Review—Aging Biology

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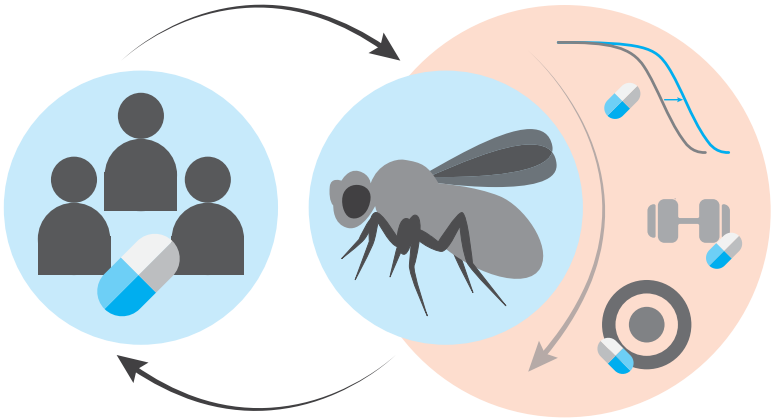

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Review—Aging Biology

Pharmacology of Aging: *Drosophila* as a Tool to Validate Drug Targets for Healthy Lifespan

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Finding effective therapies to manage age-related conditions is an emerging public health challenge. Although disease-targeted treatments are important, a preventive approach focused on aging can be more efficient. Pharmacological targeting of aging-related processes can extend lifespan and improve health in animal models. However, drug development and translation are particularly challenging in geroscience. Preclinical studies have survival as a major endpoint for drug screening, which requires years of research in mammalian models. Shorter-lived invertebrates can be exploited to accelerate this process. In particular, the fruit fly *Drosophila melanogaster* allows the validation of new drug targets using precise genetic tools and proof-of-concept experiments on drugs impacting conserved aging processes. Screening for clinically approved drugs that act on aging-related targets may further accelerate translation and create new tools for aging research. To date, 31 drugs used in clinical practice have been shown to extend the lifespan of flies. Here, we describe recent advances in the pharmacology of aging, focusing on *Drosophila* as a tool to repurpose these drugs and study age-related processes.

Introduction—Preventive Medicine Applied to Age-Related Disease

Aging is the main risk factor for chronic diseases and geriatric syndromes[1–3]. The increasing prevalence of these conditions is a serious public health challenge[4,5]. Age-related disorders are responsible for major healthcare costs and a growing burden on healthcare provision and caregiving, which threaten to become unsustainable from an economic and societal standpoint[6,7].

Major advances in modern medicine, with significant improvements in human health and life expectancy, arose from preventive approaches, such as immunization against infectious diseases and public health campaigns[8]. In geriatric medicine, personalized prevention already occurs in clinical practice. For instance, death from cardiovascular events is prevented pharmacologically by addressing risk factors such as hypercholesterolemia and hypertension. Similarly, the development of other age-related diseases, such as type-2 diabetes and certain cancer types, is delayed through dietary programs and the cessation of smoking[9–11].

Geroscience proposes the targeting of a more comprehensive risk factor: aging (Fig. 1). Treating or preventing an individual disease, if prevalent and deadly, can increase human life expectancy. However, the added years do not necessarily correlate with an improved quality of life[12], as aging will affect the whole organism and promote the development of other diseases and geriatric syndromes. Geriatric syndromes are linked to increased morbidity and reduced quality of life in the elderly[2] as well as higher mortality[13]. For instance, falls are a main cause of hospital admissions in the elderly[14]. Similar to other geriatric syndromes, falls result from multiple age-related factors, such as deteriorating muscle strength, loss of balance, and impaired vision, which illustrates the challenge of managing these conditions with an organ- or disease-focused approach. Instead, targeting aging has the potential to decrease the incidence of both age-related diseases and geriatric syndromes, extending human life with added years of health. Indeed, interventions that target aging-related processes improve healthspan in a range of animals[15,16].

Pharmacology of Aging: An Ancient Quest for a Modern Problem

The search for a compound able to target aging and extend life predates modern scientific history. Until just a few decades ago, this pursuit was still compared with medieval alchemy, even by prominent figures within the then-emerging field of aging research[17]. Initial studies in biogerontology were faced with scepticism at the idea of controlling longevity and concerns that a longer lifespan may not translate into a healthier one[18]. However, lifespan extension is often a side effect of health-promoting interventions, such as dietary restriction (DR). DR, defined as reduced food intake without malnutrition, is one of the most robust evolutionary conserved strategies to extend healthy lifespan[15]. Since the initial reports of DR in the early 20th century, researchers have strived to identify drugs that could act as DR mimetics and recapitulate its effects[19,20]. In addition, other dietary regimes, such as intermittent fasting, and genetic interventions within nutrient-sensing signaling cascades have similar benefits[21,22]. Accordingly,

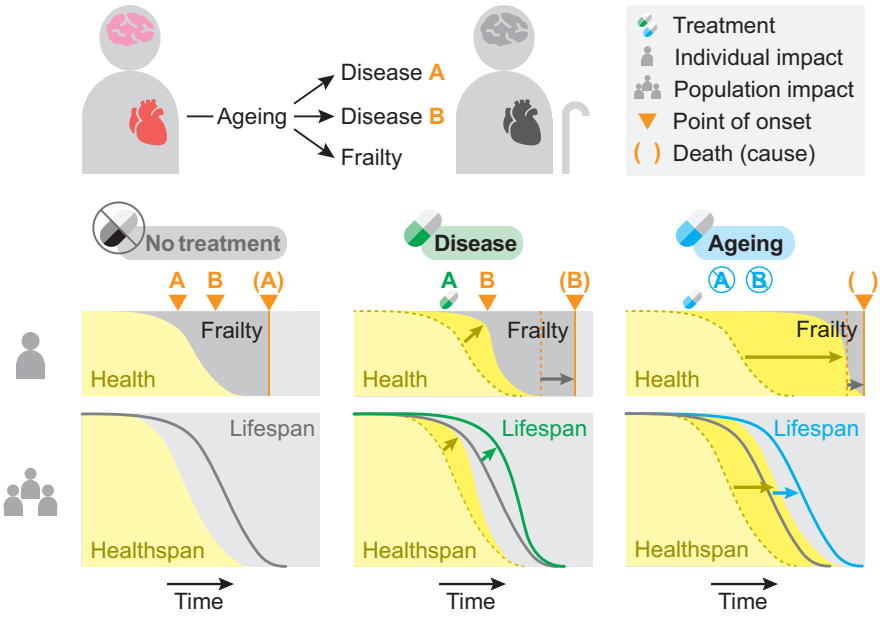

Figure 1. Approaches to health in old age: disease- and aging-centered models. In this theoretical representation, aging is the major risk factor for prevalent diseases 'A' and 'B' and causes frailty. Left untreated or treated symptomatically, a typical individual in this population will develop diseases 'A' and 'B' and die of complications related to disease 'A' in this hypothetical scenario. Aging combined with these diseases will result in a certain period of frailty. At a population level, the life expectancy or median lifespan will be limited by the prevalent disease 'A,' and the healthspan will be limited by the burden of diseases 'A,' 'B,' and aging. In the disease-centered approach to health in old age, the ideal scenario of curing or preventing disease 'A' will increase both health- and lifespan at an individual and populational level, but the period of frailty is unlikely to change due to the burden of other untreated age-related diseases—here represented by disease 'B'—and aging itself. In the aging-centered approach to health in old age, the ideal scenario of slowing or preventing aging will similarly increase both health and lifespan at an individual and populational level, by preventing or delaying the development of diseases 'A' and 'B' and, necessarily, decrease the period of frailty.

most current aging-related pharmacological interventions act—at least partly—via nutrient-sensing pathways[23].

In addition to dysregulated nutrient sensing, aging is associated with other processes that impact homeostasis with age, such as the accumulation of senescent cells or mitochondrial dysfunction[23–26]. Therapies targeting these processes also improve health in animal models[27–31].

Drug development is a slow and lengthy process, with most drugs failing to reach clinical trials[32]. This is particularly challenging in geroscience, because survival is a major endpoint of preclinical studies. Although new drug discovery is needed, a range of drugs already approved for clinical use in humans have exhibited prolongevity effects in model organisms. Studying the potential of these repurposed drugs to prevent human age-related disease may be an important first step in bringing the pharmacology of aging to the clinic, because they can be more rapidly and safely translated and may serve as proof-of-principle that targeting aging processes can improve population health. In addition, dissecting their mechanisms in animal models can help increase treatment efficacy by finding more specific longevity-modulating targets. This goal underpins the Intervention Testing Program by the U.S. National Institute on Aging[33,34] and some recent biotech companies[35,36], most of which investigate longevity in mice.

Drosophila as a Tool to Repurpose Drugs for Healthy Aging

Mammals such as mice are valuable model systems from a translational perspective due to their closer evolutionary proximity to humans, in particular to study drug metabolism, excretion, and toxicity. However, invertebrate models are important complementary tools in pharmacology by allowing quicker, cheaper, and larger-scale studies, with fewer bureaucratic restrictions[37,38]. Short-lived invertebrates are particularly useful in aging research to validate the targets of known drugs as longevity modulators, and to screen for effective drugs before further preclinical assessment of toxicity and pharmacokinetics in mammals (Fig. 2). When repurposing approved drugs, invertebrates may accelerate translation even more by providing proof-of-concept studies in the context of longevity and disease prevention[39].

Two invertebrate systems, the nematode worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, are extensively used in aging research. As one of the most prevalent occurrences across life, aging is expected to happen through analogous processes and manifest in invertebrates via shared pathways. Indeed, many pioneering discoveries in the aging field were initially made in the worm and the fly, and subsequently shown to be evolutionarily conserved in mammals[40–42].

Drosophila has a relatively short lifespan (typically ~2–3 months in the laboratory compared with ~2–3 years for mice) and exhibits strong evolutionary conservation of metabolic and signaling pathways associated with aging. Flies also allow the use of powerful genetic tools that can validate drug targets, enabling the mechanistic analysis of pharmacological interventions and the assessment of systemic physiological consequences[40].

The *Drosophila* model is historically important in the discovery and characterization of compounds that modulate longevity. Diiodomethane was the first nonnutrient shown to increase fly lifespan in the 1970s[43]. Since then, other examples with comparatively strong effects on fly survival include the metabolites phenylbutyrate[44] and α-ketoglutarate[45] as well as compounds such as trichostatin A[46,47], torin 1[48], and dihydromyricetin[49].

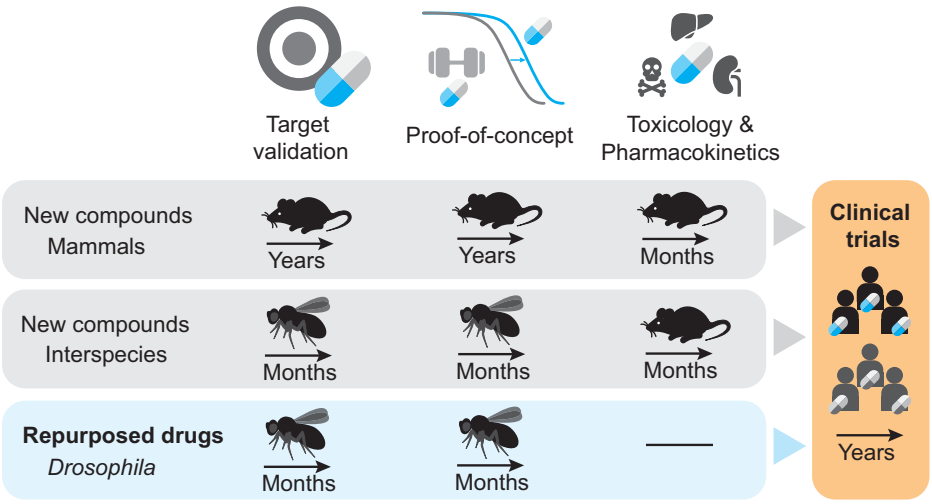

Figure 2. *Drosophila* as a tool to accelerate preclinical drug development in the context of aging. Scheme illustrating different preclinical routes for pharmacological interventions and how this process can be accelerated by a repurposing strategy and the use of *Drosophila* as an *in vivo* model system.

According to the DrugAge database, there are >600 compounds that significantly extend the lifespan of different animal models[50]. Among these, >150 compounds have been tested in *Drosophila*. Here, we specifically focus on a subset of 31 drugs that are clinically approved by the European Medicines Agency and significantly extend median/mean lifespan in flies (Table 1). The following eight drugs recently identified by a high-throughput screen will not be described in detail, as they require further validation and characterization in the context of fly survival: bupivacaine, fluspirilene, fluvoxamine, haloperidol, mianserin, promethazine, tetracycline, and thioridazine[51]. In this review, we discuss the remaining 22 clinically approved drugs present in the DrugAge database, along with an additional compound (zoledronate) from a recent study[52], which meets our inclusion criteria, and we highlight how the *Drosophila* model has contributed to advancing our understanding of their prolongevity benefits.

Most of these drugs act on nutrient-sensing pathways. To adapt to the energy supply and demands of the extracellular environment, cells sense nutrients essentially via two key pathways: the insulin/insulin-like growth factor signaling (IIS) pathway and the mechanistic target-of-rapamycin (TOR) pathway, which are activated under conditions of high glucose and amino acids, respectively. In contrast, under low cellular energy status, AMP-activated protein kinase (AMPK) promotes ATP production via the increased expression of genes and activity of proteins involved in catabolism, and energy conservation by inhibiting anabolic processes. Pharmacological modulation of components within these key metabolic cascades can impact survival (Fig. 3). The mechanisms whereby other clinically relevant drugs (i.e., not classically implicated in nutrient sensing) extend *Drosophila* lifespan are currently less explored and understood, as discussed in the respective sections.

Rapamycin and derivatives: Inhibiting the TOR pathway

Rapamycin, also known as sirolimus, is a drug that blocks the activation of the TOR kinase, more specifically acting on TOR complex 1 (TORC1). In humans, this results in the inhibition of T-cell activation and proliferation, leading to immunosuppression. Rapamycin is clinically used in immunosuppressive regimens, including the prophylaxis of kidney transplant rejection[53].

As the TOR pathway is also a key player in longevity modulation, rapamycin has been thoroughly studied in this context[54]. Rapamycin was shown to increase the survival of multiple organisms, such as worms, flies, and mice[55–58]. Importantly, rapamycin extends mammalian survival even when administered chronically later in life[34,55,59,60] and improves health by preventing aging-related phenotypes, such as liver degeneration, tendon stiffness, periodontal bone loss and inflammation, and loss of hematopoietic stem cell capacity[59,61,62].

In *Drosophila*, rapamycin extends lifespan independently of sex and fly genetic background[56], although not consistently in males[63]. Its derivative everolimus was independently found to extend mean lifespan in males[64]. Transient rapamycin treatment during fly development and early adult life is sufficient to extend lifespan as well as chronic treatment from middle and old age[65,66]. Consistent with other organisms, this effect is related to the inhibition of TORC1[56]. By inhibiting TORC1, rapamycin induces a cellular perception of amino acid deprivation, resulting in the induction of autophagy and reduced protein synthesis via S6K inhibition[56,67]. The lifespan extension is not dependent on the mRNA translation inhibitor 4E-BP, another target of TORC1, even though 4E-BP is necessary for the protective role of rapamycin in fly neurodegeneration models[68,69].

Tissue-specific effects of TOR pathway inhibition have systemic consequences sufficient to extend fly lifespan. For example, inhibitory modulation of TOR pathway gene expression in the fat body increases fly survival[70]. In the gut, rapamycin delays age-related barrier dysfunction and decreases the rate of intestinal stem cell (ISC) proliferation[58,67,71]. These effects are mediated by the indirect inhibition of polymerase III, an enzyme downstream of TORC1 that generates ribosomal RNA involved in protein synthesis[58], and the overexpression of histones H3 and H4[63,71]. The counter-intuitive synthesis of these histone proteins in enterocytes occurs via a noncanonical mechanism mediated by translation initiation factor 3 (IF-3), resulting in changes to chromatin organization in enterocyte nuclei and altered expression of autophagy-related genes. These genes include *bchs*, a cargo adaptor for selective degradation of ubiquitinated protein aggregates, which is required for rapamycin-associated lifespan extension[63,71]. Similarly, the lifespan extension after early life

Table 1. Data on clinically approved drugs that extend lifespan in *Drosophila*.

Drug	Target	Treatment Age	Strain and Sex	Food	Concentration	% Mean	% Med.	% Max.	References
Single interventions									
Aspirin	S6K?	From day 2	w^{Dah} ♀	SY	0.5 μM	-	13%	-	Song et al. (2017)[113]
	Imd?	From day 1	w^{1118}♀	-	1 mg/L (5.6 μM)	32%	-	-	Zhu et al. (2021)[119]
Celecoxib	Akt?	From day 3	w^{Dah} ♀	Holidic	0.5 μM	-	10%	-	Wu et al. (2016)[116]
			w^{Dah} ♂			-	9%	-	
		From day 42	w^{Dah} ♀			-	5%	-	
Cortisone	-	From day 1	*Canton-S* ♀	-	0.188 mg/ml (467 μM)	18%	28%	13%	Hochschild (1971)[120]
			Canton-S ♂			43%	36%	31%	
Desoxycorti-costerone	-	From day 1	*Canton-S* ♂	-	0.005 mg/ml (15 μM)	21%	29%	21%	Hochschild (1971)[120]
Everolimus	TORC1	From day -	Ore^{R} ♂	SYC	3 mM	17%	-	-	Spindler et al. (2012)[64]
Fludrocortisone	-	From day 1	*Canton-S* ♀	-	0.0075 mg/ml (18 μM)	11%	23%	9%	Hochschild (1971)[120]
			Canton-S ♂			-	1%	-6%	
Ibuprofen	-	From day -	*Canton-S* ♀	SYS	0.5 μM	-	9%	4%	He et al. (2014)[114]
			Canton-S ♂			-	17%	–8%	
		Day 30–40	*Canton-S* ♀		1 μM	-	23%	-	Proshkina et al. (2016)[115]
			Canton-S ♂			-	n/s	-	
Lamotrigine	-	From day -	*IV* ♀	BM+YP	12 mg/ml (47 mM)	17%	-	15%	Avanesian et al. (2010)[158]
			IV ♂			15%	-	18%	
Lithium	GSK-3/ Sgg	From day 2	w^{Dah} ♀	SY	1-25 mM	-	16%	18%	Castillo-Quan et al. (2016)[86]
			w^{1118}♀		25 mM	-	13%	18%	
			w^{1118}♂		25 mM	-	23%	14%	
			w^{Dah} ♀		1 mM	-	15%	10%	Castillo-Quan et al. (2019)[97]
		From day 32	w^{Dah} ♀		25 mM	-	9%	8%	Castillo-Quan et al. (2016)[97]
Metformin	Complex I *E. coli* CRP	From day 2	*Dah*♀	SY	1–10 mM	-	n/s	-	Slack et al. (2012)[106]
					25 mM	-	-11%	-	
			Dah ♂		1–50 mM	-	n/s	-	
					100 mM	-	-31%	-	
		From day 2	w^{Dah} ♀	Holidic*	0.5 mM	17%	-	-	Pryor et al. (2019)[107]
		From day 1	w^{1118}♀	SYC	5 mM	-	17%	-	Suzuta et al. (2022)[109]
Mifepristone (=RU486)	JH	From day 2	w^{1118}♀	SYC	0.16 mg/ml (372 μM)	15%	38%	-	Landis et al. (2015)[143]
			w^{1118}♀*			–1%	0%	-	
			Ore^{R} ♀			–10%	–13%	-	
			Ore^{R} ♀*			3%	4%	-	
			Canton-S ♀			5%	12%	-	
			Canton-S ♀*			15%	18%	-	
Minocycline	-	From day 1	Ore^{R} ♀	SYS	0.87 mM	-	~57%	-	Oxenkrug et al. (2012)[152]
			Ore^{R} ♂			-	~71%	-	
		From day -	Ore^{R} ♂			-	57%	-	Bonilla et al. (2012)[150]
						-	63%	-	Mora et al. (2013)[153]
		From day 3	*Canton-S* ♀	SYC	0.05 mM	-	14%	-	Lee et al. (2017)[151]
			Canton-S ♂			-	27%	-	
			w^{1118}♀			-	17%	-	
			w^{1118}♂			-	23%	-	

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Table 1. Continued.

Drug	Target	Treatment Age	Strain and Sex	Food	Concentration	% Mean	% Med.	% Max.	References
Morphine	-	From day 5	*Ore*^{R}♀	SYC	0.25 mg/ml (665 µM)	9%	-	-	Dubiley et al. (2011)[159]
			Ore^{R} ♂		0.01 mg/ml (27 µM)	16%	-	-	
***N*-acetyl-L-cysteine (NAC)**	-	From day 1	*Ore*^{R}♂	SYC	10 mg/ml (61 mM)	-	27%	25%	Brack et al. (1997)[126]
			Canton-S ♀	SY+YP	100 nM	-	–2%	–5%	Shaposhnikov et al. (2018)[128]
			Canton-S ♂			-	9%	8%	
			w^{1118}♀	SYC	1 mg/ml (6 mM)	–	6%	n/s	Niraula & Kim (2019)[127]
			w^{1118}♂			-	n/s	n/s	
Rapamycin	TORC1	From day 2	w^{Dah} ♀	SY	200 µM	-	13%	-	Bjedov et al. (2010)[56]
			w^{Dah} ♂			-	6%	-	
			yw ♀			-	4%	-	
			w^{Dah} ♀			-	14%	10%	Castillo-Quan et al. (2019)[97]
			Ore^{R} ♂	SYC	100 µM	38%	-	50%	Admasu et al. (2018)[147]
		L2/3-Pupae	w^{iso31}♀	YSCMS	200 µM	-	n/s	-	Aiello et al. (2022)[65]
		Day 0–10				-	10%	-	
		Day 10–13				-	n/s	-	
		Day 10–20				-	n/s	-	
		L2/3-Pupae	w^{iso31} ♂			-	8%	-	
		Day 0–10				-	14%	-	
		Day 10–13				-	n/s	-	
		Day 10–20				-	n/s	-	
		From day 2	w^{Dah} ♀	SY	200 µM	12%	13%	-	Juricic et al. (2022)[66]
		Day 1–30				11%	13%	-	
		Day 30–100				6%	8%	-	
		Day 45–100				3%	3%	-	
		Day 60–100				n/s	n/s	-	
		Day 1–15				7%	7%	3%	
		Day 15–30				5%	7%	3%	
		From day 2	w^{Dah} ♀		50 µM	-	20%	7%	Regan et al. (2022)[63]
			w^{iso31} ♂		200 µM	-	n/s	13%	
			Dah♀	SY	200 µM	-	3%	-	
			Dah ♂			-	4%	-	
			DGRP♀			-	4%	-	
			DGRP ♂			-	n/s	-	
Rifampicin	dFOXO?	From day 1	*Ore*^{R} ♂	SYA	50 µM	11%	-	27%	Admasu et al. (2018)[147]
Salicylamide	-	From day 1	*Canton-S* ♀	-	6.5 mg/ml (47 mM)	12%	25%	27%	Hochschild (1971)[120]
			Canton-S ♂			17%	23%	22%	
Sevelamer	-	From day -	*yw* ♂	YSCMC	1% (54 µM)	-	17%	-	Bergwitz (2012)[124]
Simvastatin	-	From day 1	*Ore*^{R}♂	SYC	240 µM	14%	-	-	Spindler et al. (2012)[121]
Trametinib	Mek	From day 2	w^{Dah} ♀	SY	15.6 µM	-	12%	-	Slack et al. (2015)[94]
						-	25%	18%	Castillo-Quan et al. (2019)[97]
		From day 30				-	4%	-	Slack et al. (2015)[94]

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Table 1. Continued.

Drug	Target	Treatment Age	Strain and Sex	Food	Concentration	% Mean	% Med.	% Max.	References
Triamcinolone	-	From day 1	*Canton-S* ♀	-	0.04 mg/ml (101 μM)	19%	25%	16%	Hochschild (1971)[120]
			Canton-S ♂			18%	23%	5%	
Zoledronate	-	From day 4	w^{Dah} ♀	YMC	1 μM	-	n/s	-	Chen et al. (2021)[52]
			w^{Dah} ♂			-	~5%	-	
		From day 40	w^{Dah} ♀		10 μM	-	17%	-	
			w^{Dah} ♂			-	5%	-	
Combined interventions									
Rapamycin Rifampicin	TORC1 dFOXO?	From day 1	Ore^{R} ♂	SY	100 μM 50 μM	63%	-	65%	Admasu et al. (2018)[147]
Rapamycin Lithium	TORC1 Sgg	From day 2	w^{Dah} ♀	SY	200 μM 1 mM	-	21%	15%	Castillo-Quan et al. (2019)[97]
Rapamycin Trametinib	TORC1 Mek	From day 2	w^{Dah} ♀	SY	200 μM 15.6 μM	-	34%	24%	Castillo-Quan et al. (2019)[97]
Lithium Trametinib	Sgg Mek	From day 2	w^{Dah} ♀	SY	1 mM 15.6 μM	-	27%	20%	Castillo-Quan et al. (2019)[97]
Rapamycin Lithium Trametinib	TORC1 Sgg Mek	From day 2	w^{Dah} ♀	SY	200 μM 1 mM 15.6 μM	-	47%	35%	Castillo-Quan et al. (2019)[97]

Summary of mean, median (med.), and maximum (max.) % lifespan extension from published studies (n/s, not significant; -, data not available). The type of food is generalized based on the major ingredients, but the relative proportion/concentrations of each component may be different between studies. All media contain agar as a setting agent, with propionic acid and/or nipagin (tegosept) as antimicrobial/fungal preservatives. SY: sugar (sucrose)–yeast-based medium; SYC: SY medium with cornmeal; SYS: SY medium with semolina; SY+YP: SY medium with fresh yeast paste; YMC: yeast–molasses–cornmeal medium; YSCS: yeast–soy flour–cornmeal–corn syrup medium; YSCMC: yeast–soy flour–cornmeal–malt–corn syrup medium; BM+YP: banana–molasses medium with fresh yeast paste; holidic: chemically defined medium that mimics SY; holidic*, modified holidic medium without sucrose; ♀*, virgin female.

transient rapamycin treatment is mediated by sustained elevated autophagy in intestinal enterocytes[66]. Overall, these studies suggest that rapamycin extends fly lifespan by promoting intestinal health. Male flies have preserved intestinal barrier function with age and lower ISC proliferation rates[72] as well as high basal levels of enterocyte autophagy that are not further increased by rapamycin, which can explain the sexually dimorphic effect of rapamycin on lifespan[63].

A side effect of rapamycin in female flies is decreased fecundity[56,58,73], although rapamycin treatment can still extend survival in sterile females[56]. More recently, rapamycin was shown to only extend the lifespan of females under sterol-limited conditions[73]. Because egg production depletes sterol availability and flies are incapable of *de novo* cholesterol synthesis, rapamycin may extend survival by inhibiting sterol-consuming processes such as egg production. Indeed, cholesterol supplementation alone extends fly lifespan, which is not further increased by the addition of rapamycin[73]. This diet–drug interaction seems to be independent of the microbiota, because the effects of rapamycin on *Drosophila* tissue aging and lifespan are unaffected under germ-free conditions[74].

Another effect of rapamycin treatment in flies is systemic fat accumulation in the form of triglycerides (TAG), which recapitulates the hypertriglyceridemia seen in humans taking rapamycin[53]. This may be a consequence of *fork head* (*fkh*) upregulation. FKH is a transcription factor whose overexpression increases nutrient transporter genes resulting in raised TAG levels in the fly, and it is required for rapamycin-related survival extension[75]. This effect may explain why rapamycin promotes resistance to starvation independently of S6K inhibition or autophagy upregulation[56]. In humans, rapamycin also reduces insulin sensitivity and glucose tolerance[76], but this adverse effect was shown to be mediated by chronic TORC2 inhibition and did not affect survival in a mammalian model[77]. In fact, insulin resistance *per se* can be uncoupled from survival in both mice and flies[77,78].

In addition to lower doses and shorter treatment duration[60], more specific TORC1 inhibitors may prevent the side effects related to chronic TORC2 inhibition and improve treatment efficacy. Recent reports from clinical trials of TORC1 inhibitors show promising results in terms of safety and efficacy for reducing infection rates in the elderly[79–81].

Lithium: Inhibiting the IIS pathway

Lithium is a mood stabilizer. Although the key mechanisms underlying its mode of action are still unclear, lithium is approved for the treatment of mood conditions, such as bipolar disorder[82]. In the UK Biobank cohort, patients treated with lithium show longer survival compared with individuals taking other antipsychotics[83]. Interestingly, lithium extends the lifespan of multiple organisms, including flies[84–86].

In *Drosophila*, low doses of lithium extend lifespan, even when administered from middle age (32 days of adulthood). This effect is greatest under fully fed conditions (i.e., on a yeast-rich diet), suggesting that lithium may partly act via mechanisms mimicking DR[86], consistent with previous evidence from *C. elegans* studies[87]. However, lithium extends lifespan beyond DR, indicating

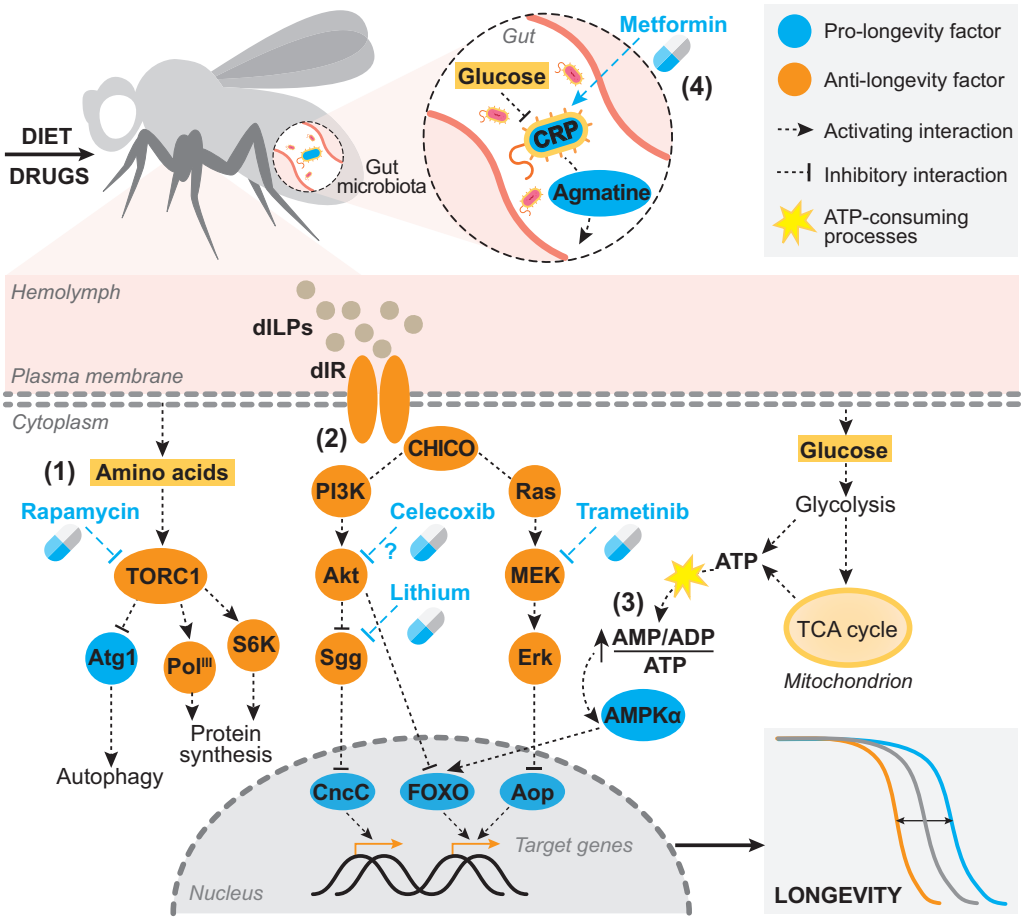

Figure 3. Simplified model of the nutrient-sensing network in *Drosophila* and its pharmacological targets. (1) The target of rapamycin (TOR) signaling pathway is activated in an amino acid-rich environment. Activated TOR complex 1 (TORC1) phosphorylates S6 kinase (S6K) and inhibits autophagy-related 1 (Atg1), promoting protein translation and inhibiting autophagy, respectively. (2) The insulin/insulin-like growth factor signaling (IIS) pathway is activated when one of the eight *Drosophila* insulin-like peptides (dILPs) binds to their sole receptor, *Drosophila* insulin receptor (dInR). Downstream of the insulin receptor substrate CHICO, activation of the PI3K/protein kinase B (Akt) phosphorylation cascade inhibits the transcription factor dFOXO, as well as the fly GSK3 ortholog *Shaggy* (Sgg), which in turn inhibits CncC, the fly ortholog of the prolongevity transcription factor NRF2. The Ras-Erk pathway is also activated downstream of CHICO, ultimately leading to the inhibition of the transcription factor Anterior open (Aop). (3) Under low-energy conditions, detected by an increase in the ratio of the adenosine nucleotides AMP and ADP to ATP, the kinase AMPK is activated and phosphorylates dFOXO at a different site. (4) Metformin induces upregulation of a bacterial transcriptional regulator, cAMP receptor protein (CRP), resulting in the production of the metabolite agmatine, which exerts prolongevity effects on the host.

additional independent or synergistic effects[86]. Lithium also increases the survival of flies fed a sucrose-rich diet, which is linked to changes in lipid metabolism[86,88].

The lifespan extension by lithium in flies relies on its ubiquitous inhibition of Shaggy (Sgg), the fly ortholog of glycogen synthase kinase-3 (GSK-3), and the activation of cap'n'collar C (CncC), the fly ortholog of nuclear factor erythroid 2-related factor (NRF-2). The prolongevity effect seems to be hormetic, because strong inhibition of Sgg/GSK-3 is detrimental for survival[86]. Increased lithium bioavailability due to different food composition might thus explain the shortening of female fly lifespan in an independent study, where flies of similar genetic background were fed equivalent doses of the drug[89]. This consideration prompts the need for further research exploring lithium–diet interactions, which is important given the particularly narrow therapeutic window[86].

GSK-3 is upregulated in many disease states, such as neurodegeneration, type-2 diabetes, inflammatory conditions, and some types of cancer[90]. More selective GSK-3 inhibitors are in development and being assessed in clinical trials for Alzheimer's disease and progressive supranuclear palsy[91]. These drugs could also be repurposed for longevity modulation and may avoid the influence of lithium on mood, interactions with other molecular targets, and possible long-term side effects, such as renal damage[88,92].

Trametinib: Inhibiting the Ras-Erk pathway

Trametinib is an inhibitor of the Ras pathway by targeting the mitogen-activated protein kinase kinases (MEK1/2) and used as a chemotherapeutic agent for tumors in patients with the BRAF V600E activating mutation, commonly found in melanomas[93].

In *Drosophila*, trametinib extends lifespan by preventing the activation of the downstream MEK target, extracellular signal-regulated kinase (Erk), both when supplemented from early adulthood (2 days old) or midlife (30 days old). This inhibition is not accompanied by a compensatory overactivation of the upstream Ras pathway or the PI3K/Akt pathway[94].

Trametinib lowers fecundity, which is expected because loss-of-function female Ras mutants are sterile[94]. Similar to rapamycin, reduced egg laying may improve survival by avoiding the depletion of essential micronutrients, an example of trade-off

between fertility and longevity[95]. However, trametinib extends lifespan when administered from a late age when fecundity is already decreased, which makes this scenario less likely. Furthermore, despite affecting fecundity, trametinib does not alter feeding[94], suggesting that its beneficial effects on lifespan are not via induction of DR.

Similar to rapamycin, trametinib may extend lifespan by promoting gut health. Although an initial study suggested that trametinib does not protect from age-related gut barrier dysfunction or alter proliferation rates of ISCs at either 15 or 65 days of age in female flies[94], subsequent studies using the same fly background, sex, and drug dose showed gut barrier protection by trametinib at 60 days and decreased proliferation of ISCs at 35 days[72]. However, the prolongevity effects of trametinib are inconsistent in males and seemingly unrelated to gut homeostasis[96]. Similar to rapamycin, trametinib indirectly decreases RNA polymerase III activity and its prolongevity effect is partially mediated by the polymerase III repressor Maf1[96]. These studies suggest a convergent mechanism through which life-extending drugs act on specific RNA synthesis pathways to preserve female gut health.

Combination therapy: Rapamycin, lithium, and trametinib

Because the three drugs described previously—rapamycin, lithium, and trametinib—target different interconnected pathways (Fig. 3), understanding whether they act separately or via the same mechanisms within a network is crucial. This question was addressed in a study showing that the triple drug combination extends lifespan by almost 50%, and results in prolongevity benefits beyond those of additive effects, suggesting synergy[97]. This synergistic effect is consistent with observations in *C. elegans*, where concomitant interventions in the TOR and IIS pathways had higher effects on survival than the sum of their individual effects[98].

Pharmacologically, this synergy may result from improvements in different health determinants that favor each other, or the neutralization of respective negative drug side effects. For instance, lithium treatment reverses the accumulation of TAG associated with rapamycin, accordingly decreasing rapamycin-induced resistance to starvation. In addition, lithium further extends the lifespan of long-lived IIS mutant flies lacking the *Drosophila* insulin-like peptides (dILPs) 2, 3 and 5, counteracting the potential activation of Sgg as a result of lowered IIS signaling, which would partly limit survival[97]. Trametinib improves insulin resistance in obese mice[99] and may prevent this side effect associated with chronic rapamycin treatment in flies.

Feeding behavior is unaltered by the triple drug combination, and reduced fecundity is mainly a consequence of trametinib, as no further change was observed when the other two drugs were included[97], which makes the trade-off scenario unlikely to explain the effects of the combination. Systemic levels of each drug were unaltered by the triple combination[97], suggesting the absence of pharmacokinetic effects in flies causing increased bioavailability of a certain drug. This study supports the principle of an intervention that combines multiple active substances targeting different aging-related processes to extend healthspan and lifespan.

Metformin: Modulating the metabolism of microbiota

Metformin is the first-line treatment for type-2 diabetes. It is a cheap and well-tolerated drug that increases insulin sensitivity in peripheral tissues and decreases liver glucose production, thereby decreasing hyperglycemia with a good safety profile, i.e., with a low risk of hypoglycaemia. Beyond its antidiabetic properties, epidemiological and animal model studies indicate that metformin can improve health markers and survival, including in late-onset interventions in mammals[100–102]. A series of clinical trials are planned to assess if metformin also prevents the incidence of age-related diseases in humans[103].

Metformin has multiple modes of action, with part of its metabolic effects reported to occur via the inhibition of complex I in the mitochondrial respiratory chain and the indirect activation of AMPK[104,105]. Despite evidence of prolongevity benefits in other model organisms, such as worms and rodents, metformin was initially reported not to extend lifespan in *Drosophila*, even though *in vivo* AMPK activation and decreased TAG levels were observed as expected[106]. However, a subsequent study showed that metformin can extend fly lifespan under defined nutritional and microbial conditions[107]. Consistent with earlier findings in *C. elegans*[108], the microbiota was found to be essential for the prolongevity effects of metformin in flies. Metformin leads to the production of the bacterial metabolite agmatine, which is essential for survival benefits in both the worm and fly host[107], although how bacteria-derived agmatine leads to lifespan extension in the host remains to be elucidated. A further independent study reported lifespan extension by metformin in flies of a different strain[109]. This effect was related to the prevention of ubiquitinated protein aggregates in adult muscle, linked to muscle autophagy induction[109].

Overall, the case of metformin exemplifies the importance of the gut microbiota in modulating different aspects of nutritional physiology and pharmacological metabolism to impact on host longevity[104,105,110], which adds another layer of complexity to the study of drug interventions in aging.

NSAIDs in longevity: Aspirin, salicylamide, ibuprofen, and celecoxib

Nonsteroidal anti-inflammatory drugs (NSAIDs) are first-line treatment options in a wide range of medical conditions, mostly related to their anti-inflammatory benefits. They include non-selective cyclooxygenase (COX) inhibitors, such as aspirin and ibuprofen, as well as the selective COX-2 inhibitor celecoxib[111].

Although the existence of COX enzymes in *Drosophila* remains unclear, the NSAIDs aspirin, ibuprofen, and celecoxib have all been shown to increase fly survival[112–116], which makes the fly system particularly useful to assess the action of these drugs on other targets and characterize the effect of those targets on lifespan modulation.

Aspirin has been shown to extend lifespan in different model organisms, including mammals[117]. In humans, a recent report indicated that aspirin does not prolong disability-free survival of healthy individuals over the age of 70[118]. However, aspirin was previously shown to improve aspects of numerous age-related diseases, such as certain types of cancer, type-2 diabetes, atherosclerosis, and neurodegenerative diseases[113], and it may prevent disease if administered from earlier in life. Aspirin extends lifespan in *Drosophila* and decreases fecundity without altering food intake[112,113,119], indicating that DR does not play a role in the longevity effect. In fact, similar to rapamycin, aspirin-treated flies have higher TAG content and increased starvation resistance. Aspirin was recently shown to prevent the dysbiosis of commensal microbiota in the fly gut as well as

age-related gut leakage and ISCs over-proliferation, partly through the downregulation of the inflammatory Imd pathway[119]. Salicylamide, another salicylic acid derivative, was also found to extend *Drosophila* lifespan, but the mechanisms remain unexplored[120].

The effects of ibuprofen on fly survival are controversial. Initially, ibubrofen was reported to increase lifespan moderately in both sexes when supplemented to the diet at 0.5 or 1 μM[114]. In another independent study, 1 μM ibuprofen extended the survival of females, but not males, when given for 10 days during middle age (from the age of 30 days) but not from early adulthood[115]. In fact, ibuprofen treatment in males was found to impair physical activity, a parameter of healthspan, by an unknown mechanism[115]. Therefore, the role of ibuprofen in modulating fly survival and the context of its prolongevity effects still require further clarification.

Celecoxib, in the form of 2,5-dimethyl-celecoxib (DMC), can extend *Drosophila* lifespan in a sex-independent way, even when administered from later timepoints. The ability of DMC to modulate lifespan was shown to require both IIS and TOR signaling and to be dependent on Akt inhibition[116].

Nonselective COX inhibition by aspirin and ibuprofen can have serious side effects in the long term, such as gastrointestinal bleeding and nephrotoxicity, to which the elderly are particularly susceptible[111]. Therefore, drugs specifically aimed at COX-independent targets that promote longevity and health must be developed to improve their potential for translation. Long-term COX-2 inhibition is also associated with life-threatening complications. The celecoxib-derivative DMC lacks COX inhibitory function, which may allow the use of this drug in the context of longevity[116].

Simvastatin and zoledronate: Mevalonate pathway inhibition

Simvastatin belongs to the statin class of drugs and is a competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, part of the mevalonate pathway responsible for cholesterol biosynthesis. In the clinic, simvastatin is used to treat pathologically elevated cholesterol levels, providing protection against cardiovascular disease. Zoledronate is a bisphosphonate that inhibits the mevalonate pathway downstream of HMG-CoA by targeting the enzyme farnesyl pyrophosphate synthase (FPPS). This results in the inhibition of osteoclastic bone resorption in humans, making zoledronate suitable for diseases such as osteoporosis.

Both simvastatin and zoledronate extend male *Drosophila* median lifespan when administered continuously from early life[52,121]. Zoledronate also extends both male and female survival when given from a later timepoint[52]. Both compounds improve health markers with age: simvastatin reduces age-related cardiac arrhythmias[121] and zoledronate improves climbing activity and the maintenance of intestinal epithelium function with age[52].

Unlike mammals, flies lack several enzymes required for *de novo* cholesterol biosynthesis, and therefore the beneficial effects of simvastatin on *Drosophila* health and survival must be cholesterol independent. The mevalonate pathway is also important for protein prenylation and as a precursor for the generation of sterol hormones and isoprenoids. By targeting HMG-CoA reductase, simvastatin could potentially decrease juvenile hormone (JH) signaling or levels of coenzyme Q, which have both been implicated in longevity[122,123].

The potential role of protein prenylation in determining fly survival has been demonstrated, with pharmacological inhibition of isoprenyl transferases extending *Drosophila* lifespan[121]. Although the detection of prenylated proteins in fly samples was not technically possible, decreased prenylation of Ras family small GTPases was observed in the liver of mice after simvastatin treatment (188 mg per kg of food)[121]. However, in an independent study, simvastatin at low and high doses (20 and 120 mg per kg of food, respectively) did not exhibit a prolongevity effect in either male or female mice[34]. These discordant results suggest that the beneficial effects of statins on survival may be dose-, disease-, and/or species-specific and require further investigation.

Sevelamer

Sevelamer is an anion exchange resin that binds phosphate and was developed to reduce elevated serum phosphate in individuals with chronic kidney disease. In flies, sevelamer treatment (1% w/v in the diet) results in lifespan extension, which is abolished when food is supplemented with excess (30 mM) phosphate[124]. Intriguingly, sevelamer does not change the concentration of phosphate circulating in the fly hemolymph[124]. As the study did not include lifespan data under low dietary phosphate conditions, it is difficult to conclude that reduced phosphate uptake is responsible for the sevelamer-mediated increased longevity. Further investigations are warranted to explore other potential actions of this drug as well as the effects of phosphate independently on physiology and survival. For instance, sevelamer in humans is able to reduce blood uric acid levels and inflammatory factors such as IL-6[125], which have been implicated in longevity modulation[23,78].

N-acetylcysteine

N-acetylcysteine (NAC) is administered in humans to treat paracetamol overdose and as a mucolytic for conditions such as cystic fibrosis. Several studies have assessed the effects of NAC treatment on fly survival, with mixed outcomes depending on the sex and genetic background[126–128]. NAC was initially reported to extend the lifespan of *Oregon R* males when either 1 or 10 mg/mL were supplemented to the food[126]. Subsequently, NAC was shown to only extend the survival of *Canton S* male flies but not females[128]. Conversely, NAC was recently found to extend lifespan in females but not males of the w^{1118} strain at a dose of 1 mg/mL, while a higher concentration of 10 mg/mL was toxic[127]. The reason for this variability between sexes and genetic backgrounds is unclear and may be influenced by other factors such as differences in the basal diet composition between the studies (see Table 1).

All three reports claim that the modulation of survival by NAC is through its antioxidant activity, both directly by detoxifying reactive oxygen species (ROS) and indirectly by upregulating antioxidant systems. Recent findings suggest that NAC does not alter H_2O_2 flux but rather suppresses complex I-linked respiration in female flies, while typically maintaining a reduced glutathione pool at lower doses (1 mg/mL)[129]. NAC was shown to elevate the transcript levels of catalase and phospholipid-hydroperoxide glutathione peroxidase in the whole body, and more specifically in the head and abdomen of females flies, although these results again seem to be strain-specific[127,128]. The activity of these enzymes, known to be important ROS scavengers, was also increased in response to NAC and correlated with enhanced resistance to paraquat in one report[127]. However, a recent study in *C. elegans* shows dose-dependent shortened survival following NAC administration associated with the inhibition of the worm NRF-2 ortholog and

suggests that scavenging naturally occurring ROS may be harmful by inhibiting healthy redox signaling[130]. Indeed, NAC can induce reductive stress at high concentrations[129].

Overall, considering the discrepancy of these results, further studies should dissect the interacting factors or conditions that allow NAC to extend lifespan and explore the role of antioxidant factors and specific redox signaling pathways, which may uncover new and more consistent targets for pharmacological intervention in this context[131].

Corticosteroids

Corticosteroids are synthetic analogs of the steroid hormones produced in the cortex of adrenal glands. They can have either glucocorticoid or mineralocorticoid properties, or both to varying degrees. Corticosteroids are prescribed to ameliorate a wide range of medical conditions. Glucocorticoids can be used for their immunosuppressive and anti-inflammatory properties in autoimmune or inflammatory disorders involving effectively any organ. Mineralocorticoids regulate electrolytes and water balance and are used in combination with glucocorticoids as replacement hormonal therapy, for example, in the case of adrenal insufficiency[132].

Both types of corticosteroid are reported to extend fly lifespan[120]. The glucocorticoid triamcinolone extends the survival of both males and females. Hydrocortisone and its pro-drug cortisone, which possess both glucocorticoid and mineralocorticoid activities, similarly extend fly lifespan independently of sex[120]. Other glucocorticoids, fluprednisolone and dexamethasone, were reported to have no impact or even to shorten fly lifespan[120], despite appearing as drugs that significantly extend lifespan in the DrugAge database[50]. Conversely, the mineralocorticoids fludrocortisone and desoxycorticosterone are absent from the database but are reported to significantly extend lifespan[120]. Although these drugs were proposed to maintain membrane stability and thereby slow aging, no mechanistic evidence was provided[120].

Flies were recently found to have a receptor that responds to cortisone, encoded by the estrogen-related receptor (ERR) gene[133]. Both cortisone and dexamethasone may act in the fly by conserved immunosuppressive mechanisms to increase susceptibility to infection[133,134]. Interestingly, an endogenous ligand for the fly ERR has yet to be identified and, therefore, further research is needed to understand the role of steroid hormones in fly physiology[133].

The anti-inflammatory effects of glucocorticoids may decrease the age-related low-grade inflammation known to occur in mammals, in part related to the overproduction of pro-inflammatory factors and aged innate immunity cells[135]. The decreased corticoid production with age in mice correlates with the surge of this systemic pro-inflammatory profile and was proposed to be one of its drivers[135]. However, in humans, daily glucocorticoid production varies with age in a U-shaped pattern[136]. At older ages, total levels actually increase, and are related to circadian changes, with the peak diurnal rhythm shifted to later in the day, and higher concentrations in the late evening and early night[137]. This circadian shift may be detrimental for health, for example, by affecting quality of sleep[137]. Together with known adverse effects of chronic glucocorticoid intake, such as osteoporosis and dyslipidaemia, this suggests that glucocorticoids may be of limited use to improve human health in old age. Similarly, chronic intake of mineralocorticoids in the absence of adrenal insufficiency will likely be detrimental due to the retention of sodium and water, and increased risk of hypertension, a major risk factor for cardiovascular events. Despite this, further research into maintaining a normal circadian production of these hormones over time and their beneficial specific targets for fly survival may uncover new strategies to preserve human health in old age.

Mifepristone

Mifepristone (or RU486) is another synthetic steroid that acts instead as an antagonist to human progesterone and type II glucocorticoid receptors and is clinically used to terminate pregnancy[138].

In fly research, mifepristone is used as the inducing agent for the GeneSwitch conditional gene expression system[139–141]. This inducible method allows mifepristone-dependent temporal changes in gene expression in flies with the exact same genetic background and has been widely applied in longevity studies. More recently, this system has been reported to be leaky under some conditions (i.e., to induce expression of the transgene in the absence of the drug), dependent on the upstream activating sequence and driver lines used[142,143].

In addition to potential interference by endogenous ligands at the GeneSwitch receptor in the absence of the drug, mifepristone itself has been reported to affect fly metabolism and lifespan[138,142–145]. This effect was first described in mated female flies but not in nonmated females or males, and it was found to be genotype-dependent[143]. In an independent study, mifepristone was shown to decrease food intake in low-yeast conditions (0.1% w/v) and to exacerbate lifespan-shortening undernutrition, but had little to no effect on either food intake or lifespan of flies at higher yeast content (5% w/v), independent of their sex[142]. The studies where mifepristone showed a positive effect on survival used food with an intermediate yeast content (~2.5% w/v), without change in food intake[138,143,144]. Although later reported to also moderately extend nonmated female lifespan[138,144], mifepristone was shown to mainly extend survival of mated females to nonmated levels by counteracting the negative effects of mating, which are related to innate immune system activation by bacterial factors. Mifepristone is proposed to prevent these effects by antagonizing JH signaling downstream of male sex peptide in the midgut[138,144,145].

Overall, these studies suggest that when using the GeneSwitch system in the context of longevity, experiments should be adequately controlled or interpreted taking into account the potential indirect effects of mifepristone[141]. The action of mifepristone also appears to depend on the nutritional context and varies according to the composition of the fly food. Considering the specific conditions under which mifepristone itself affects survival, it is unclear whether this drug acts on aging or aging-related disease mechanisms that can be translated to the clinic.

Rifampicin

Rifampicin is clinically used as an antibiotic to treat infections caused by a broad range of bacteria. It binds specifically to the β subunit of bacterial RNA polymerases to inhibit transcription, while having little to no activity against human polymerases[146].

In *Drosophila*, rifampicin alone was shown to only moderately extend male lifespan but displayed a strong synergistic effect with rapamycin and even more in triple combination with allantoin to extend mean survival by up to 77% compared with the short-lived controls[147]. The mechanism of action was not explored in the fly model, but in *C. elegans*, rifampicin was dependent on *daf-16*, the worm ortholog of the forkhead box-O transcription factor (FOXO), for the survival outcome[147]. In a recent study, rifampicin

is proposed to prevent the activation of the innate immune system by gut microbiota[148]. Therefore, further research into innate immunity activation and survival of flies may reveal new pharmacological targets for improved health in old age.

Minocycline

Minocycline, a second-generation tetracycline, is a broad-spectrum antibiotic prescribed for conditions such as acne vulgaris. Its bacteriostatic action is related to the inhibition of protein synthesis in bacteria by binding to the bacterial 30S ribosomal subunit[149]. Beyond its antibiotic effect, minocycline is shown to improve health in animal models of several neurodegenerative and inflammatory diseases. Multiple mechanisms have been proposed related to different molecular targets interacting with the drug. These include its antioxidant properties, calcium chelation, and the ability to inhibit proinflammatory enzymes[149].

In *Drosophila*, minocycline extends the survival of both males and females from different fly strains. This effect correlates with a delay in the age-related loss of motor activity[150–153]. Interestingly, minocycline also extends the lifespan of germ-free flies[151], suggesting its prolongevity action is at least partially independent of the microbiota. This drug does not alter feeding rates or fecundity, indicating that its effects on survival are not caused by DR or a reproductive trade-off[151]. Oxidative stress resistance is enhanced by minocycline in different species[149]. However, as discussed with NAC, its ROS scavenging effect might be detrimental under homeostatic conditions. The transcription factor FOXO seems to be necessary for minocycline-associated oxidative stress resistance and lifespan extension, because these effects are lost in *foxo*-null mutant flies. Consistent with this observation, minocycline increases FOXO expression in thoracic muscle and the fat body[151] and prevents the accumulation of ubiquitinated protein aggregates in flight muscles by upregulating autophagy in a FOXO-Hsp70-dependent manner[154]. FOXO overexpression in skeletal muscle[155] and fat body[156,157] increases fly lifespan. However, minocycline further extends the lifespan of flies overexpressing FOXO ubiquitously[151], prompting additional studies not only to characterize the interaction of minocycline with this transcription factor but also to dissect other potential molecular targets impacting longevity.

Lamotrigine

Lamotrigine is used in the clinic as an anticonvulsant to treat conditions such as epilepsy as well as a mood stabilizer for individuals with bipolar disorder[82]. In *Drosophila*, lamotrigine is reported to increase survival in both males and females[158]. However, lamotrigine also reduces fly locomotor activity, suggesting that the lifespan extension may not be accompanied by healthspan benefits[158], which weakens its potential for translation. Nevertheless, future studies providing mechanistic insight into how lamotrigine promotes fly survival may help find more specific targets that do not compromise parameters of health.

Morphine

Morphine is an opiate commonly used in the clinic as pain-relief (antalgic) therapy. In *Drosophila*, morphine is reported to extend lifespan, primarily in males, even when administered later in life[159], although no mechanistic insight was provided. The adverse effects of morphine in humans, such as respiratory depression, constipation, gain of tolerance, and physical dependence, undermine its potential to be repurposed for health benefits or disease prevention. However, understanding how morphine promotes survival in flies may uncover novel aging-related targets to benefit human health.

Future Directions and Challenges

Drug repurposing can facilitate clinical translation[160], but screening compounds for age-related disease prevention is still challenging. These issues are not specific to *Drosophila*, but addressing them will make the fly a more reliable model for preclinical studies and drug translation in the context of aging. Here, we discuss the following challenges: 1) the use of survival as a proxy for aging, 2) the lack of comprehensive health scores, 3) false hits and the conservation of drug targets, 4) the diet dependency of survival effects, and 5) the downside of combination treatments.

1. *The use of survival as a proxy for aging*

In the absence of universal and robust biomarkers, population survival is the most widely used parameter as a proxy for aging, which is an important limitation to consider. While targeting aging should extend population survival, this is not sufficient to classify an intervention as geroprotective or antiaging. Life-extending interventions may promote fly health and survival independently of aging[161]. Semantically, interventions that extend lifespan must be termed prolongevity or prosurvival until further evidence. Rejuvenating antiaging therapies will be more potent and should be the ultimate goal of biogerontology, but these will only emerge from a better understanding of aging itself[162].

Among other factors, survival within a population depends on individual genetic predisposition to develop disease. Therefore, it is important to use genetically heterogeneous populations when testing longevity interventions[163]. In a genetically heterogeneous population, individuals may still share the same predisposition to develop a disease with age that limits the life expectancy of the population. Treating these aging-unrelated factors to protect against the development of that disease can theoretically extend median lifespan. However, other age-related conditions will eventually develop resulting in smaller changes to the maximum lifespan of the population. Directly treating aging itself should instead lower the risk for all age-related diseases by definition, and thus extend both median and maximum lifespan (Fig. 1), which is necessary to classify a drug as a geroprotector.

2. *The lack of comprehensive health scores*

To further assess the efficacy of longevity interventions in both preclinical and clinical studies, drugs should improve comprehensive health scores[164]. If aging is associated with a loss of organ function and homeostasis over time, measuring functional and systemic homeostasis markers is necessary to screen for antiaging compounds. These scores could replace survival as the primary endpoint of early clinical studies, which may accelerate the translation of interventions focused on aging. Health scores to assess aging interventions should combine functional, imaging, and biochemical parameters. Some are already applied in geriatric medicine: for example, frailty indexes measure performance in activities of daily living, while mental state scores examine cognitive function[165,166]. These scores can be expanded to include more detailed physical and psychological performance tests combined

with imaging signs of tissue aging and molecular biomarkers of normal organ function to create a global view of individual health.

In this context, it is equally important to standardize healthspan and frailty measures in invertebrate animal model studies, as attempted in mammals[167]. For instance, the creation of a *Drosophila* health and frailty index (e.g., based on physiological and behavioral assays including physical activity, movement and feeding, as well as markers of organ function) would be valuable to assess the effects of interventions on health the same way across studies. This would complement lifespan information when screening for drugs that target aging and could be used as exclusion criteria when screening for geroprotectors.

3. *False hits and the conservation of drug targets*

Drug screening in animal models can result in false negative and false positive hits. In addition to applying comprehensive health scores, a way to increase confidence in positive hits is to show conservation of the molecular target in functionally equivalent tissues.

A false negative occurs when a drug fails to extend the lifespan or improve the health of a model organism, while able to prevent the consequences of aging in humans. Aging is associated with systemic loss of homeostasis, which is maintained differently across species depending on respective organ composition and physiology. Consequently, aging can manifest differently between species[168]. Particular organs can even be key drivers for age-related disease in a given species. For instance, preventing human thymic involution may ameliorate age-related immune dysfunction and improve human health in old age. However, fundamental aging processes can be the same across different organs. Building on the previous example, thymus involution occurs partially through stem cell exhaustion, which is a consequence of aging seen across multiple organs and species[23,169].

Even though flies lack certain organs present in humans, they do have many functionally similar organs with comparable anatomical distribution and physiology. Flies are useful to uncover interventions that act on conserved aging processes, which are conceptually more likely to affect multiple organs and therefore be more potent targets. Overall, false negatives are hard to predict and avoid, as prior knowledge of these human-specific aging processes is required, but they also have fewer consequences for drug development compared with false positives in terms of time and financial investment.

A false positive happens when a drug extends the lifespan of a model organism via mechanisms that prevent survival-limiting pathophysiological processes specific to that model[161]. To prevent false positives, it is important to show that the drug target is conserved and that potential organ-specific effects occur in tissues that have at least a functional equivalent in humans. *Drosophila* research is equipped with powerful genetic tools to easily and precisely validate beneficial drugs and targets in a tissue-specific manner. This can be accomplished by: 1) engineering knock-in mutants where the proposed target is unresponsive to the drug, cancelling its effects; 2) knocking down or overexpressing the proposed target to simulate action of the drug on the endpoint, without further pharmacological additive effects; and 3) using tissue specific or inducible drivers to apply the above genetic constructs in a localized or temporally defined manner.

Measuring drug bioavailability in the fly hemolymph—the circulating extracellular fluid analogous to blood—is technically possible[97] and should be considered in future studies to ensure that a drug has the ability to reach target tissues. For example, FOXO and AMPK increase fly lifespan when genetic interventions are restricted to the gut[170,171]. Drugs that act on these pathways may similarly have gut-specific effects with systemic consequences for health. However, if drug absorption is not shown, additional systemic effects might be missed.

4. *The diet dependency of survival effects*

Drugs can interact with the diet to impact longevity in several ways. Food composition can affect the outcome of an intervention by influencing drug bioavailability[172]. At a biological level, the presence/absence of specific nutrients can determine whether a drug is mechanistically able to extend lifespan[73,108]. Furthermore, as exemplified by metformin and rapamycin, diet and drugs can jointly modulate microbiota and host cell metabolism with systemic effects[105]. Therefore, it is critical to report comprehensive details of media recipes for all drug studies and possibly standardize diets across laboratories to make findings more comparable and reproducible[172–174].

Diet alone is a key factor in chronic disease prevention[175]. Integrating dietary information with drug interventions targeting aging and their metabolic consequences may uncover synergies that promote survival more potently. In *Drosophila*, this can be accomplished by using chemically defined media, where the constituents of the fly diet can be altered individually[172,174] and by performing multilevel screens integrating nutritional and pharmacological information with metabolic and microbiome data[107].

While fine-tuning the nutritional context to improve drug efficacy and favor longevity is important, an ideal antiaging drug should be effective under a broad range of dietary conditions. In fact, interventions that target nutrient-sensing pathways may promote health and survival of animal models independently of aging, by ameliorating the side effects of overnutrition when reared in the laboratory compared with the wild. Similarly, DR mimetics may improve human health solely by preventing the consequences of overfeeding. Drugs targeting aging must show additional health and longevity benefits even under DR conditions.

5. *The downside of combination treatments*

Combining therapies that target each of the currently described aging-related processes may be a potent strategy to deal with such a pervasive problem as aging[23]. Within the same hallmark of aging, targeting different components can similarly be beneficial. For example, the triple drug combination study[97] shows that multiple nutrient-sensing-related drugs acting on different processes can synergise, possibly by counteracting respective side effects. Another example of potential synergy in age-related drugs is the combination of metformin with rapamycin in mammals, even though further conclusive data are required[176].

An issue with combination therapies is that polypharmacy itself is a geriatric syndrome. Drug interactions, side effects, and dosage are particularly important problems in the clinical management of elderly patients[177]. Combining different active substances into one pill, as proposed previously[178], may work in an optimized experimental cohort, but translation to humans, especially to the elderly, will face challenges related to altered metabolic rates, enzyme activities, or kidney function. Individuals may also be taking other disease-targeted drugs that can interfere with their respective bioavailability, leading to the abolition of benefits or to toxicity. A poly-pill strategy may avoid some of these

Table 2. Proposed criteria for drug screening in *Drosophila* preclinical studies of aging pharmacology.

Challenges	Proposed Criteria
Survival as endpoint	• Extends both median and maximum lifespan
	• Effective in genetically heterogeneous populations
Health scores	• Improves frailty index scores
	• Improves imaging signs of tissue aging
	• Improves markers of organ function
Target validation	• Target mutation in the binding site cancels drug effect
	• Target overexpression/knockdown simulates drug effect
	• Conservation of the target across species
	• Tissue-specific effects (if any) are seen in analogous organs
Diet dependency	• Drug effect is observed with different media
	• Drug effect is not limited by the absence of nutrients in the diet
	• Effect is present when flies are maintained under dietary restriction
Polypharmacy	• Identification of common upstream or downstream targets
	• Improved integration of personalized data

challenges if applied early in life and/or in a personalized manner, but substantial progress is needed to ensure effectiveness and safety (Table 2).

Another approach is to find divergent or convergent aging targets. There is evidence that different processes of aging interact, which means that a single target may have multiple beneficial effects. For example, pharmacologically targeting components of nutrient-sensing pathways can ameliorate age-related loss of proteostasis[74], cellular senescence[179], and altered intercellular communication[180].

Conclusion

Longevity can be modulated pharmacologically. As a short-lived metazoan, *Drosophila* is a useful tool to dissect how drugs increase lifespan and study their biological targets *in vivo*. However, there is still a long road ahead for the pharmacology of aging to deliver on its promise. Further studies are needed to discover new fundamental mechanisms of aging and validate interventions as reliable promoters of healthy aging before clinical translation. Drug repurposing can be an important initial step in bringing the pharmacology of aging to the clinic. *Drosophila* enables rapid preclinical proof-of-concept experiments in this context. These studies will shape the future of the field, potentially paving the way for rejuvenating antiaging drug discovery.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data Selection

Drugs were selected from the DrugAge database[50] (https://genomics.senescence.info/drugs), based on the following inclusion criteria: 1) median/mean lifespan extension of *Drosophila melanogaster* by at least 1%, 2) statistically significant effect, and 3) clinically authorized according to the European Medicines Agency, Article 57(2) of Regulation (EC) No. 726/2004 EMA/518502/2018 (https://www.ema.europa.eu/en/human-regulatory/post-authorisation/data-medicines-iso-idmp-standards/public-data-article-57-database). Information was accessed on 20 June 2022, revised on 3 May 2024.

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