REVIEW

Developing criteria for evaluation of geroprotectors as a key stage toward translation to the clinic

Alexey Moskalev,1,2,3 Elizaveta Chernyagina,3 Vasily Tsvetkov,3,4 Alexander Fedintsev,2 Mikhail Shapiroshnikov,1 Vyacheslav Krut’ko,5 Alex Zhavoronkov6,7 and Brian K. Kennedy8

1Engelhardt Institute of Molecular Biology of Russian Academy of Sciences, Moscow 119991, Russia
2Institute of Biology of Komi Science Center of Ural Branch of Russian Academy of Sciences, Syktyvkar 167982, Russia
3Moscow Institute of Physics and Technology, Dolgoprudny 141700, Russia
4The Research Institute for Translational Medicine, Pirogov Russian National Research Medical University, Moscow 117997, Russia
5Institute for Systems Analysis, Russian Academy of Sciences, Moscow 117312, Russia
6D. Rogachev FRC Center for Pediatric Hematology, Oncology and Immunology, Samory Machela 1, Moscow 117997, Russia
7The Biogerontology Research Foundation, 2354 Chynoweth House, Trevissome Park, Blackwater, Truro, Cornwall TR4 8UN, UK
8Buck Institute for Research on Aging, Novato, CA 94945, USA

Summary

In the coming decades, a massive shift in the aging segment of the population will have major social and economic consequences around the world. One way to offset this increase is to expedite the development of geroprotectors, substances that slow aging, repair age-associated damage and extend healthy lifespan, or healthspan. While over 200 geroprotectors are now reported in model organisms and some are in human use for specific disease indications, the path toward determining whether they affect aging in humans remains obscure. Translational research efforts would benefit from the formation of a scientific consensus on the following: the definition of ‘geroprotector’, the selection criteria for geroprotectors, a comprehensive classification system, and an analytical model. Here, we review current approaches to selection and put forth our own suggested selection criteria. Standardizing selection of geroprotectors will streamline discovery and analysis of new candidates, saving time and cost involved in translation to clinic.

Key words: aging; criteria of geroprotectors; geroprotectors; healthspan; lifespan.

Introduction

Aging is a major risk factor for a number of chronic diseases, including cancer, type II diabetes, atherosclerosis, hypertension, myocardial infarction, stroke, and neurodegenerative diseases. In animal models, treatments that extend lifespan often protect against these chronic diseases and there is a reason to believe that a similar approach might work in humans. Therefore, the geroscience concept, which aims to prolong the healthy state of the human body, is likely to become a key paradigm of biomedicine in developed countries in coming decades (Seals et al., 2015) (http://www.nature.com/nm/journal/v21/n12/full/nm.4004.html). The aforementioned diseases and conditions may 1 day be prevented or at least delayed with the growing range of chemicals capable of slowing the processes of aging. These have been termed geroprotectors.

The search for new geroprotectors is a dynamic area of biomedicine. In their 2009 review, Kapoor et al. (2009) listed 24 geroprotectors known at that time. Today, more than 200 substances belong to this group, each reported to slow aging and/or increase lifespan in a variety of organisms, including yeasts, nematodes, fruit flies, and rodents, according to the Geroprotectors.org database (http://geroprotectors.org/) (Moskalev et al., 2015).

Despite such an impressive rate of discovery, not a single geroprotector has yet reached the pharmaceutical market as a recognized intervention targeting aging (http://www.nature.com/nm/journal/v21/n12/full/nm.4005.html). There are several nonexclusive reasons (http://www.nature.com/nm/journal/v21/n12/full/nm.4005.html). First, there is no unified mechanistic concept of aging, and primary triggers of aging are still poorly understood. As a result, molecular targets for candidate drugs are often unknown, complicating studies in the clinic. Second, there is no comprehensive system of objective human aging biomarkers. Biomarkers are critical to the translation of geroprotectors from simple model organisms to preclinical stage and then to clinic. Third, aging is not recognized as a disease or a complex of syndromes; thus, pharmaceutical companies are disinclined to create and evaluate geroprotectors (http://journal.frontiersin.org/article/10.3389/fgene.2015.00205/full). Finally, and perhaps most importantly, the scientific community has no consensus view on the concept of geroprotectors, on selection criteria for potential geroprotectors, or on the development of appropriate classification schemes, efficiency ratings, and approaches for predicting and modeling geroprotective properties. Development of such criteria would aid in the search for new geroprotectors and translate these substances into clinic more effectively.

Evidence indicates that a subset of the key causes and mechanisms of aging are ancient and evolutionarily conserved (Smith et al., 2007; Moskalev, 2010). Thus, targets for interventions can be identified through bioinformatics approaches and lifespan comparing data from multiple species, especially with regard to known longevity pathways. According to the GenAge database (Tacutu et al., 2013), there are 1825 genes whose knockout, knockdown, or overexpression is known to result in an increase in lifespan. A purposeful search of substances that
affect the activity of these genes and their encoded proteins will significantly expand the pool of potential geroprotectors.

Creating a system of criteria for ranking and grouping geroprotectors in accordance with their effect on life expectancy (mean, median, maximum), their molecular targets, the mechanisms of aging involved and affected age-associated pathologies, the transcriptomic and metabolomic changes, and the closeness of chemical structures will significantly enhance efforts to target healthspan and lifespan. Such a system will also facilitate chemical structure prediction and targeted synthesis of new geroprotectors. If this strategy can be employed successfully, it will create an alternate and cheaper route to small molecule identification compared with high-throughput screening, which is difficult in the context of aging.

Once identified, classified, and tested, geroprotectors have great potential for the prevention and treatment of age-related pathologies, acting on a major and common cause of these diseases – the aging process. Ultimately, they may also help in achieving a substantial extension of human healthspan, the fully active and disease-free period of life.

In a recent review, Longo et al. (2015) selected a subset of the most promising interventions that could be tested in humans for their effects to slow aging and increase healthy lifespan. However, there is not complete consensus about the general principle of criteria for geroprotectors selection. In the following review, we will propose and give a detailed description of a set of primary and secondary criteria for potential geroprotectors. Providing consistency and uniformity in these areas will greatly accelerate progress in the aging research field. Based on the primary criteria, we selected potential candidates for human aging-suppressive interventions.

Systematic evaluation criteria for geroprotector identification

To date, more than 200 substances that extend the life of model organisms have been reported in the literature. It is evident that future studies in this direction will be more intensive, and the number of such substances will increase markedly. The emerging challenge, in turn, will be to streamline translational research to keep pace with laboratory advances. Reducing the cost and improving the efficiency with which increasingly large amounts of data from model organisms can be applied to humans will be critical to progress in the development of human geroprotectors.

At present, there are many different definitions of geroprotectors in the scientific literature. This lack of consensus creates uncertainty, especially given that significant differences exist in research methods among aging researchers, and contributes to difficulties in evaluating and comparing results. In addition, differences in experimental conditions, model organisms, and genetic background within a species further add to the difficulty of interpreting and/or comparing data. All of these issues must be weighted and taken into account in any large-scale analysis. In this regard, we have identified the following important tasks at the present time: introduction of the concept of geroprotector and development of criteria for classifying a substance as a potential geroprotector, development of a single analytical model of a geroprotector based on these criteria, and consolidation of various research initiatives with the help of this model.

The concepts of ‘geroprotector’ and ‘gerontology’ were introduced by Ilya Mechnikov (Metchnikoff, 1910). The term ‘geroprotector’ is literally translated as an agent ‘protecting against aging’. In essence, this means ‘deterring the aging process and thus prolonging life’. Numerous synonyms including ‘anti-aging drugs’, ‘longevity therapeutics’, ‘geroprotector’, ‘aging-suppressant’, and others are also used in the scientific literature (Spindler et al., 2012).

For many years, the ability to increase lifespan has served as the main criterion for a geroprotector. Therefore, any substance, method, or exposure that increases lifespan is considered geroprotective. This paper focuses on substances with geroprotective properties. In real-life situations, a researcher often has to deal with substances for which potential effects on increasing lifespan are apparent, but direct experimental evidence is lacking. For example, a substance might be known to have a positive impact on the specific mechanisms of aging or the risk of diseases associated with aging. In such cases, we propose to use the terms ‘potential geroprotector’ or ‘candidate geroprotector’. For instance, recently, a new class of drugs, senolytics, are reported to selectively kill senescent cells. Dasatinib showed notable senolytic potential, but its effects on lifespan are not yet known (Zhu et al., 2015).

The criteria of a geroprotector should be represented by a system that describes their desired properties and characteristics in the most complete manner. Creating such a system is not a trivial task, as it cannot be limited to one or two criteria. For example, an increase in lifespan may be accompanied by deterioration of quality of life and functional capabilities of organism, as has been reported to be the case of certain long-lived mutants of C. elegans (Bansal et al., 2015). The real goal for translation into humans should be an increase in healthy lifespan or healthspan. Therefore, it is important to incorporate functional measures of aging wherever possible.

Thus, the task of creating a system of geroprotector criteria is becoming all the more urgent. The compliance of a substance with at least the majority of such criteria would allow the claim that a candidate drug is indeed a geroprotector. Our proposed system of criteria for geroprotectors is divided into primary and secondary groups. While the basic criteria must be met unconditionally for any candidate geroprotector, the additional criteria can accelerate the procedures for identifying the geroprotective properties, reduce the cost of such procedures, or establish the translatability of the results into humans.

Primary selection criteria for potential geroprotectors

Increased lifespan

The criterion of increasing lifespan is undoubtedly the most significant main criterion for geroprotectors. At the population level, increased lifespan manifests as reduced mortality. In an ideal situation, positive changes in all characteristics of the survival curve are observed. Those characteristics include mean lifespan, median lifespan, maximum lifespan, age of 90% mortality, and rate of aging. Many authors consider mortality rate doubling time (MRDT) as a measure of the rate of aging. This variable is derived from the Gompertz equation, MRDT = \(0.693/G\), where G is the exponential (Gompertz) mortality rate coefficient (Finch, 1990). An increase in MRDT is expected to reflect a decrease in the rate of aging.

In reality, the increase in lifespan is not always accompanied by positive changes in the quality of life, and a more subtle analysis of the effects of a geroprotector is required. For this reason, the introduction of additional criteria for geroprotectors is warranted and discussed below.

Amelioration of human aging biomarkers

Biomarkers of aging are molecular, cellular, and physiological parameters of the body that demonstrate reproducible quantitative or qualitative changes with age. Candidate geroprotectors should ideally reverse these
biomarkers to a younger state or slow down the progression by which they change with age. The criterion for geroprotectors associated with biomarkers of aging is of particular importance for the translation of results into humans (Longo et al., 2015). Studies of human longevity under the influence of a candidate geroprotector are extremely lengthy and costly. Thus, the analysis of lifespan must be carried out on animals, but one way that the effects of a candidate geroprotector on human aging can be analyzed is on the basis of changes in various biomarkers during the geroprotective therapy.

A human aging biomarker should be minimally invasive, reproducible and should reflect main aging mechanisms. The most comprehensive list of human aging biomarkers is available online in the database Digital Ageing Atlas (http://ageing-map.org) (Craig et al., 2015). While there are no single definitive biomarkers for aging, a range of different measures have been proposed and are worthy of consideration. For example, when selecting geroprotectors relevant to criteria of amelioration of aging biomarkers, we can take into account the results of studies on cultures of human cells in vitro (expression of telomere-related genes, beta-amyloid-lowering effect, low levels of advanced glycation end products and oxidative damage, reduced level of lipofuscin) or in human clinical trials (prevent neurodegeneration, hypertension, reduce blood glucose concentrations, anti-inflammatory properties, triglyceride-lowering effect, improve insulin sensitivity, prevent hair loss, improve immune function in the elderly, delay skin aging). Interestingly, a recent study evaluated a collection of candidate biomarkers longitudinally in a relatively young population and developed a criterion for predicting biological age that was predictive of functional parameters in 38-year-olds (Belsky et al., 2015). If these measures or a similar set prove reliable across multiple human cohorts, the testing of geroprotectors may be greatly accelerated.

Acceptable toxicity
Most geroprotectors show a preventive effect only when used at relatively high concentrations over long periods of time. To evaluate toxicity, studies on model organisms are commonly used (FDA 1996). Acute toxicity, characterized by median lethal dose, LD50, refers to adverse effects occurring after administration of a single dose of a substance or multiple doses given within 24 h (United Nations 2009). Other toxicity measures include identifying doses that are toxic to a specific target organ, induce carcinogenicity, reduce fertility, or increase the level of germ cell mutagenicity (United Nations 2009). Acceptable toxicity for geroprotectors should require significant (several orders of magnitude) differences between lifespan-extending dose and toxic dose.

Minimal side effects at therapeutic dosage
Some substances that prolong the life of model animals in certain concentrations have multiple adverse side effects. For instance, dyslipidemia, anemia, insulin resistance, increased susceptibility to infections, hypertension, and gastrointestinal disorders have been reported in some cases. The achievement of a geroprotective effect implies in the simplest scenario the use of geroprotectors for many years. It is reasonable to expect that over the years of use some unwanted adverse effects of these drugs will be observed in addition to the expected results. These adverse effects, with passage of time, may reduce both the quality of life and the effectiveness of a geroprotector in the prevention of aging. Therefore, it is desirable to ensure that the number and severity of side effects caused by candidate geroprotectors in doses sufficient to achieve positive effects in humans is minimal.

Improving health-related quality of life
The aging process is associated with decreased metabolic efficiency, as well as reduced mental and physical activity. Furthermore, an age-related rising incidence of illnesses and disability associated with chronic diseases may significantly diminish health-related quality of life. Potential geroprotectors should improve at least a subset of these parameters. Potential geroprotectors should improve physical, mental, emotional, and social functioning of the treated individual. Some geroprotectors are reported to stimulate cognitive function and show an antidepressant-like effect when used in therapy, for instance preventing sleep disorders.

Secondary selection criteria for potential geroprotector

Evolutionary conservatism of target or mechanism of action
An evolutionarily conserved target increases the possibility that geroprotective effects identified in simple models will reproduce in mammals. The targets of many known geroprotectors are evolutionarily conserved. For example, TOR kinase, the target of inhibition by rapamycin, is highly conserved across the range of species from unicellular yeast to humans (Dann & Thomas, 2006). Similar evolutionary conservation is evident for AMPK (Nayak et al., 2006), as well as NFκB (Chuang et al., 2013) and IGF-1R/Akt (Song et al., 2014).

Reproducibility of geroprotective effects on different model organisms
This criterion is not identical to the previous one. Once the geroprotective effect of a substance is identified by screening for lifespan in one species, an attempt can be made to reproduce this effect in other species, even in the absence of a known conserved target. Initial screens for potential lifespan-extending compounds are usually done in short-lived invertebrates. Enhanced lifespan in a second invertebrate likely increases the chances that effects will also be evident in humans. Of course, data in mammals or human cells are even more valuable. In short, geroprotectors that impact lifespan in multiple animal models of aging should be given increased emphasis.

Simultaneous influence on several aging-associated causes of death in mammals
Aging is driven by an intrinsic process or set of processes that enable the onset of chronic diseases. Candidate geroprotectors should be able to delay the development of one or several age-associated pathologies. Thus, even today’s geroprotective substances, which extend the lifespan in animal models, can be evaluated by their reported positive therapeutic effects on the causes of human mortality and considered for potential use as geroprotectors in humans.

Increase in stress resistance
At present, a large body of accumulated evidence suggests that interventions associated with enhanced longevity also often confer stress resistance (Miller, 2009). Pathways and mechanisms linked to both stress resistance and longevity include the insulin/IGF-1 (Longo & Fabrizio, 2002) (Holzenberger et al., 2003), TOR (Lin et al., 2014) and NF-xB (Helenius et al., 1996) signaling pathways, DNA repair (Moskalev et al., 2013), free radicals detoxification (Cutler, 2005), molecular chaperones (Morley & Morimoto, 2004), and epigenetic control of gene expression (Saunders & Verdin, 2009). In their recent review, Epel and Lithgow (Epel & Lithgow, 2014) suggested that reduction in stress resistance is a common feature for all of the nine hallmarks of aging proposed earlier by López-Otín et al. (2013). These hallmarks include the
loss of proteostasis, epigenetic changes, genomic instability, telomere attrition, altered intercellular communication, dysregulated nutrient sensing, mitochondrial dysfunction, cell senescence, and stem cell exhaustion. Hence, it is a reasonable assumption that potential geroprotectors act to increase the body’s resistance to adverse environmental factors. As with other categories, we do not rule out the possibility that a geroprotector will not confer stress resistance; however, we believe this to be a strong enough correlation to date to include in our analytics. Thus, the criterion of increasing stress resistance can serve as one of indicators of the drug’s specific activating effect on the mechanisms of longevity associated with the resistance to stress.

**Identification of candidates**

Longevity studies on animal models and human aging studies can provide sufficient support to initiate clinical trials. Limited investigations have been performed to date, however. The impact of the mTOR inhibitor RAD001 (Everolimus) on immunosenescence was evaluated, and it has shown promising effects (Mannick et al., 2014). Another study proved metformin was able to reduce diabetes-related and all-cause mortality, myocardial infarction, and any diabetes-related end point in individuals with type 2 diabetes (Scarpello, 2003; Wang et al., 2014). Recently scientists have set a new clinical trial to investigate whether metformin would be able to reduce mortality of healthy adults (https://clinicaltrials.gov/show/NCT02432287).

According to our analysis of the literature, approximately 200 compounds that can extend the lifespan of animal models have now been discovered. The extent to which they comply with the proposed criteria described above varies widely. Analysis of published data with the use of developed criteria did, however, reveal candidates that fit all of the main criteria (e.g., acarbose, deprenyl, d-glucosamine, dihydrogencristine methanesulfonate, ellagic acid, fenofibrate, glutathione, metformin, spermidine, tyrosol, and vinpocetine), and we suggest that they are tractable candidates for human interventions. Notably, we have compiled data in the literature for this analysis and have tried to impose minimal judgments on data quality. While this has the advantage of reducing bias imposed by the authors, readers are encouraged to examine the direct literature for specific compounds of interest.

**Acarbose**

Acarbose is an α-glucosidase inhibitor used to treat diabetes mellitus. Treated with this compound, male UM-HET3 mice had 22% longer lifespan (Harrison et al., 2014). It has acceptable acute toxicity – mouse LD50 oral is 24 gm kg−1 (Tomasulo, 2002). Acarbose is considered to be well-tolerable drug with adverse effects not significantly differing from placebo (Hotta et al., 1993). Acarbose reduces blood glucose concentrations, blood pressure, triglycerides, the progression of intima media thickness, and incidence of cardiovascular events and of newly diagnosed hypertension, and it demonstrates beneficial effects on overweight individuals and downregulates biomarkers of low-grade inflammation (Hanefeld & Schaper, 2008).

**Deprenyl**

Deprenyl is a selective MAO-B inhibitor used to treat major depression and early-stage Parkinson disease. It prolongs lifespan in male rat (Kitani et al., 1993). No significant acute toxicocological data for this compound were identified in literature search (mouse LD50 intraperitoneal 200 mg kg−1) (Kane et al., 1988). Deprenyl is also well tolerated (Robottom, 2011). Deprenyl protects human neurons from apoptosis induced by various kinds of insults by interfering with early apoptotic signaling events and may be applicable to delay the deterioration of neurons during advancing aging (Maruyama & Naoi, 1999; Magyar et al., 2004).

**D-glucosamine**

D-glucosamine is an amino sugar and a potent drug for the treatment of arthritis, although its effectiveness is controversial (Burdev & McNeil, 2012). Nevertheless, it is able to promote lifespan of nematodes and C57BL/6NJr mice (Weimer et al., 2014). This compound has very low acute toxicity and is safe for everyday use (Reginster et al., 2001). Current use of glucosamine was associated with a significant decreased risk of death from cancer (HR 0.87 95% CI 0.76-0.98) and with a large risk reduction for death from respiratory diseases (HR 0.59 95% CI 0.41-0.83) (Pocobelli et al., 2010; Bell et al., 2012). Glucosamine supplementation can significantly decrease risk of lung cancer in humans (Brasky et al., 2011). A meta-analysis has shown that glucosamine has lowest risk of adverse effects compared with other treatments (Diarecin and NSAIDs) (Kongtharvenskul et al., 2015). The oral supplementation of glucosamine can potentially improve cutaneous aging in human and reduce the appearance of visible wrinkles and fine lines of the skin (Murad & Tabibian, 2001).

**Dihydrogencristine methanesulfonate**

Dihydrogencristine methanesulfonate is an fungally derived alkaloid salt and a potent vasodilator (Valli et al., 1984). It is one of the 60 compounds identified in a screen for increased longevity in C. elegans (Ye et al., 2014). In two studies (Aranda et al., 1992; Milvio, 1992), dihydrogencristine was shown as safe and well tolerated with rare side effects including mild gastralgia, nausea, and dyspepsia in aged patients with senile dementia of Alzheimer type. Dihydrogencristine methanesulfonate in some studies has statistically significant positive effects on symptoms of age-related cognitive dysfunction (Wadworth & Chrisp, 1992).

**Ellagic acid**

Ellagic acid is a natural polyphenol found in numerous edible plants. Its ability to prolong C. elegans lifespan is proposed to be due to a hormetic effect (Saul et al., 2011). This substance has also low acute toxicity – rat LD50 oral > 20 gm kg−1 (Tomasulo, 2002). Study on rats showed a potentially good tolerability (Tasaki et al., 2008). Ellagic acid prevents collagen destruction and inflammatory responses caused by UV-B-induced photoaging in HaCaT keratinocytes and human dermal fibroblasts (Baie et al., 2010).

**Glutathione**

Glutathione is a tripeptide with notable antioxidant effect. Glutathione prevents aging-associated oxidation of proteins in the crystalline lens (Kamel, 1993). It is also able to promote C. elegans lifespan (Shibamura et al., 2009) and has rather low acute toxicity – mouse LD50 oral 5 gm kg−1 (Tomasulo, 2002). A recent study showed that glutathione was well tolerated with few side effects (Richie et al., 2015).

**Metformin**

Metformin is an oral anti diabetic drug of the biguanide class that is widely prescribed as an early-stage drug for type II diabetes. It enhances
longevity is a range of model organisms: *C. elegans* (Cabreiro et al., 2013), *D. melanogaster* (Slack et al., 2012), and *M. musculus* (Martin-Montalvo et al., 2013). However, it should be noted that there are studies showing no effect or only a very small effect of metformin in rats and rodents with normal genetics and longevity (https://www.ncbi.nlm.nih.gov/pubmed/20304770, http://www.ncbi.nlm.nih.gov/pubmed/23900241). It has acceptable acute toxicity – mouse LD50 oral 1450 mg kg\(^{-1}\) (Tomusola, 2002) – and is well tolerated (Giugliano et al., 1993). Serious but rare adverse effects include lactic acidosis (mostly linked to alcoholism due to depletion of NAD+ stores), heart failure, respiratory disease (due to inadequate oxygenation of tissues), and impaired renal function. Metformin treatment confers insulin sensitivity, leads to weight loss, and improves lipid profiles (Salpeter et al., 2008).

### Spermidine

Spermidine is a natural polyamine compound found in animal tissues. It is reported to promote lifespan of yeast, flies, worms, human cells, and mice (Eisenberg et al., 2009). Spermidine has acceptable acute toxicity – mouse LD30 oral 1 gm kg\(^{-1}\) (Oyanagui, 1984). Administration of spermidine, whose intracellular concentration declines during human aging, markedly extends the lifespan of human immune cells. In aging human cells, spermidine treatment triggers epigenetic deacetylation of histone H3 through inhibition of histone acetyltransferases, enhances autophagic flux, and suppresses oxidative stress and necrosis (Eisenberg et al., 2009).

### Tyrosol

Tyrosol is a natural phenolic antioxidant, a derivative of phenethil alcohol. It is able to increase both mean and maximum lifespan in *C. elegans* (Canuelo et al., 2012) and is well tolerated (Tuck & Hayball, 2002). Tyrosol can afford considerable protection against aging-associated heart deterioration (Owen et al., 2000). Several studies showed a cardioprotective role of tyrosol (Samuel et al., 2008; Smol’iakova et al., 2010; Sun et al., 2015). Tyrosol’s cardiovascular benefits are likely due to its ability to prevent oxidation of LDL (Covas et al., 2006; Castaner et al., 2012). Also, tyrosol ameliorated hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats (Chandramohan et al., 2015). A human study of tyrosol-rich products (white wine and virgin olive oil) demonstrated an anti-inflammatory effect (Migliori et al., 2015). Tyrosol has a potent anti-allergic effect by inhibiting the degranulation of mast cells and expression of inflammatory cytokines (Je et al., 2015). Tyrosol has very low acute toxicity (LD50 was found to be 2700 and 1700 mg kg\(^{-1}\) in mice after intrastranial and intraperitoneal injection, respectively, and 7079 mg kg\(^{-1}\) in rats after intragastric administration). No toxicity was observed after 3 months of chronic intragastric administration of p-tyrosol at the doses of 200 mg kg\(^{-1}\) in male rats and 10 mg kg\(^{-1}\) in dogs (Saratikov & Krasnov, 2004).

### Nonpharmacological interventions

There are also several nonpharmacological interventions affecting lifespan and aging biomarkers. Among them are physical exercise (PE) and caloric restriction (CR). CR delays aging both in rodents and primates (http://tpx.sagepub.com/content/24/6/742.full.pdf) and improves aging biomarkers in humans including BMI, systolic and diastolic blood pressure, glucose, insulin, and lipids (http://biomedgerontology.oxfordjournals.org/content/5). Moreover, CR decreases risks of aging-associated diseases, such as diabetes and cardiovascular disease (http://www.ncbi.nlm.nih.gov/pubmed/10630589). However, this intervention has potential side effects on human health, which limits its implementation (http://www.sciencedirect.com/science/article/pii/S0003207214000422). In humans, CR side effects may be lowered by intermittent fasting (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3946160).

Intermittent fasting (IF) is a pattern of eating that cycle between a period of fasting and nonfasting. CR and IF have similar effects on cardiovascular biomarkers (http://www.fasebj.org/content/20/6/631.full). CR and IF effects may be caused by reduction in specific nutrients, for example, proteins and especially amino acids such as methionine (https://www.ncbi.nlm.nih.gov/pubmed/8429371, http://online.liebertpub.com/doi/abs/10.1089/rej.2009) or tryptophane.

As methionine restriction effects also depend on other factors (http://journals.plos.org/plosgenetics/article?id=10.1, http://www.fasebj.org/cgi/content/meeting_abstract/25, http://www.ncbi.nlm.nih.gov/pubmed/19291087).
Criteria of geroprotectors, A. Moskalev et al.

956092/), we suppose that combination of this diet with geroprotectors may have a cumulative effect on extending lifespan.

In animal models, PE has shown ability to increase survival, but not the maximal lifespan (http://www.ncbi.nlm.nih.gov/pubmed/1542046, http://www.ncbi.nlm.nih.gov/pubmed/4055572). Nevertheless, PE has impact on healthspan by decreasing risk of age-related diseases, including cardiovascular disease, diabetes, and osteoporosis (http://www.ncbi.nlm.nih.gov/pubmed/22210414). Moreover, it may be used in combination with CR to improve both maximal lifespan and healthspan (http://www.ncbi.nlm.nih.gov/pubmed/9049716).

Discussion

Our goal is to establish a set of meaningful criteria by which to promote the identification of successful geroprotectors. Positive identification of candidates would help to direct efforts and resources, maximizing the chances of identifying interventions that extend human healthspan.

It should be noted that all currently known geroprotectors are characterized by relatively weak effectiveness. A positive impact on lifespan rarely exceeds 40% in invertebrates and is often less in mammals (Spindler, 2012; Lucanic et al., 2013). In contrast, much larger effects have been observed with genetic interventions, for instance a 10-fold enhancement induced by mutations in a PI3K subunit gene (Ayyadavera et al., 2008). It is possible that the most promising path to increase the efficiency of geroprotectors lies in the increase in their specificity to molecular targets. High specificity and efficiency may be achievable through a combination of targeting and screening approaches.

Increasing abundance of genomic, transcriptomic, proteomic, metabolomic, and metagenomic data, together with the development of bioinformatics approaches, makes geroprotector screening approaches based on searching for gene signatures, identifying aging-associated pathways more efficient. These in silico approaches may lead to the development of new classes and combinations of geroprotectors that would have a more robust effect on health span and lifespan. Early attempts at using metabolic transformation algorithms aiming to mimic the young metabolic state using known drugs showed promising results (Yizhak et al., 2013). Approaches using large-scale human multi-omic data derived from healthy patients of various ages and patients with pathologies and data linked to the effects of large number of drugs are currently being explored (Zhavoronkov et al., 2014).

At present, the view that aging is a systemic process represented by a set of several overlapping pathways is increasingly taking hold. In our opinion, it is worth discussing the pathological networks of genes of aging. Affecting the most important nodes of such networks would likely provide geroprotection. Therefore, the traditional target-based approach may be surpassed by systems-based approaches, with the goal of influencing a combination of several targets that are of key importance in the aging process (Carretero et al., 2015).

From this perspective, it may be advisable to combine known geroprotectors along with the development of new drugs. As aging involves many intracellular signaling pathways, the joint action of several geroprotectors aimed at different targets or processes may have an additive or synergistic effect on life expectancy. For example, according to our earlier data, the combined effect of TOR, PI3K, and NF-kB pathway inhibitors increased Drosophila lifespan to a greater extent than the individual drugs alone (Danilov et al., 2013). In another example, the combined action of TOR and JNK inhibitors on the rotifer Brachionus manjavacas increased the average lifespan by an additional 65% compared with each inhibitor alone (Snell et al., 2014). Thus, use of the right combinations of geroprotectors may lead to a substantially greater pharmacological effect on life expectancy.

Another aspect important when testing geroprotectors individually and in combinations is personalization and tissue specificity. While many geroprotectors are expected to be efficacious across multiple species in heterogeneous populations, side effects may vary significantly. Ideal geroprotectors regimen should include a set of companion diagnostic markers to ensure personalization on the tissue-specific and system levels and adjusted to multiple parameters including age, gender, and lifestyle.

There is also the possibility of repurposing older medications for new geroprotective indications (Ye et al., 2014). The most effective cures for old age may already be on the market, but they remain unknown because their geroprotective properties have not yet been studied. If such drugs are already approved by the FDA, it makes for an easier path to test them in humans. Metformin may be a classic example and a recent retrospective study of diabetic patients taking metformin indicated that the drug might be delaying the onset of other aging pathologies (Bannister et al., 2014). Such approaches could save substantial time and resources usually spent on drug testing and approval. Accepted hallmarks for geroprotectors can play a leading role in this process.

Acknowledgment

The authors thank Dr. Leslie C. Jellen of Insilico Medicine for edits to the manuscript.

Funding

Russian Science Foundation (Grant/Award Number: 14-50-00060). B.K.K. is an Ellison Medical Foundation Senior Scholar in Aging.

Conflict of interest

The authors declare no potential conflict of interests.

References

Aranda B, Dumoulin P, Groothold G (1992) Controlled study of the effect of dihydoroergocristine on organic brain psychosyndrome. Arzneimittelforschung 42, 1406–1409.

Ayyadavera S, Alla R, Thaden JJ, Shmoookler Reis RJ (2008) Remarkable longevity and stress resistance of nematode PI3K-null mutants. Aging Cell 7, 13–22.

Bae JY, Choi JS, Kang SW, Lee YJ, Park J, Kang YH (2010) Dietary compound dihydroergocristine on organic brain psychosyndrome. Arzneimittelforschung 60, 1765–1767.

Bannister CA, Holden SE, Jenkins-Jones S, Morgan CL, Halcox JP, Schernthaner G, Mukherjee J, Currie CJ (2014) Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. Diabetes Obes. Metab. 16, 1165–1173.

Bansal A, Zhu LJ, Yen K, Tissenbaum HA (2015) Uncoupling lifespan and healthspan in Caenorhabditis elegans longevity mutants. Proc. Natl Acad. Sci. USA 112, E277–E286.

Bayer R, Piewa S, Borcescu E, Claus W (1988) Filterability of human erythrocytes – drug induced prevention of aging in vitro. Arzneimittelforschung 38, 1765–1767.

Bell GA, Kantor ED, Lampe JW, Shen DD, White E (2012) Use of glucosamine and chondroitin in relation to mortality. Eur. J. Epidemiol. 27, 593–603.

Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, Harrington H, Israel S, Levine ME, Schaefer JD, Sugden K, Williams B, Yashin AI, Poulton R, Moffitt TE (2015) Quantification of biological aging in young adults. Proc. Natl Acad. Sci. USA 112, E4104–E4110.
Brasky TM, Lampe JW, Slatore CG, White E (2011) Use of glucosamine and chondroitin and lung cancer risk in the VITamins And Lifestyle (VITAL) cohort. *Cancer Causes Control* 22: 1335–1342.

Burdett N, McNeil JD (2012) Difficulties with assessing the benefit of glucosamine sulphate as a treatment for osteoarthritis. *Int. J. Evid. Based Healthc*. 10, 222–226.

Cabeiro F, Au C, Leung KY, Vergara-Irigraray N, Cocheme HM, Noori T, Weinkove D, Schuster E, Greene ND, Gems D (2013) Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. *Cell* 153, 228–239.

Canela A, Gilbert-Lopez B, Pacheco-Linan P, Martinez-Daza E, Siles E, Miranda-Vizuete A (2012) Tyrosol, a main phenol present in extra virgin olive oil, increases lifespan and stress resistance in *Caenorhabditis elegans*. *Mech. Ageing Dev.* 133, 563–574.

Carretero M, Gomez-Amaro RL, Petrascheck M (2015) Pharmacological classes that extend lifespan of *Caenorhabditis elegans*. *Front. Genet.* 6, 77.

Castaner O, Covas MI, Khyemenets O, Nysssonen K, Konstantinidou V, Zunft HF, de la Torre R, Munoz-Aguayo D, Vila J, Fito M (2012) Protection of LDL from oxidation by olive oil polyphenols is associated with a downregulation of CD40/ligand expression and its downstream products in vivo in humans. *Am. J. Clin. Nutr.* 95, 1238–1244.

Chandramohan R, Pari L, Rattiniam A, Sheikh BA (2015) Tyrosol, a phenolic compound, ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats. *Chem. Biol. Interact.* 229, 44–54.

Cholnoky E, Domok L (1976) Summary of safety tests of ethyl apovincamine. *Arzneimittel-Forschung* 26, 1938–1944.

Chuang KH, Peng YC, Chien HY, Lu ML, Du HI, Wu YL (2013) Attenuation of LPS-induced lung injury by glucosamine in rats. *Am. J. Respir. Cell Mol. Biol.* 49, 110–111.

Covas MI, de la Torre K, Farre-Albaladejo M, Kaikkonen J, Fito M, Lopez-Sabater C, Pujadas-Bastardes MA, Joglar J, Weinbrenner T, Lamuela-Raventos RM, de la Torre R (2006) Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. *Free Radic. Biol. Med.* 40, 608–616.

Craig T, Smelick C, Tacutu R, Wuttke D, Wood SH, Stanley H, Janssens G, Savitskaya E, Moskalev A, Arkng K, de Magalhaes JP (2015) The Digital Ageing Atlas: integrating the diversity of age-related changes into a unified resource. *Nucleic Acids Res.* 43, D873–D878.

Cutler RG (2005) Oxidative stress and aging: catalase is a longevity determinant. *Biochem. Pharmacol.* 69, 153–1546.

Dann SG, Thomas G (2006) The amino acid sensitive TOR pathway from yeast to Drosophila. *Cell. Mol. Life Sci.* 63, 2296–2309.

Gasparini G, Cardinale M, Buse S, Yoga P, Spaccapietra S, Conoto F, Fontana L, Noone M, Verilli A, Vichi M, Neri G, Partridge L (2009) A diet rich in nuts extends lifespan and protects against age-related diseases in mice. *Nature* 421, 182–187.

Hotta N, Kakuta H, Sano T, Matsumae H, Yamada H, Kitazawa S, Sakamoto N (1993) Long-term effect of ascorbolic on glycemic control in non-insulin-dependent diabetes mellitus: a placebo-controlled double-blind study. *Diabet. Med.* 10, 134–138.

Je IG, Kim DS, Kim SW, Lee S, Lee HS, Park EK, Khang D, Kim SH (2015) Tyrosol suppresses allergic inflammation by inhibiting the activation of phosphoinositide 3-kinase in mast cells. *PloS ONE* e0129829.

Johnson SC, Rabinovitch PS, Kaeberlein M (2013) mTOR is a key modulator of aging and age-related disease. *Nature* 493, 338–345.

Kamieli A (1993) Glutathione levels of the human crystalline lens in aging and its antioxidant effect against the oxidation of lens proteins. *Biol. Pharm. Bull.* 16, 870–875.

Kane JM, Dudley MW, Sorensen SM, Miller FP (1988) 2,4-Dihydroxy-3H-1,2,4-triazole-3-thiones as potential antiageing agents. *J. Med. Chem.* 31, 1253–1258.

Kapoor VK, Dureja J, Chadha R (2009) Synthetic drugs with anti-ageing effects. *Drug Discov. Today* 14, 899–904.

Kotani K, Kanai S, Sato Y, Ditta M, Ivy GO, Carrillo MC (1993) Chronic treatment of (-)-deprenyl prolongs the life span of male *C. elegans* 344 rats. Further evidence. *Life Sci.* 52, 281–288.

Kongtharvonskul J, Anothaisintawee T, McEvoy M, Atii J, Woratanarat P, Thakkinstian A (2015) Efficacy and safety of glucosamine, diacerein, and NSAIDs in osteoarthritis knee: a systematic review and network meta-analysis. *Eur. J. Med. Res.* 20, 24.

Lamming DW, Ye L, Sabatini DM, Baur JA (2013) Rapamycin and mTOR inhibitors as anti-ageing therapeutics. *J. Clin. Invest.* 123, 980–989.

Lin YH, Chen YC, Kao TY, Lin YC, Hsu TE, Wu YC, Ja WW, Brummel TJ, Kapahi P, Yuh CH, Yu LK, Lin ZH, You RJ, Jiang YH, Wong HD (2014) Diacylglycerol lipase regulates lifespan and oxidative stress response by inversely modulating TOR signaling in *Drosophila* and *C. elegans*. *Aging Cell* 13, 755–764.

Longo VD, Fabrisio P (2002) Regulation of longevity and stress resistance: a molecular strategy conserved from yeast to humans? *Cell. Mol. Life Sci.* 59, 903–908.

Longo VD, Antebi A, Bartke A, Barzilai N, Brown-Borg HM, Caruso C, Curiel TJ, de Cabo R, Franceschi C, Gems D, Ingram DK, Johnson TE, Kennedy BK, Kenyon C, Small K, Kopchick JJ, Leppardinger G, Madoa F, Mirsola MG, Mitchell JR, Passarino G, Rudolph KL, Sedivy JM, Shadel GS, Sinclair DA, Spindler SR, Suh Y, Vlajkovic M, Fontana L (2015) Interventions to slow aging in humans: are we ready? *Aging Cell* 14, 497–510.

López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. *Cell* 153, 1194–1217.

Lucanic M, Ligthow GJ, Alavez S (2013) Pharmacological lifespan extension of invertebrates. *Aging Res. Rev.* 12, 445–458.

Magyar K, Palfi M, Tabi T, Kalasz H, Szende B, Szoko E (2004) Pharmacological aspects of (-)-deprenyl. *Curr. Med. Chem.* 11, 2017–2031.

Mannick JB, Del Giudice G, Lattanzio M, Valiante NM, Staegastgna J, Huang B, Lonetto MA, Maeker HT, Kovarik J, Carson S, Glass DJ, Klickstein LB (2014) mTOR inhibition improves immune function in the elderly. *Sci. Transl. Med.* 6, 268ra179.

Martín-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Schiebye-Koch M, Gomes AP, Ward TM, Minor RK, Blouin MJ, Schwab M, Pollak M, Zhang Y, Yu Y, Becker KG, Bohr VA, Ingram DK, Sinclair DA, Wolf NS, Spindler SR, Bernier M, de Cabo R (2013) Metformin improves healthspan and lifespan in mice. *Nature* 504, 2192.

Maruyama N, Naoi M (1999) Neuroprotection by (-)-deprenyl and related compounds. *Mech. Ageing Dev.* 111, 189–200.

Medvedik O, Lamming DW, Kim KD, Sinclair DA (2007) MSN2 and MSN4 link cellderived and TGF-β to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae*. *PloS Biol.* 5, e261.

Methcinnkoff E, Mitchell PC (1910) The *Prolongation of Life: Optimistic Studies*. New and Revised Edition. New York & London: G. P. Putnam's Sons.
Zhavoronkov A, Buzdin AA, Garazha AV, Borisov NM, Moskalev AA (2014) Signaling pathway cloud regulation for in silico screening and ranking of the potential geroprotective drugs. Front. Genet. 5, 49.

Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ilkeno Y, Hubbard GB, Lenburg M, O’Hara SP, LaRusso NF, Miller JD, Roos CM, Verzosa GC, LeBrasseur NK, Wren JD, Farr JN, Khosla S, Stout MB, McGowan SJ, Fuhrmann-Stroissnigg H, Gurrar AU, Zhao J, Colangelo D, Dorronsoro A, Ling YY, Barghouthy AS, Navarro DC, Sano T, Robbins PD, Niedernhofer LJ, Kirkland JL (2015) The Achilles’ heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 14, 644–658.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Table S1 Summary of the effects on lifespan, healthspan, and potential side effects of the pharmacological geroprotector candidates, ordered on the base of evolutionary conservation of lifespan effects in different models.