New biomarkers and interventions in aging

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In animal models, agents that extend lifespan often protect against these diseases and there is the reason to believe that a similar approach might work in humans.
Geroprotectors

• The pharmacological agents that decrease the rate of aging and extend lifespan are called geroprotectors.

• This term was first coined by the Nobel Prize winner Ilya Mechnikov.

• First attempts of life extension via pharmacological treatment were described in the pioneer investigations of Thomas Gardner experiments performed on mice and flies in 1946.
Geroprotectors.org: a new, structured and curated database of current therapeutic interventions in aging and age-related disease

The DrugAge database of aging-related drugs

Diogo Barardo, Daniel Thornton, Harikrishnan Thoppil, Michael Walsh, Samim Sharifi, Susana Ferreira, Andreja Anžič, Maria Fernandes, Patrick Monteiro, Tjaša Grum, Rui Cordeiro, Evandro Araújo De-Souza, Arie Budovsky, Natali Araujo, Jan Gruber, Michael Petrascheck, Vadim E. Fraifeld, Alexander Zhavoronkov, Alexey Moskalev, João Pedro de Magalhães

DrugAge: Database of Ageing-Related Drugs

DrugAge Database of Anti-Ageing Drugs

The DrugAge database contains an extensive compilation of drugs, compounds and supplements (including natural products and nutraceuticals) with anti-ageing properties that extend longevity in model organisms. Our focus is on drugs/compounds potentially impacting on ageing, and therefore drugs/compounds extending lifespan in disease-prone animals (e.g., cancer models) are excluded.

Browse & Search

Search DrugAge for any term (case insensitive) or browse all the data.

Search Organization

Drug Search

Retrieve specific drug data from DrugAge.
Criteria of geroprotector

- Nowadays we have more than 200 experimental geroprotectors (Moskalev et al., 2016).
- For translation of this knowledge we have to come to an agreement what should be considered applicable to humans anti-aging drugs.
- It cannot be limited to one or two criteria, e.g. life extension. The goal for translation into humans should be an increase in healthspan as well. Sometimes 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*.
- Therefore it is important to incorporate functional measures of aging wherever possible.
Developing criteria for evaluation of geroprotectors as a key stage toward translation to the clinic

Alexey Moskalev¹,²,³,*, Elizaveta Chernyagina³, Vasily Tsvetkov³,⁴, Alexander Fedintsev², Mikhail Shaposhnikov¹, Vyacheslav Krut'ko⁵, Alex Zhavoronkov²,⁶,⁷ and Brian K. Kennedy⁸,*

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Primary selection criteria for potential geroprotector

1. Increased lifespan

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, and rate of aging.
**NSAIDs effects on lifespan**

The suppression of gene expression of Pkh2/Pdk1 using RNA interference led to an increase in life span of female fruit flies. At the same time females with RNAi Pkh2/Pdk1 not observed positive effect of valdecoxb on lifespan. Thus, the geroprotective effect of NSAIDs is possibly mediated by Pkh2/ypk1/lem3/tat2 signaling pathway.

He et al., 2014; Danilov et al., 2015

**Effects of ibuprofen on yeast, worm, fly lifespan**

TAT2 - Tryptophan permease
• The increase in lifespan is not always accompanied by positive changes in the quality of life, and additional criteria for geroprotectors is needed, and discussed below.
Primary selection criteria for potential geroprotector

2. 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. Ideally, geroprotectors should reverse these biomarkers to a younger state or slow down the changes with age.
• A human aging biomarker should be minimally invasive, reproducible and reflect main aging mechanisms.

• While there are no single definitive biomarkers for aging, a range of different measures have been proposed and are worthy of consideration.
• For example, studies on cultures of human cells *in vitro* (expression of telomere-related genes, beta-amyloid-lowering effect, low levels of advanced glycation endproducts and oxidative damage, reduced level of lipofuscin, etc.) 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, etc.).
AGING.AI - Biochemistry
- Blood Biochemistry & Cell-count based predictor of chronological age (complete/published)
- 41 & 33 parameters
- Population & diet-specific
- 4.5-7 year Mean Absolute Error
- Collaborations with Canada (Alberta), Korea (2 hospital networks, getting to National level), Malaysia (through Asia Genomics), Kazakhstan (emerging collaboration, national-level), Russia (Invitro, emerging collaboration with Moscow city)
Top 5 features:

- Albumin (Liver function)
- Glucose (Metabolic function)
- Alkaline phosphatase (Liver function)
- Erythrocytes (Respiratory function)
- Urea (Renal function)
Deep Biomarkers Of Human Aging

Aging.AI ¹⁰
- 41 input parameters
- $r = 0.91$
- $	ext{Rsq} = 0.82$
- MAE = 5.5 years

Aging.AI ²⁰
- 33 input parameters
- $r = 0.79$
- $	ext{Rsq} = 0.63$
- MAE = 6.2 years

www.impactaging.com

Deep biomarkers of human aging: Application of deep neural networks to biomarker development

Evgeny Putin ¹,², Polina Mamoshina¹,³, Alexander Aliper¹, Mikhail Korzinkin¹, Alexey Moskalev¹,⁴, Alexey Kolosov⁵, Alexander Ostrovskiy⁵, Charles Cantor⁶, Jan Vijg⁷, and Alex Zhavoronkov¹,³
Table 3. List of assessed clinical parameters ranked by their Pearson correlation coefficients.

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal of two cIMTs measured for left and right carotid (cIMTmin)</td>
<td>0.684</td>
<td>5.76e-23</td>
</tr>
<tr>
<td>Maximal of two Stenosis values (STEN_A)</td>
<td>0.596</td>
<td>2.04e-22</td>
</tr>
<tr>
<td>Central blood pressure (difference between systolic and diastolic)</td>
<td>0.505</td>
<td>6.52e-22</td>
</tr>
<tr>
<td>Augmentation Index (Aix)</td>
<td>0.481</td>
<td>3.90e-13</td>
</tr>
<tr>
<td>Pulse wave velocity (PWV)</td>
<td>0.456</td>
<td>2.23e-13</td>
</tr>
<tr>
<td>Total number of atherogenic plaques in left and right carotids</td>
<td>0.453</td>
<td>4.23e-17</td>
</tr>
<tr>
<td>B-type natriuretic peptide</td>
<td>0.364</td>
<td>0.0006</td>
</tr>
<tr>
<td>Von Willebrand factor</td>
<td>0.358</td>
<td>2.47e-08</td>
</tr>
<tr>
<td>IGF-1</td>
<td>-0.331</td>
<td>0.0004</td>
</tr>
<tr>
<td>C-peptide</td>
<td>0.324</td>
<td>0.0007</td>
</tr>
<tr>
<td>Telomere length</td>
<td>-0.319</td>
<td>0.015</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>0.318</td>
<td>4.52e-05</td>
</tr>
<tr>
<td>Urea</td>
<td>0.269</td>
<td>6.74e-05</td>
</tr>
<tr>
<td>Presence of hypertension</td>
<td>0.264</td>
<td>0.0005</td>
</tr>
<tr>
<td>Presence of Diabetes</td>
<td>0.263</td>
<td>3.42e-06</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.258</td>
<td>0.01</td>
</tr>
<tr>
<td>Telomerase activity</td>
<td>-0.246</td>
<td>0.0016</td>
</tr>
<tr>
<td>Presence of obesity</td>
<td>0.245</td>
<td>0.012</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.239</td>
<td>0.006</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.228</td>
<td>0.0015</td>
</tr>
<tr>
<td>ESR</td>
<td>0.225</td>
<td>0.0025</td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td>0.224</td>
<td>0.0015</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.218</td>
<td>0.0022</td>
</tr>
<tr>
<td>BMI</td>
<td>0.194</td>
<td>0.0064</td>
</tr>
<tr>
<td>Hips ratio</td>
<td>0.193</td>
<td>0.0069</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.188</td>
<td>0.01</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.164</td>
<td>NS</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>0.157</td>
<td>0.036</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.156</td>
<td>0.029</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>0.149</td>
<td>0.046</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.146</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin after 2h</td>
<td>0.141</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.131</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td>0.13</td>
<td>NS</td>
</tr>
<tr>
<td>LDL</td>
<td>0.125</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.114</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.106</td>
<td>NS</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>-0.104</td>
<td>NS</td>
</tr>
</tbody>
</table>
Using machine learning, we arrived on a set of four predictors, all of which reflect the functioning of the cardiovascular system.

In Arterial Indices models, results of carotid artery duplex scan that show the thickness of the intima media complex and quantitatively describe the degree of stenosis are combined with pulse wave velocity and augmentation index measurements performed by applanation tonometry.

In our cohort, the age of men was determined with MAE = 6.91 years (adjusted $R^2 = 0.55$), and the age of women with MAE = 5.87 years (adjusted $R^2 = 0.69$).
Primary selection criteria for potential geroprotector

3. Acceptable toxicity
Since most geroprotectors show a preventive effect only when used at relatively high concentrations over long periods of time. The acceptable toxicity of geroprotector suggests significant (several orders of magnitude) differences between lifespan extending and toxic dose.
Primary selection criteria for potential geroprotector

3. Minimal side effects
Some substances that in certain concentrations prolong the life of model animals have multiple adverse side effects. For instance, dyslipidemia, anemia, insulin resistance, increased susceptibility to infections, hypertension, and gastro-intestinal disorders have been reported in some cases.
Primary selection criteria for potential geroprotector

4. Improving health-related quality of life
Geroprotective effects on aging will appear many years after the onset of exposure. It is important that the potential geroprotector improves the quality of life from the very beginning, for example, quality of sleep (melatonin) or severity of depression (ibuprofen)
Secondary selection criteria for potential geroprotector

5. Evolutionary conservatism of target or mechanism of action
6. Reproducibility of geroprotective effects on different model organisms
7. Simultaneous influence on several aging-associated causes of death in mammals
8. Increase of stress resistance
Candidates

• Analysis of data of our database (>200 compounds) with the use of developed criteria did, reveal candidates that fit all of the main criteria (e.g. D-glucosamine, dihydroergocristine methanesulfonate, ellagic acid, fenofibrate, glutathione, metformin, spermidine, tyrosol, and vinpocetine) and we suggest that they are tractable candidates for human interventions.
GeroScope

• is a computational tool that can aid prediction of novel geroprotectors from existing human gene expression data.
EVOLUTION OF GENE EXPRESSION ANALYSIS

Expression of individual genes

Full transcriptome

Signaling pathways

Differential signaling pathway activation analysis

1980+

1995+

2000+

2010+

illuminax

affymetrix

QIAGEN

THOMSON REUTERS

INGENUITY SYSTEMS

gen

NuMedii

Ayasdi

INSILICO MEDICINE

Deep Learning

2015+
To verify the predictive potential of the Geroscope software, the top substances rated by the program were added to non-transformed human embryonic lung fibroblasts at the senescence stage ("old") in 50 μM concentrations and incubated for 3 days. Fibroblasts from several passages earlier, in a pre-senescent state ("young") served as control.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Substance</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>young</td>
<td>-</td>
<td>Y</td>
</tr>
<tr>
<td>old</td>
<td>-</td>
<td>O</td>
</tr>
<tr>
<td>old</td>
<td>Nordihydroguaiaretic acid</td>
<td>A</td>
</tr>
<tr>
<td>old</td>
<td>Myricetin</td>
<td>B</td>
</tr>
<tr>
<td>old</td>
<td>N-acetyl-L-cysteine</td>
<td>G</td>
</tr>
<tr>
<td>old</td>
<td>Fasudil (HA-1077)</td>
<td>H</td>
</tr>
<tr>
<td>old</td>
<td>PD-98059</td>
<td>I</td>
</tr>
<tr>
<td>old</td>
<td>Epigallocatechin gallate</td>
<td>J</td>
</tr>
</tbody>
</table>
It can be seen that all substances, except A (nordihydroguaiaretic acid), strongly reduced beta-galactosidase staining of senescent fibroblasts. Substance I (MEK inhibitor PD98059) had the most pronounced effect.
Flow cytometry analysis revealed that most of the test substances slightly increased the viability of senescent cells. Interestingly, substance G (NAC) increased it almost to the level of pre-senescent cells, whereas substance A (nordihydroguaiaretic acid) decreased the viability. All test substances decreased the mean size of senescent cells, and most of them also decreased the variation in cell size. The changes in cell granularity were comparable to the changes in cell size.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Viability</th>
<th>Size</th>
<th>Granularity</th>
<th>Auto-fluorescence</th>
<th>Beta-galactosidase</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nordihydroguaiaretic acid (A)</td>
<td>-</td>
<td>-/=</td>
<td>-/=</td>
<td>+/=</td>
<td>-/=</td>
<td>---</td>
</tr>
<tr>
<td>Myricetin (B)</td>
<td>+/-</td>
<td>-</td>
<td>-/=</td>
<td>-</td>
<td>--</td>
<td>---</td>
</tr>
<tr>
<td>N-acetyl-L-cysteine (G)</td>
<td>+++</td>
<td>-/=</td>
<td>-/=</td>
<td>-/=</td>
<td>--</td>
<td>+++</td>
</tr>
<tr>
<td>Fasudil (HA-1077) (H)</td>
<td>+</td>
<td>-/=</td>
<td>-/=</td>
<td>+++</td>
<td>--</td>
<td>-</td>
</tr>
<tr>
<td>PD-98059 (I)</td>
<td>+</td>
<td>--</td>
<td>---</td>
<td>--</td>
<td>---</td>
<td>+++</td>
</tr>
<tr>
<td>Epigallocatechin gallate (J)</td>
<td>=</td>
<td>---</td>
<td>--</td>
<td>---</td>
<td>--</td>
<td>---</td>
</tr>
</tbody>
</table>

- Substance A had almost no effect on senescent phenotype, but decreased both short- and long-term survival.
- Substance B had mild rejuvenating effect as judged by cell phenotype, but severely compromised long-term survival.
- Substance G had very mild rejuvenating effect but dramatically increased short- and long-term survival.
- Substance H also had very mild rejuvenating effect but did not dramatically affect survival.
- Substance I had very strong rejuvenating effect and increased both short- and long-term survival.
- Substance J also had very strong rejuvenating effect but dramatically decreased long-term survival.
- Overall, these results indicate that substance I possesses the strongest rejuvenating and pro-survival properties out of the substances tested.
Xanthophyll, accessory pigment in the chloroplasts of brown algae, giving them a brown or olive-green color.
Fucoxanthin increases lifespan of *Drosophila melanogaster* and *Caenorhabditis elegans*

Ekaterina Lashmanova, Ekaterina Proshkina, Svetlana Zhikrivetskaya, Oksana Shevchenko, Elena Marusich, Sergey Leonov, Alex Melerzanov, Alex Zhavoronkov, Alexey Moskalev.

**Lifespan and Oxidative Stress Resistance Increase**

**Locomotor Activity and Fecundity Effects**

**Changes in expression of stress resistance genes**
Analysis of fecundity and fertility

Control and experimental females of different ages

Young Canton-S males

1:1 (n = 50)

Mating during 24 h

Egg laying during 24 h (one time a week)

Number of eggs (fecundity)

10-15 days

Number of imagoes (fertility)
Effects of fucoxanthin treatment on age-dependent dynamics of fecundity and fertility

Fecundity

Number of eggs per female

Age (days)

0 10 20 30 40 50

0 5 10 15 20 25

Fertility

Progeny number per female

Age (days)

0 10 20 30 40 50

0 5 10 15 20 25 30

Fertility/Fecundity

Age (weeks)

0 1 2 3 4 5 6 7 8

0 0.2 0.4 0.6 0.8 1 1.2

control  fucoxanthin (1 µM)

*p<0.05, Student’s t-test
As previously shown by Rera et al. (2012) the intestinal permeability can be used as a physiological marker of *Drosophila* aging.
The error bars show standard error of the proportion

* $p<0.01$, Fisher’s exact test
Analysis of locomotor activity and sleep/rest parameters

Drosophila Activity Monitor (Trikinetics, USA)

n = 1 fly × 48 glass tubes

Analysis of locomotor activity during 24 h
(12 h:12 h light-dark cycle)

Estimation of average total daily locomotor activity and sleep/rest parameters

Sleep/rest - contiguous ≥5 min with no activity
Age-dependent dynamics of total daily locomotor activity

The error bars show standard errors. *p<0.05 **p<0.01, Student’s t-test
Age-dependent dynamics of sleep/rest parameters

The white and gray background colors indicate a 12 h:12 h light-dark cycle, respectively. The error bars show standard error of the proportion. *p<0.05, **p<0.01, Fisher’s exact test.
The top 57 genes, involved in 17 KEGG pathways were identified.