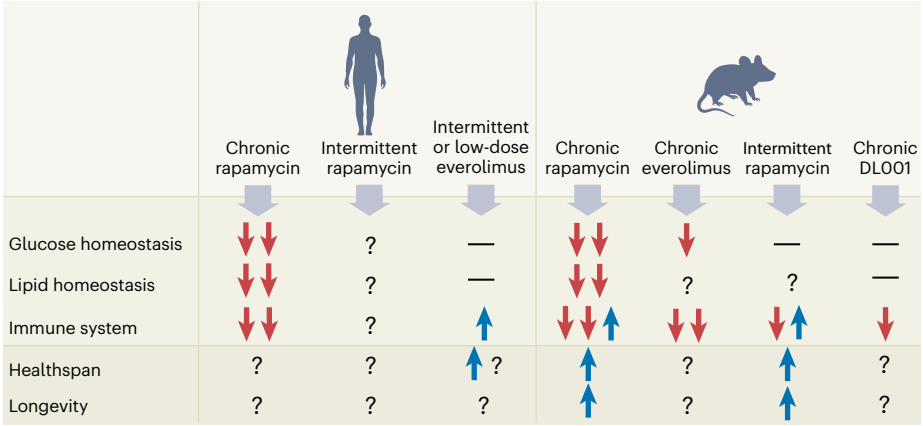

Fig. 3 | The known and unknown effects of rapalog-dosing regimens on metabolic health, the immune system, healthspan and longevity in humans and mice. Chronic (daily) dosing of rapamycin is associated with impaired blood glucose regulation and hyperlipidemia in humans and mice, while everolimus in mice has somewhat reduced effects on glucose homeostasis. Intermittent or low-dose regimens of rapamycin or everolimus are associated with reduced side effects, while the mTORC1-selective rapalog DL001 does not impair blood glucose control or alter circulating lipid levels. Blue up arrow, improvement; red down arrow, impairment; dash indicates no change; question mark indicates unknown.

Researchers are capitalizing on this knowledge to develop new but still early-stage mTORC1-selective drugs based on molecular and structural information about mTORC1 activation (Fig. 2). One of the best examples of these is the compound is NR1, which binds the mTORC1 activator RHEB and prevents it from allosterically activating mTORC1 (ref. 164). LRS has been proposed to function as a leucine sensor for mTORC1, acting as a GAP for RagD, and several recent compounds, for example, (*S*)-4-isobutyloxazolidin-2-one and BC-LI-0186, have been identified that inhibit mTORC1 by interfering with LRS sensing or activity[178,183–186]. BC-LI-0186 inhibits mTORC1 activity in vivo in mice and slows tumor growth in a mouse model of non-small-cell lung cancer[187]. As outlined in Fig. 2, small molecules that interfere with amino acid sensors or cholesterol sensors that normally signal nutrient availability could potentially be developed as mTORC1-selective inhibitors.

Newer rapalogs have been discovered that are more selective for mTORC1 than rapamycin. One company that screened a library of modified rapalogs identified a compound, DL001, with significantly greater selectivity for mTORC1 than rapamycin[120]. As expected, mice treated with DL001 had reduced glucose intolerance, dyslipidemia and immune disruption as compared to mice treated in parallel with rapamycin[120]. Multiple other companies are also working to bring more mTORC1-selective rapalogs to the clinic[188]. Preclinical trials of one such compound, NV-20494, have reportedly shown efficacy in a mouse model of polycystic kidney disease and in vitro in human three-dimensional cell culture[189]. Finally, Rapalink-1, a compound in which an mTOR kinase inhibitor is linked to rapamycin and delivered at a low dose, has shown the ability to inhibit mTORC1 kinase activity selectively[190]. A potential issue with this approach for diseases of aging is that mTOR kinase inhibitors result in broader mTORC1 inhibition than rapamycin[53], and it remains to be determined whether mTOR kinase inhibitors recapitulate the lifespan benefits of rapamycin in preclinical species.

Conclusions

There is rapidly growing interest in using mTOR inhibitors to promote healthy aging and to treat, delay or reverse numerous age-related diseases. While there is incredibly strong preclinical evidence in mice that rapamycin can extend lifespan and healthspan, excitement about rapamycin has outpaced rigorous evidence that rapalogs are both safe and efficacious for diseases of aging in humans. There are many unanswered questions from the trials that have been conducted thus far, but a few general lessons can be taken from the clinical trials of mTOR inhibitors that have been performed thus far. As highlighted in Fig. 3, in both humans and mice, treatment with low or intermittent doses of rapamycin or everolimus or treatment of mice with the mTORC1-selective inhibitor DL001, is much better tolerated than the high doses of mTOR inhibitors currently approved for organ transplant and oncology indications, with fewer metabolic side effects and less immunosuppression. In addition, low doses of mTOR inhibitors have been shown to have some beneficial effects on the function of aging human organ systems, in particular, the immune system.

There remains much work ahead to bring mTOR inhibitors into the clinic for age-related conditions, and, as highlighted in Fig. 3, many open questions remain. While the safety profile of low-dose rapamycin and rapalogs in humans appears promising, the long-term safety and efficacy of low-dose regimens remain to be determined. A much better understanding is needed of the specific dose and duration of mTOR inhibitors that both maximize efficacy and minimize risk. In humans, higher doses (for example, 3 mg per day) of mTOR inhibitors such as everolimus inhibit T cell function and are therefore are used to suppress immune-mediated organ transplant rejection in patients. By contrast, a sixfold lower dose of everolimus for 6 weeks was associated with improved immune function as assessed by vaccination response. Thus, both dose and duration may contribute to whether mTOR inhibition has positive or negative effects on healthy aging, but, generally speaking, the lower the dose of a drug, the fewer expected side effects. Moreover, animal dosing regimens that extend lifespan cannot be directly translated to human doses due to differences between species in factors such as bioavailability, half-life, metabolism, plasma protein binding and tissue distribution. These factors need to be taken into consideration when estimating the human doses that may extend healthspan or lifespan.

There is also a need to identify both the specific aging population and the specific aging-related conditions that show the greatest benefit from a safe mTOR-inhibitor regimen. An important question for future research is discovering why mTOR inhibitors have sex-specific or sex-biased benefits, as pharmacological treatment with rapamycin or genetic inhibition of insulin–insulin-like growth factor 1 (IGF1)–PI3K–AKT–mTOR–S6K1 signaling typically (but not always) extends the lifespan of female mice a greater amount than it extends the lifespan of male mice[191–193]. We also need to define new regulatory paths for aging-related conditions such as frailty and sarcopenia and to develop new mTOR inhibitors that improve on the safety and efficacy of currently approved mTOR inhibitors. Over the next 5 years, we

expect results from a rapidly expanding list of human clinical trials as well as work in canines and non-human primates to shed light on the viability of mTOR inhibition as a therapy for aging-related conditions. New mTORC1-specific molecules may help to widen the therapeutic window for rapalogs, limiting undesirable side effects resulting in whole or in part from inhibition of mTORC2. Collectively, we expect that researchers will soon be able to determine whether clinicians can safely and effectively bring mTOR inhibitors to the geriatric bedside.

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Author contributions

J.B.M. and D.W.L. wrote the manuscript and prepared the figures.

Competing interests

J.B.M. is CEO and cofounder of Tornado Therapeutics, which is developing safer, more effective mTOR inhibitors to extend human healthspan, and a former CMO of resTORbio. D.W.L. has received funding from and is a scientific advisory board member of Aeovian Pharmaceuticals, which seeks to develop new, selective mTOR inhibitors for the treatment of various diseases.

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