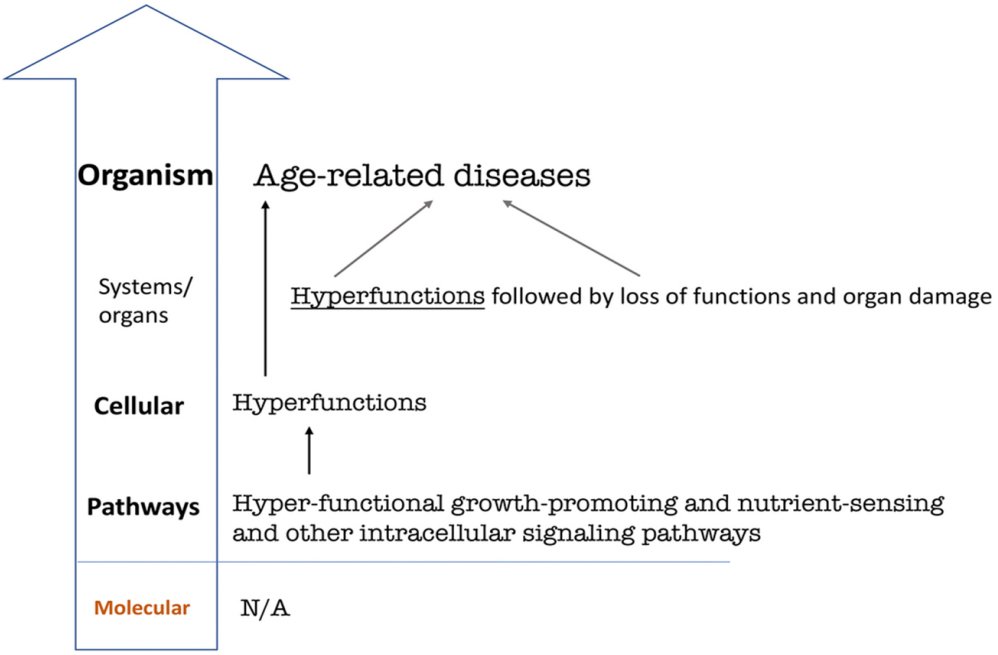

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Figure 4: Hierarchical hallmarks of aging based on hyperfunction theory, universal from "Hallmarks of cancer and hallmarks of aging" by Mikhail Blagosklonny.

Mikhail Blagosklonny's Publications in Aging (Albany NY)

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Editorial

Targeted cancer therapy: The initial high concentration may slow down the selection for resistance

Mikhail V. Blagosklonny

ABSTRACT

Unfortunately, any targeted therapy is, always, started with low levels of the drug in the organism, selecting for drug resistance. One should propose that initial drug levels must be maximized, and durations may be minimized, ideally, as portions of preemptive combination of targeted drugs.

To ensure rapid selection of drug resistance in the cell culture, we first treat cancer cells with low drug concentrations, increasing drug concentrations over time. I hope no further explanation is needed.

In patients, treatment with targeted drugs is also mistakenly designed to expedite the selection of resistance, a fact that may shock my readers. Initially, cancer cells are exposed to low drug concentrations, which are then increased. This is achieved simply by administering the same daily dose to patients from day 1. For example, Cabozantinib is taken once daily. Cabozantinib has a long terminal plasma half-life (~120 hours) and accumulates 5-fold by day 15 with daily dosing.

I reiterate: the concentration increases 5-fold by day 15 due to its long half-life and consistent daily dosing of one tablet.

Low initial concentrations can be avoided by taking 5 tablets on the first day and then continuing with 1 tablet every week, for example. Another hypothetical regimen is 3 tablets for every 3 days and then discontinuing it. Levels of Cabozantinib remain high for the next one to two weeks (post-treatment remained activity).

Consider my case: I have multiple brain metastases driven by METex14, effectively targeted by capmatinib, the most selective and effective MET inhibitor. Capmatinib selects for resistant secondary mutations in the METex14 that can be targeted by Cabozantinib, although it is less effective and selective than capmatinib. It is possible that one of the metastases contains at least one capmatinib-resistant cell with secondary MET mutations, which could eventually make the metastasis resistant to capmatinib.

In my case, I propose using 5 tablets of Cabozantinib for 1 day every two months. Only 1 day every 2 months.

The mutant cell is exposed to relevant concentrations of Cabozantinib for one to two weeks (post-treatment remained activity).

Another example. Afatinib (EGFR/HER2-4 inhibitor) is used to treat EGFR-mutant-dependent lung cancer (it is not my case, as mine is METex14-dependent). Afatinib has a half-life of 37 hours. Steady-state is achieved within 8 days of once-daily dosing, with overall accumulation ratios of 2.0–2.7 for C max and 2.5–3.4 for AUC. Instead, we should achieve Steady-state on day 1. In some cases, the first day levels should be higher than ever further and the course of treatment may be brief.

In my case, the most common off-target mechanism of resistance involves overexpression of EGFR and HER2-4. I prefer very high dose treatment with afatinib for just 1 days in combination with capmatinib. After stopping afatinib, its levels remain high for several additional days (post-treatment remained activity), and capmatinib must continue to be used. Afatinib alone is ineffective in my case (METex14), but afatinib targets potential resistance to capmatinib. (Note: Without capmatinib, METex14-positive cells would not be killed by the off-target resistant drug. A combination of two drugs must be used, including high levels of afatinib post-treatment).

Another example: trametinib (an MEK inhibitor) is usually given 1 tablet (2 mg) every day from day 1. The estimated elimination half-life is 3.9 to 4.8 days. Trametinib accumulates with daily repeat dosing with the accumulation ratio of 6.0. Steady state was achieved by day 15. This is reminiscent of Cabozantinib.

One may suggest that steady-state-like levels (or even higher levels) may be required for the very beginning of treatment. Especially, in brief applications use. For drugs with long half-life, the drug may be given only on day 1 because high levels of the drug remain for a long time in the organism, no matter what. These are post-treatment levels and I call this the reversal level curve (Figure 1). I suggest to use reversal schedules in targeted drug combinations (Figure 2). I've depicted the schema I have used for me for very specific reasons that are beyond the topic of this editorial (My battle with cancer: exceptional chapters from part II). It is worth mentioning that inhibition of MEK and mTOR should

be used together, because rapamycin may activate MEK, and Trametinib may activate the mTOR pathway.

In conclusion, to decelerate the acquisition of drug resistance, targeted therapy must immediately target the tumor at a higher than steady-state drug concentration. The courses may be brief, if the doses are high, but that is a topic for another story. Importantly, as previously discussed, targeted drugs should be used in appropriate combinations [1, 2].

Starting treatment with a higher dose is common in medicine. For instance, starting with high doses is typical for antibacterial antibiotics to avoid resistance.

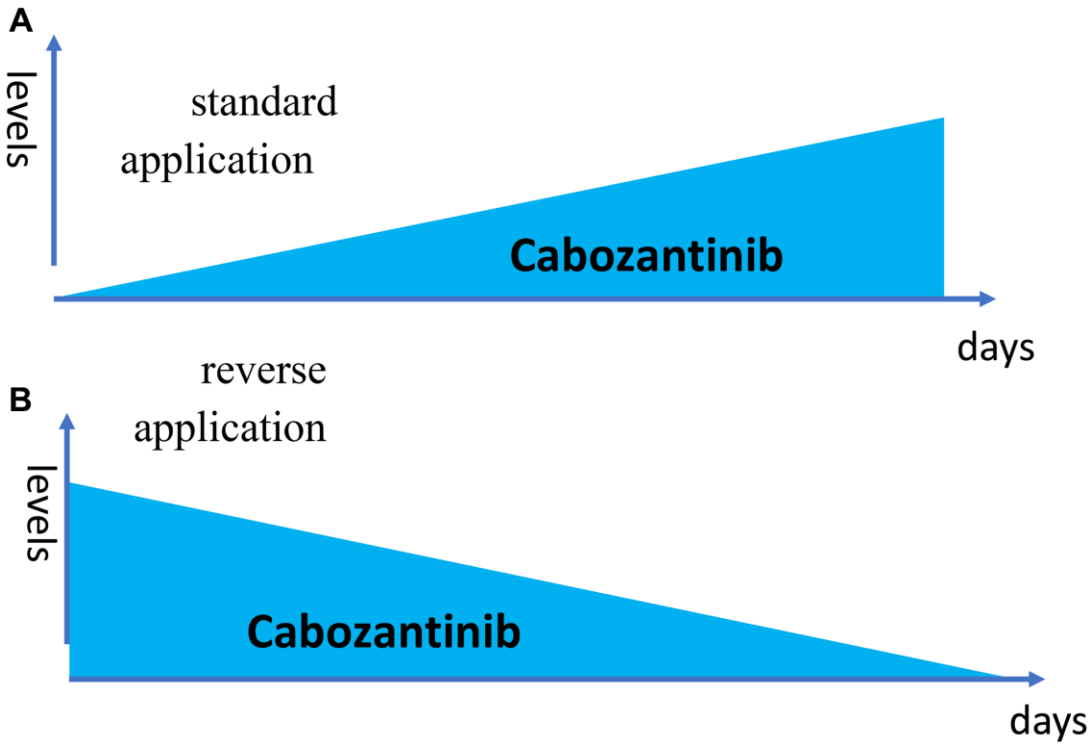

Figure 1. Reverse dose application. (**A**) The drug is given every day 1 tablets. (**B**) The drug is given on day 1 only 6 tablets (just one as an example).

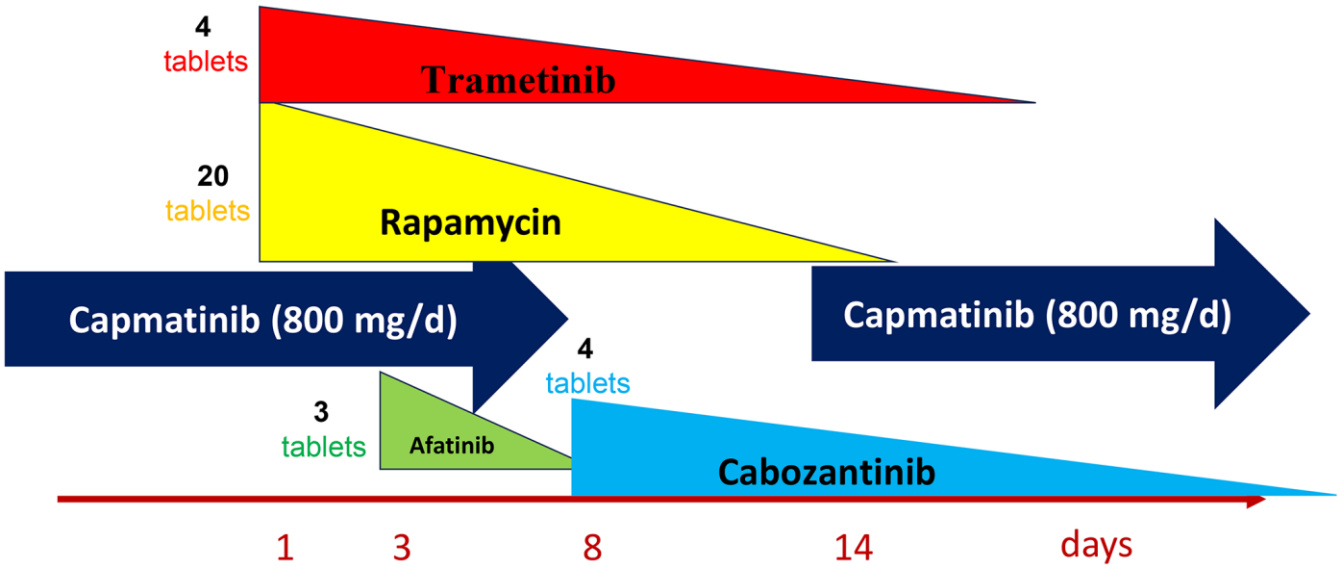

Figure 2. One of my targeted combinations. Capmatinib – standard everyday use. All other medications - reverse dose application -are given on day 1 only. The number of tablets on day1 are shown.

Often, antibiotics are used at double (or higher) doses on the first day, as a high single dose, or even intravenously.

Also, rapamycin is given at a loading dose on the first day, which is three times higher than the maintenance dose in organ transplant patients. For another example, dexamethasone is often started with a load.

Appendix

"My battle with cancer. Part 1." Oncoscience. 2024 Jan 3; 11: 1-14.
Abstract:
In January 2023, diagnosed with numerous metastases of lung cancer in my brain, I felt that I must accomplish a mission. If everything happens for a reason, my cancer, in particular, I must find out how metastatic cancer can be treated with curative intent. This is my mission now, and the reason I was ever born. In January 2023, I understood the meaning of life, of my life. I was born to write this article. In this article, I argue that monotherapy with targeted drugs, even when used in sequence, cannot cure metastatic cancer. However, preemptive combinations of targeted drugs may, in theory, cure incurable cancer.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10765422/

Forthcoming "My battle with cancer: exceptional chapters from part II."
Abstract:
For a divine reason, I was destined for cancer with multiple brain metastases: to create the book "My Battle with Cancer," a far-reaching endeavor for which I was born. Surprisingly to others, I experience moments—no, entire days—of happiness and joy. My journey through cancer, as both a patient and a researcher, has gifted me insights into cancer cures, born from a mind that remains active, often even in sleep. Unfortunately, one thing happened on May 20, 2024, hurting me, but this is not a part of this book. Nevertheless, I am finishing the book with the hope of increasing the lifespan to the normal duration for future incurable cancer patients. Among other topics, I propose preemptive combinations of targeted drugs to prevent resistance to the key cancer driver and thus sustain long-lasting remission; classification of targeted combinations; intermittent targeting; reverse dose applications; increasing targeting of brain metastases; and the harmful effects of standard WBRT, Avastin, and immunotherapy-induced hyper-progression.

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Review

Cellular senescence: when growth stimulation meets cell cycle arrest

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ABSTRACT

At the very moment of cell-cycle arrest, the cell is not senescent yet. For several days in cell culture, the arrested cell is acquiring a senescent phenotype. What is happening during this geroconversion? Cellular enlargement (hypertrophy) and hyperfunctions (lysosomal and hyper-secretory) are hallmarks of geroconversion.

Epigraph

"Growth stimulation leads to cellular senescence when the cell cycle is blocked" [1].

Arrest is not yet senescence

Not anything that causes arrest causes senescence. For example, serum withdrawal, contact inhibition, nutrient starvation and rapamycin cause reversible arrest (quiescence) instead of senescence. What these conditions have in common is that they inhibit cellular mass or volume growth and specifically inhibit the mTOR pathway. (Of note: in the cell culture, quiescent cells will eventually succumb to senescence, because even rapamycin does not suppress geroconversion completely).

To induce senescence, DNA-damaging agents p21 and p16 cause cell-cycle arrest. Freshly arrested cells do not have senescent phenotype. During several days, the arrested cells acquire a large, flat morphology, beta-Gal positivity and Senescence-Associated Secretory Phenotype (SASP) [2–4]. The acquisition of senescent phenotype in arrested cells is known as gerogenic conversion or geroconversion [4–8].

Geroconversion is a continuation of cellular growth, when the cell cycle is blocked [1]. It may also partially occur in proliferating cells and is overstimulated in cell culture conditions. Cellular mass (volume) growth is driven in part by growth-promoting pathways such as mTOR [6]. And this is how the anti-aging activity of rapamycin was predicted, before life-extension was shown in animals [9].

Despite the obvious (acquisition of senescent phenotype takes time via active process), the existence of geroconversion is largely ignored by scientific community. One of the reasons is that in cell culture, geroconversion occurs automatically, unless actively prevented by rapamycin, serum and nutrient withdrawal, contact inhibition, severe hypoxia and some other factors (discussed later). In 2011, it was pointed out that "In cell culture, cell cycle arrest typically leads to senescence, because the cell is overstimulated by serum, nutrients, oncogenes and so on. Therefore, cell cycle arrest is sufficient to cause senescence, especially in cancer cells. This is why arrest of cell cycle is confused with senescence" [10].

Growth stimulation drives senescence during cell cycle arrest

Nutrients, mitogens or growth factors (GF), hormones (e.g., insulin and testosterone), cytoplasmic oncoproteins, oxygen and other factors stimulate growth-promoting pathways such as mTOR and MAPK, which stimulate both cellular mass growth, cyclin D induction and cell cycle progression. In the

absence of growth stimulation (e.g., GF or serum withdrawal), MAPK and mTOR are deactivated. This slows down both cellular mass growth and cell cycle progression, and the cell becomes quiescent. Re-addition of growth factors allows quiescent cells to re-start proliferation [5, 6].

In proliferating cells, mTOR drives cellular mass growth, and this growth in cell size is balanced by cell division (Figure 1A). In quiescent cells, mTOR is deactivated, and the cell cycle is arrested. What would happen if the cell cycle were arrested, but mTOR is still active?

This condition can be caused by induction of CDK inhibitors (p21 and p16), which block the cell cycle, without affecting growth-promoting pathways such as mTOR and MAPK [6].

When the cell cycle is arrested by p21/p16, then mTOR drives growth in the absence of cell division, causing cellular hypertrophy (a large, flat cell morphology), lysosomal hyperfunction (beta-Gal-staining) and other hyperfunctions such as SASP (Figure 1B). It also increases tissue-specific hyperfunctions [6, 11].

Overactivated mTOR causes compensatory resistance to growth factors and insulin, via the pS6K1/IRS feedback loop [12, 13].

In cell culture, p21 and p16 cause cell-cycle arrest fast, but, at the moment of the arrest, the cells are not yet senescent. During the next 3–5 days, the arrested cells acquire the senescent phenotype [2–4]. This process is called geroconversion.

In a typical cell culture, cells are overstimulated by nutrients, serum and oxygen and grow in low cell density, making mTOR maximally active. For example, DMEM contains 5-fold higher than normal blood levels of glucose, higher than even in diabetic patients [13]. This is why it is sufficient to induce cell cycle arrest to induce senescence, unless mTOR-driven geroconversion is actively suppressed by serum withdrawal and contact inhibition, which deactivate mTOR [5, 14].

Pseudo-DNA-damage response in senescent cells

Molecular damage is not required for geroconversion (like it is not required for growth). For example, p21 and p16 (CDK inhibitors) and cause cell-cycle arrest without causing DNA damage: p21 and p16 directly bind to CDKs to arrest cell cycle. Then still active mTOR, MAPK and other growth-promoting pathways convert this arrest to senescence (geroconversion).

During geroconversion, overactivated kinases such as ATM phosphorylate H2AX, even in the absence of DNA damage [15]. As suggested by Rybak et al. [16] although DNA double-strand breaks always induce γH2AX, the reverse is not true: γH2AX is not an unequivocal marker of these breaks [16–18].

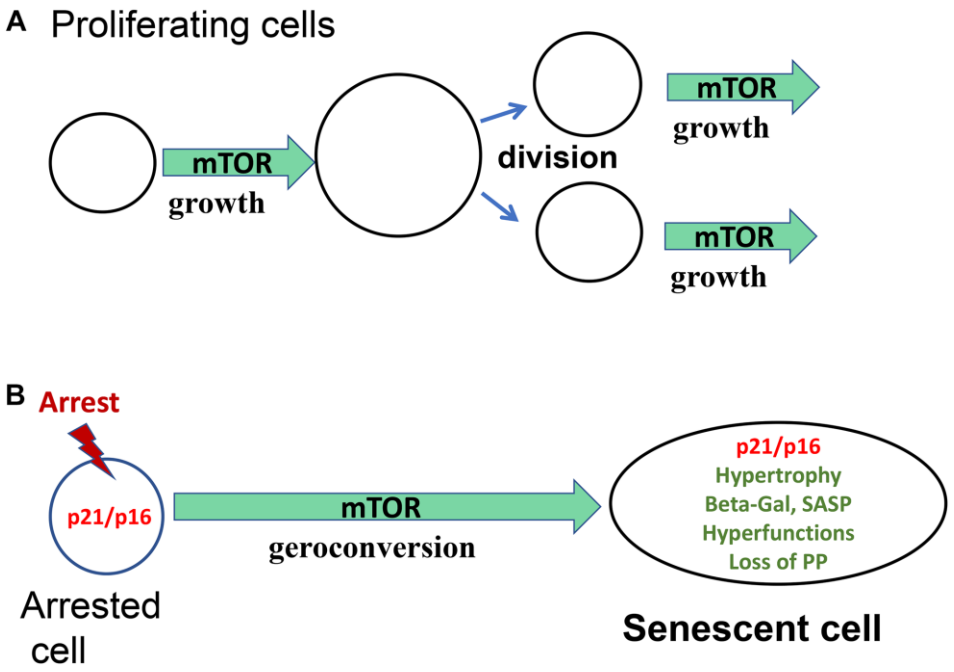

Figure 1. Geroconversion as a form of growth. (**A**) Proliferating cells. Cellular enlargement (growth) is followed by cell division. mTOR is shown as one of the drivers of growth. (**B**) Arrested cells. In the arrest cell (p21 and p16) cellular enlargement is followed by cell division. mTOR is shown as one of the drivers of geroconversion.

So, detection of γH2AX indicates that the cell may be senescent but does not indicate that it is necessarily caused by DNA damage. Unfortunately, it is not known to most scientists.

Acute DNA damage can cause arrest, but it's not yet senescence

Acute DNA damage by radiation and DNA-damaging drugs activates DNA damage response (DDR). While DNA damage response (DDR) causes cell-cycle arrest, it is growth-promoting pathways such as mTOR that convert this arrest to senescent phenotype. (Figure 1B).

[Note: Life-long, gradual accumulation of DNA damage (accumulation of mutations) does not lead to cell-cycle arrest, but, in contrast, contributes to unlimited proliferation, robustness and immortality in cancer cells].

Once again, acute DNA damage or DDR in proliferating cells can lead to cellular senescence, because proliferating is associated with high activity of growth-promoting pathways necessary for gero-conversion. When DDR causes arrest, these growth-promoting pathways drive geroconversion [19]. In serum-starved quiescent cells, mTOR is inactive and DNA damage cannot cause senescence. Growth stimulation with serum then drives geroconversion [19].

In the organism, acute DNA damage, or DDR, can lead to cell senescence by arresting proliferating cells. This is an age-independent cellular senescence that may occur at any age. This is also called non-adaptive cell senescence [20].

In contrast, age-dependent cellular senescence may be driven by life-long hyperfunction of growth-promoting pathways, especially in arrested (post-mitotic) cells.

Proliferative potential

At first, the freshly arrested cells retain proliferative potential (PP) and can re-start proliferation, if cell-cycle arrest is lifted. Following geroconversion, senescent cells cannot proliferate, even when cell-cycle arrest is lifted. The senescent cell may re-enter the cell cycle but cannot progress further or die in mitosis [2–4]. Loss of PP is a marker of the senescent phenotype, and rapamycin partially prevents loss of PP, as it partially prevents other markers of senescent phenotype such as cell hypertrophy, beta-Gal and SASP. Proliferative *potential* should not be confused with proliferation. For example, rapamycin inhibits proliferation but preserves PP. When p16 and p21 were induced for one day and then switched off, the cells resumed proliferation. If p16 was switched off after six days, cells remained phenotypically senescent and could not restart proliferation [2, 3]. Serum starvation [1, 19, 21] and mTOR inhibitors [1, 4, 22], prevent loss of PP during arrest, caused by switchable p21/p16 and the synthetic CDK4/6 inhibitor Palbociclib (PD0332991).

The irreversibility of cell cycle arrest should not be confused with Loss of PP. For example, Doxorubicin, a DNA-damaging drug, can render cell-cycle arrest irreversible, because doxorubicin cannot be easily washed out from the cell. If arrest is irreversible, it is impossible to know whether the cell retained (or not) the proliferative potential.

Cell hypertrophy (enlargement) as a marker of senescence

The large senescent morphology is the most noticeable feature of senescence in cell culture [23] and in the organism [24]. And it is not coincidental. Geroconversion is a continuation (quasi-program) of cellular growth [25]. At the beginning of gero-conversion in p21-arrested cells, cellular mass (protein per well) is increased exponentially, and then growth becomes linear in p21-arrested cells [26]. In agreement, Neurohr et al. showed that within 9 days after doxorubicin-induced arrest, cell size increased linearly 8-fold [21]. Similarly, linear increase in cell volume was observed during arrest caused by the CDKi Palbociclib, and this increase was completely prevented by serum starvation [21]. Rapamycin partially decreases hypertrophy during cell-cycle arrest caused by either p21 or synthetic CDK inhibitors [4, 26]. Pan-mTOR inhibitors more potently suppressed hypertrophy than rapamycin [27, 28].

Thus, hypertrophy is only partially rapamycin-sensitive [26, 27].

Excessive cell growth as a marker of geroconversion

Geroconversion can occur not only in arrested but also in proliferating cells, if growth stimulation is excessive. For example, stem cells are small, and their size is increased with aging [29], and excessive growth stimulation drives stem cell geroconversion [7, 8].

It was even suggested that an increase in cell size by itself can cause senescence [21, 29, 30]. According to the geroconversion concept, excessive activation of growth-promoting pathways (MAPK, mTOR, etc.,) drives both excessive growth and other hyperfunctions (SASP, lysosomal hyperfunction (beta-Gal), hyper-differentiation). Furthermore, overactivated MAPK and

mTOR pathways may induce p53/p21 and cycle arrest [31]. Following cell-cycle arrest, growth becomes even more excessive. Excessive growth and other manifestations of geroconversion are difficult to dissociate, because the manipulations that decrease growth (serum/nutrient starvation, rapamycin) also block MAPK/mTOR network that drives ALL manifestations together. This may suggest that cell size drives senescence rather than hyperfunctional growth-signaling drives senescence-associated hyprertrophy. As suggested, excessive mitogen/growth-stimulation may lead to hypermitogenic arrest [32] and then full-blown cell senescence [9, 31].

Geroconversion as terminal differentiation

Geroconversion can also be viewed as hypertrophic differentiation. For example, chondrocytes, responsible for bone growth in length, become hypertrophic and undergo senescence [33–36]. Like geroconvesrsion, terminal differentiation is an active process associated with decrease of proliferative potential [37], possible beta-Gal-positivity [38] as well as hypertrophy [39, 40] and increase of cellular functions, mainly tissue-specific functions. Geroconversion can be called gerogenic differentiation. This topic links the organismal/body growth program, hypertrophic differentiation, and geroconversion as a quasi-program of cellular growth and developmentally programmed cellular senescence [20].

Developmentally programmed cell senescence

While cell senescence is a quasi-programmed in aging, it may be programed in development [20, 41–45]. During mammalian embryonic development, senescent cells are cleared by macrophages, resulting in tissue remodeling [41].

Oncogene-induced senescence

Hyper-mitogenic stimulation may trigger cell-cycle arrest and simultaneously promote size growth [32, 46–49].

How should we define cellular senescence?

Cellular senescence is neither functional decline nor caused by chronic accumulation of molecular damage. In contrast, cellular senescence is characterized by universal hyperfunctions such as SASP plus tissue-specific hyperfunctions (senescent beta-cells as an example). Second, whether accumulation of molecular damages (mutations) lead to cancer, cancer cells tend to be immortal. A common definition of cellular senescence as permanent loss of proliferative potential does not recapitulate the most important features of the senescent phenotype, such as hypertrophy and hyperfunctions (e.g., SASP).

Cell senescence is a proliferation-like state in non-proliferating cells. Growth-promoting pathways, including mTOR and MEK/MAPK, drive both growth and geroconversion. When actual growth is completed, growth-promoting pathways drive cellular senescence (Figure 1). Thus, a program of growth becomes a quasi-program of senescence. (Quasi- means pseudo- or "resembling but not real"). Senescent cells resemble proliferating cells but do not proliferate [5]. As "Growth stimulation leads to cellular senescence when the cell cycle is blocked" the molecular hallmark of senescent cells is presented: high levels of p21/p16, phospho-S6 and cyclin D1 [50]. Cell senescence is associated with constitutive, proliferative-like activity of nutrient-sensing and growth-promoting pathways such as mTOR in non-proliferative (arrested) cells.

David Gems and Carina Kern suggested replacing the term cellular senescence with remodeling activation, and SASP with RASP [20]. The key word is activation. According to hyperfunction theory, cellular senescence (or remodeling activation) can be viewed as hyperactivation, hyperfunction, hypertrophy, hyper-differentiation.

In 2003, I proposed "that simultaneous stimulation of mitogen-activated pathways and downstream inhibition of cyclin-dependent kinases leads, ultimately, to cell senescence" [32]. In other words, senescence occurs when growth stimulation meets cell cycle arrest. In agreement, Rapamycin and other rapalogs (Everolimus and Ridaforolimus), pan-mTOR inhibitors [27, 28] and, to a lesser extent, MEK, PI3K, mdm-2 and S6K inhibitors all slow down geroconversion in mammalian cells [1, 22, 26, 51–55].

Numerous studies further confirmed that mTOR is involved in the senescence phenotype [56–69].

Regardless of whether cellular senescence contributes to organismal aging or not, the geroconversion cell culture model is a prototype of the hyperfunction theory of quasi-programmed aging. The geroconversion model introduces the notion of a quasi-program of growth and hyperfunction. Regardless of mechanistic link (or its absence) between cellular senescence and organismal aging, they are analogies. The same pathways that drive geroconversion are involved in organismal aging and age-related diseases. The same drugs that slow down geroconversion also extend lifespan, as tested in animals so far. Targets of gerostatics (e.g., mTOR, PI3K) are involved in aging of animals from worm to mammals. Therefore, gerostatics are anti-aging drugs.

The model of geroconversion is useful to discover anti-aging drugs.

Organismal aging as quasi-program of developmental growth

Like geroconversion is a continuation of cellular growth, the organismal aging is a continuation of developmental growth (see Figure 1 in reference [70]). Aging is not programmed, it is quasi-programmed. A quasi-program is a purposeless continuation of programs that were not turned off upon their completion. This has been discussed in detail [9, 50, 71–75].

Growth and aging are driven by overlapping signaling pathways. As suggested in 2007, "mTOR stands out because (a) it is a hub in the signaling network, (b) it is conserved from plants to animals (c) its inhibitors, rapamycin (Sirolimus) and everolimus, are clinically available drugs" [76]. To be clinically useful, the hyperfunction theory is mTOR-centric.

CONFLICTS OF INTEREST

The author declares no conflicts of interest related to this study.

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Research Perspective

Are menopause, aging and prostate cancer diseases?

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ABSTRACT

There is no doubt that prostate cancer is a disease. Then, according to hyperfunction theory, menopause is also a disease. Like all age-related diseases, it is a natural process, but is also purely harmful, aimless and unintended by nature. But exactly because these diseases (menopause, prostate enlargement, obesity, atherosclerosis, hypertension, diabetes, presbyopia and thousands of others) are partially quasi-programmed, they can be delayed by slowing aging. Is aging a disease? Aging is a quasi-programmed disease that is partially treatable by rapamycin. On the other hand, aging is an abstraction, a sum of all quasi-programmed diseases and processes. In analogy, the zoo consists of animals and does not exist without animals, but the zoo is not an animal.

Prostate cancer

Prostate cancer is an age-related disease. Every man would be diagnosed with prostate cancer, except that most men do not live long enough, dying from other age-related diseases. The frequency of prostate cancer detected by autopsy is 30-fold higher than mortality from prostate cancer so that "more men die with prostate cancer than because of it" [1]. Among men aged 70–79, a tumor is found by autopsy in 36% of Caucasians and 51% of African-Americans [1, 2]. The older the man, the higher frequency of autopsy-detected prostate cancer. The frequency of high-grade prostate cancer doubles every ten years [1].

Puberty is critical for susceptibility to prostate cancer later in life [3]. Older age at sexual maturation is linked to a decreased risk of prostate cancer later [4, 5]. Thus, prostate cancer is partially quasi-programmed (it will be discussed later) in puberty and would develop almost in everyone, if other causes of death did not exist.

Prostate enlargement or BPH

Benign prostatic hyperplasia (BPH) is the most common age-related disease in men. An enlarged prostate can block the urethra, leading to an inability to urinate and kidney damage and, if left untreated, to death. Benign prostatic hyperplasia can be detectable by the age of 30. Between 30 and 50 the prostate grows in size, with a doubling time of 4.5 years. Between 51 and 70 years old, the doubling time is around 10 years [6]. Thus, the prostate is enlarged in every aging man, and therefore it is a "normal" disease, occurring in everyone, often asymptomatic.

Early in puberty, the prostate doubles in size, and its secretory function is increased to produce prostate fluid. During puberty, the prostate reaches the required size and function, but it continues to grow without purpose, becoming eventually hypertrophic, hyperplasic and hyper functional. The disease is quasi-programmed, a continuation of the developmental growth and reproductive program that was not switched off upon its completion. Quasi-programs are purely harmful and unintended by nature, but they are a continuation (or a byproduct) of essential programs, so natural selection is powerless to eliminate them. (Note: The force of natural selection is negligible late in life, so selection is very weak against quasi-programs. Natural selection is maximally strong for growth and reproductive programs, and quasi-programs are by-products).

Cellular hyperfunctions drive prostate growth and, ultimately, benign prostate hyperplasia (BPH). Hyperproliferation of epithelial and stromal cells, leukocyte infiltration, inflammation and other hyper-functions lead to BPH. Hypersecretory phenotype (hyperfunction) also known as senescence-associated secretory phenotype (SASP) contributes to the development of BPH [7, 8]. Prostatic inflammation (hyperfunction) stimulates prostatic growth and progression of symptoms [9].

mTOR drives cellular size growth, hyper-inflammation, senescent and hyper-secretory phenotypes [10–17]. Therefore, rapamycin (Rapatar) prevents prostate hypertrophy and hyperplasia and reduces inflammation in rat models of BPH [18].

Atherosclerosis

Atherosclerosis is driven by hyperfunction of numerous cell types, acting locally and distantly. Thus, activation of endothelial cells, smooth muscle cells (SMC) and macrophages contributes to the formation of atherosclerotic plaque. Hypertrophy and hyperplasia of SMC and hypertrophic transformation of macrophages (foam cells) are hallmarks of atherosclerosis. Hyperfunctional blood platelets interact with the arterial wall, accelerating atherosclerosis and thrombosis. Adipocytes and hepatocytes hyperproduce atherogenic lipoproteins and cytokines. Hyperlipidemia, hyper-glycemia, hyperinsulinemia, and hypertension contribute to atherosclerosis. Atherosclerosis is associated with all other diseases of aging, especially hypertension, type II diabetes and obesity.

Atherosclerosis originates in childhood and progresses throughout life [19]. It occurs in everyone. It is a hallmark of aging and a "normal disease".

Clinical manifestations of atherosclerosis, cardio-vascular diseases, are the main causes of death in humans. The path from cellular hyperfunction that causes atherosclerosis, hypertension and thrombosis to myocardial infarction is shown in Figure 2 in ref. [20].

Rapamycin (sirolimus) and its analog (everolimus) attenuate atherosclerosis in mice [21] and rabbits [22]. According to a prospective randomized controlled trial, rapamycin (sirolimus) decreased carotid atherosclerosis in humans [23].

Menopause

Some age-related diseases are so program-like that they are considered to be the norm. Menopause happens in every woman (the average age at menopause is 51, according to the North American Menopause Society), and therefore it is not commonly viewed as a disease. But atherosclerosis and prostate enlargement (and all age-related diseases) also happen in everyone. One may argue that menopause is not as deadly as cancer. However, it is deadlier than osteoarthritis and Alzheimer's disease. Menopause promotes cardiovascular diseases (CVD) osteoporosis, obesity, type II diabetes and other diseases [24, 25]. Needless to say, loss of reproductive function is highly disadvantageous from an evolutionary point of view (we will discuss the grandmother hypothesis in the next section).

One may argue that menopause occurs too early in life compared with prostate cancer and Alzheimer's disease, for instance, to be called disease. However, premature menopause is considered a disease. By arbitrary definition, it occurs before the age of 40 years, or two standard deviations in years before the mean menopausal age of the study population [26].

Regulation of the menstrual cycle is very intricate and vulnerable, and hormonal hyperstimulation can disrupt the cycle. Even low doses of estradiol and progesterone are contraceptive. The famous contraceptive *"Plan B*", a progestin, disrupts the menstrual cycle and prevents pregnancy by a single dose. (Note: in comparison, the regulation of a male reproduction function is much simpler, explaining why men do not lose it as much as women do with age).

Not surprisingly, hyperfunction of the hypothalamic-pituitary-ovarian axis eventually dysregulates the system and causes ovarian failure (see Figure 3 in ref. [27]). The menstrual cycle is tightly-regulated by numerous hormones, cell types and organs. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), produced by the pituitary gland, stimulate ovulation and the production of estrogens and progesterone by the ovary. For example, FSH stimulates follicles, production of ova and estrogens. Before puberty, the levels of both FSH and estrogens are low. To start the menstrual cycle, production of FSH is increased, stimulating the ovaries and estrogen production. Activation of follicles from the dormant pool serves as the source of fertilizable ova. With age, levels of FSH continuously increase, hyper-stimulating the ovaries [28], causing more follicles to be recruited simultaneously (see Figures 3–4 in ref. [27]).

Hyper-stimulation of follicle recruitment leads to follicular depletion and ovarian failure.

Thus, stimulation of FSH is initiates puberty, and its continuous hyperfunction accelerates menopause. Since

the quasi-program of menopause is a continuation of puberty, mTOR, a central regulator of the onset of puberty, accelerates the onset of both puberty [29] and menopause in animals [30–32].

By activating mTOR, obesity accelerates ovarian follicle development and follicle loss in rats [33]. By inhibiting mTOR, calorie restriction delays puberty and extends reproductive lifespan in rodents [34, 35]. Overactivated mTOR activates the entire primordial follicle pool, and, subsequently, all primordial follicles become depleted in early adulthood, causing premature ovarian failure (POF) in mice [30–32].

Rapamycin preserves the follicle pool reserve and prolongs the ovarian lifespan in female rats [36] and mice [34, 37, 38]. mTOR is overactivated in the peripheral blood cells of women with premature ovarian insufficiency [39].

Critique of the grandmother (great-great-grandmother hypothesis) hypothesis

As we discussed in the previous section, menopause is a byproduct of the reproductive program that initiates puberty. The same process that turns the menstrual cycle on in puberty becomes hyperfunctional, damaging the reproductive system and (unintentionally) switching it off. As are all age-related diseases, menopause is purely harmful and provides no benefits.

Some prominent gerontologists, however, hypothesize that menopause is adaptive and intended by natural selection to prevent older women from reproduction and thus redirect their efforts to help daughters to raise grandchildren [40]. Unless a daughter is a modern working mom, rather than a prehistorical female, this hypothesis makes no sense.

First, the natural age of grandmothers is 28, whereas menopause occurs at 51. Then the hypothesis should be renamed as great-great-grandmother hypothesis. The genetic similarities of a woman with great-grandchildren are less than with nephews and nieces.

Second, the best possible help would be breast feeding. However, post-menopausal women cannot get pregnant and therefore cannot lactate. If nature selects for caring for grandchildren, elderly women should produce milk or become pregnant to produce it.

Third, only maternal grandmothers increase grandchildren's survival, whereas paternal mothers decrease it. The presence of paternal grandmothers (mothers-in-law) is detrimental to grandchild survival or well-being [41–44]. In most societies, a wife would likely live with a parental grandmother.

Fourth, only a minority of pre-historical females lived long enough to become great-great-grandmothers. Even 300 years ago in England, only 25% of people survived to the age of 26. How many would survive until menopause? It is commonly argued that hunter-gatherers lived as long as modern people. Although the maximal lifespan can be the same, due to accidental causes of death, the median lifespan of any species in the wild is much shorter than in a protected environment (laboratory animals and modern humans). It does not matter how long some survivors live after menopause, what is important is that most died before it.

If only one of these arguments is correct, the grandmother hypothesis has little value. The list can go on [45]. Some observations cannot be reconciled with the grandmother hypothesis. Older women have an increased chance of giving birth to twins and triples [46]. Furthermore, the outcome of such pregnancies in older mothers are better than in younger mothers [47, 48]. Why is declining fertility is associated with the increasing twinning rate? It is in agreement with hyperfunction theory. Hyperstimulation with FSH leads to multiple ovulation and a higher incidence of twins and triplets with age [46].

If menopause were adaptive, it would be conditional in the presence of grandchildren, but not in their absence. Conditional control is easy to achieve, even a single spike of sex steroids is sufficient to do the trick (this is exactly what a single pill of birth control pill like "*plan B*", a progestin, does).

If nature equipped women with menopause to take care of grandchildren, why then does it impair their vision? Presbyopia, or age-related farsightedness, develops in humans by the age of female menopause. Is presbyopia an adaptive program as well? Like menopause, presbyopia is quasi-programmed; the ability to focus on near objects declines from childhood to adulthood, and its continuation culminates in presbyopia. By the time of menopause, presbyopia occurs in everyone. It is purely harmful and is treated by glasses (as a disease should be treated).

Male fertility gradually decreases with aging. Men do not have menopause, because men do not have a vulnerable menstrual cycle to start with (similarly, women do not have BPH).

Finally, consider a parody "grandfather hypothesis" that prostate hyperplasia develops in order to make men urinate in the middle of the night and thus protect

grandchildren from lions. I hope it is not taken seriously, just as the bizarre grandmother hypothesis should not be either.

Age-related diseases happen, potentially, in everyone

It is difficult to define a disease, especially an age-related disease [49, 50]. For example, osteoporosis and obesity were not officially recognized as diseases until 1994 and 2013, retrospectively. Whether we define age-related alterations as a disease depends on political, cultural, financial, medical and social reasons.

The main objection to considering age-related diseases such as menopause and presbyopia as diseases is that they happen to everyone. However, disease does not need to be rare to be a disease. For example, everyone may be sick with influenza during their lifetime, but it does not make it any less a disease. Furthermore, no definition of disease includes the requirement that it should not affect everyone.

All age-related diseases happen either in everyone (for example, prostate enlargement in men and atherosclerosis) or would happen in everyone (Alzheimer's disease and cancer), if one does not die from a competing disease. For example, a human may suddenly die from myocardial fibrillation due to coronary atherosclerosis at the age of 60, but if one were saved and properly treated, they may be diagnosed with Alzheimer's disease, and die from cancer at the age of 80.

Age-related diseases are quasi-programmed

Age-related diseases occur to everyone, and, therefore, no one is immortal.

They happen in everyone because they are quasi-programmed in development, a continuation of growth and reproductive programs. External (environmental) factors and genetic predispositions also play a role, making certain age-related diseases manifest at different times or even not manifest at all in a lifetime.

For example, hypertension is a continuation of developmentally increased blood pressure from the newborn (blood pressure 64/41 mmHg) to the adult. Hypertension can also be viewed as a quasi-program of growth upon its completion. In fact, accelerated postnatal growth leads to higher blood pressure later in life [51]. Yet, external factors such as alcohol and smoking may accelerate the development of hypertension [52].

Cancers are the least quasi-programmed among all aging-related diseases because of the critical role of (a) external factors (e.g., smoking) that cause mutations and (b) inherited genetic susceptibility. In prostate enlargement, in comparison, environmental and genetic factors play a lesser role, and the prostate becomes hyperplastic and hypertrophic in everyone.

External factors and genetic variations may accelerate and aggravate quasi-programmed diseases. In humans, the role of external factors and genetic variability may obscure quasi-programmed nature of diseases. In genetically identical *C. elegans* at identical conditions, age-related diseases are clearly quasi-programmed [53–57].

Age-related diseases are hyper-functional

Age-related diseases are driven by hyperfunctions on different levels: from signal-transduction pathways, to cells and tissues, to systems and organs. These hyperfunctions eventually damage tissues and organs, causing secondary loss of function. Hyperfunction is a function that was not turned off upon its completion [58]. (Note: Hyperfunction is not necessarily an absolute increase in function but may even be a decrease if it is still higher than optimal for longevity [59]). For example, mTOR drives cellular growth, but when the cell cycle is blocked, and mTOR is not turned down, then it drives the senescence phenotype associated with hyperfunctions such as SASP and proinflammation [60]. Cellular hyperfunctions are tissue-specific: osteoclasts resorb the bone, thus leading to osteoporosis; fibroblasts and immune cells cause proinflammation, associated with most age-related diseases; constriction of arterial SMC causes coronary artery spasm; blood platelets form clots. On systemic levels, hyperfunctions include hyperinsulinemia, hypertension, hyperglycemia, hyperlipidemia and others.

Cellular hyperfunction inevitably leads to age-related diseases and then to organ failure and secondary functional decline [61]. For example, hyperfunctional cells promote atherosclerosis, hypertension, arterial spasm, thrombosis, culminating in myocardial infarction, which, in turn, causes loss of function [20, 62].

mTORC1-dependent beta-cell hyperfunction culminates in beta-cell exhaustion (diabetes) [63–65]. Ovarian overactivation leads to follicular exhaustion and menopause [27, 30–32, 34, 36–38].

Hyperfunctional phases of pre-diseases are often asymptomatic, while their consequences – loss of function – are always symptomatic. Even classic diseases, such as hypertension, may have mild symptoms until damage occurs (stroke, myocardial infraction, heart or renal failure).

Functional decline in athletic performance [66] can precede official age-related diseases. Such an early-life decline is not caused by recognized age-related diseases. Early-life hyperfunctions are unrecognized. They are asymptomatic, until causing mild functional decline in athletic performance in everyone. Secondary loss of function can be observed early in life due to unnamed hyperfunctions.

Is aging a disease?

According to conventional views, aging is a risk factor for developing disease. It is believed that aging can be healthy (without diseases) and that humans can die either from aging or from diseases. It was claimed, "aging should be strongly considered not to be a disease and as such should not be treated" [67].

According to hyperfunction theory, aging is not a risk factor, aging is the sum of all age-related diseases. There is no aging without these diseases. So-called "healthy" aging is slow aging observed in centenarians, who develop diseases later in life. But no centenarian dies from old age, all die from age-related diseases [68–70].

Like quasi-programmed diseases, aging is a natural continuation of developmental programs that were not switched off upon their completion. Aging is the sum of all quasi-programmed diseases. As David Gems put it, aging versus disease is a false dichotomy [71].

Aging is natural. Natural process is a disease, if it leads to death or functional decline [50, 71, 72]. A natural process, such as atherosclerosis, is a disease, whereas an unnatural process, such as a car accident, is not a disease. All age-related diseases are natural, and therefore we are mortal.

Aging is driven, in part, by hyperfunctional signaling pathways, such as the nutrient-sensing and growth-promoting mTOR pathway. Inhibition of the mTOR pathway by genetic, pharmacological and other means extends lifespan in numerous species and decelerates development of age-related diseases [73–75].

As suggested in 2006, "Once development is completed, a program for development is not switched off, thus becoming a quasi-program for aging. This hyper-functional quasi-program is manifested as diseases of aging, leading to organ damage and secondary decline." [58]. (Note: Secondary decline is the most visible manifestations of advanced aging).

So, is aging a disease?

On one hand, aging is a progressing disease with 100% mortality rate. It can be treated (as a disease) with rapamycin, for instance. Diseases can be prevented by slowing down aging [58]. Potential anti-aging drugs could be tested by slowing diseases. Disease or not, aging is as treatable as a disease [76].

However, aging is not a specific disease, but the sum of all age-related diseases, including both life-limiting (e.g., diabetes, cancer and CVD) and non-life-limiting (e.g., osteoarthrosis and gray hair). It is a form of complex disease syndrome [71]. Using an analogy, is the American people a human? Is it a man or a woman? The people consist of all men and women; each of them is a human. But the people are not a human, neither a man nor a woman. Similarly, aging consists of all quasi-programmed alterations, age-related pre-diseases and diseases, early unrecognizable diseases that manifested as early functional decline, cosmetic conditions, and others. The aging process is the common mechanism of all diseases.

Given that aging is a sum of all age-related diseases, it can be called aging syndrome, or aging.

Aging seems mysterious, if one is studying so-called "healthy" or "successful" aging. One can subtract disease after disease until nothing is left. No aging. It is like subtracting every man and woman from the American people until nothing is left. Aging looks quasi-programmed, because it consists of quasi-programmed diseases that are driven by hyperfunctions that culminate into organ/system failure (and secondary loss of function). Aging behaves as the sum of all diseases. And this sum can be prevented by inhibiting the common mechanism that we call aging. Aging is driven by the same processes as diseases: from over stimulated signal-transduction pathways to cellular hyperfunction, systemic hyperfunction leading to organ failure (secondary functional decline). To understand aging, we should depict the pathogenesis of overlapping age-related diseases driven by hyperfunctional signals and cells towards organ damage. Aging is a collection of processes that drive quasi-programmed diseases. Preventive medicine that targets early hyperfunctional stages of a group of overlapping diseases is an anti-aging medicine. Aging can be understood through the development of all quasi-programmed diseases. Treatments that prevent age-related diseases partially prevent aging and *vice versa* [71, 77].

CONFLICTS OF INTEREST

The author declares no conflicts of interest related to this study.

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Review

Hallmarks of cancer and hallmarks of aging

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ABSTRACT

A thought-provoking article by Gems and de Magalhães suggests that canonic hallmarks of aging are superficial imitations of hallmarks of cancer. I took their work a step further and proposed hallmarks of aging based on a hierarchical principle and the hyperfunction theory.
To do this, I first reexamine the hallmarks of cancer proposed by Hanahan and Weinberg in 2000. Although six hallmarks of cancer are genuine, they are not hierarchically arranged, i.e., molecular, intra-cellular, cellular, tissue, organismal and extra-organismal. (For example, invasion and angiogenesis are manifestations of molecular alterations on the tissue level; metastasis on the organismal level, whereas cell immortality is observed outside the host).
The same hierarchical approach is applicable to aging. Unlike cancer, however, aging is not a molecular disease. The lowest level of its origin is normal intracellular signaling pathways such as mTOR that drive developmental growth and, later in life, become hyperfunctional, causing age-related diseases, whose sum is aging. The key hallmark of organismal aging, from worms to humans, are age-related diseases. In addition, hallmarks of aging can be arranged as a timeline, wherein initial hyperfunction is followed by dysfunction, organ damage and functional decline.

Hallmarks of cancer: comparing apples and oranges

As depicted by Hanahan and Weinberg in 2000 [1], the circle schema of six hallmarks of cancer somewhat compares apples and oranges https://els-jbs-prod-cdn.jbs.elsevierhealth.com/cms/attachment/428dbc2e-657c-429d-98f4-9910c7df1678/gr1_lrg.jpg.

The hallmarks themselves are exact, but they are not equal. For example, limitless replicative potential (cell immortality) cannot be directly compared to sustained angiogenesis. Cell immortality is revealed outside the host (extra-organismal level), for example, in cell culture where clonal cell lines can proliferate indefinitely without interaction with normal tissues. In contrast, sustained angiogenesis requires interaction of cancer cells with normal cells of several tissues. Angiogenesis can be only understood on the tissue level.

Second, cancer cells undergo Darwinian-type selection [2] for resistance to anti-growth signals, resistance to apoptosis and self-sufficiency in mitogenic signals. This trio represents three out of six hallmarks of cancer [1]. They can be combined in one super-hallmark: resistance to growth-limiting conditions [3]. (Note: The definition of oncogenic resistance to growth-limiting conditions was discussed previously [4]). Not only resistance to apoptosis and anti-growth signals but also self-sufficiency in mitogenic signals render cells resistant to growth-limiting conditions. Examples of growth-limiting conditions include lack of external mitogenic signals, cytostatic cytokines such as TGF-beta, cytotoxic carcinogens such as tobacco smoke, anti-cancer drugs, contact inhibition, glucose deprivation, cellular senescence, hypoxia, absence of nutrients and

growth factors [5, 6]. For example, glucose deprivation selects for oncogenic Ras [6].

Whereas normal cells do not proliferate in growth-limiting conditions, cancer cells do. Resistance to growth-limiting conditions provides an immediate selective advantage. But what immediate advantages can be provided by cellular immortality? The cell cannot tell the future, that it will live in cell culture one day. Cellular immortality is selected indirectly as derived hallmarks [3], because the same mutations that provide resistance to growth-limiting conditions also make cells immortal, angiogenic, invasive and metastatic [1, 7, 8]. Cellular immortality, angiogenesis, invasion and metastasis are derived hallmarks.

Third, molecular alterations (e.g., DNA mutations) are absent in the six-hallmark circle by Hanahan and Weinberg [1]. As discussed by Gems and de Magalhães, the hallmarks do not include mutations (or genetic instability) because this hallmark is implicitly taken for granted [9]. In fact, Hanahan and Weinberg called it an enabling hallmark in their revised paper published in 2011 [7].

In 2005, I explicitly included the molecular hallmark (mutations) and suggested the hierarchical principle to arrange these hallmarks from molecular to organismal levels [5].

Hierarchical model of hallmarks of cancer: arranging the oranges

Here I present the hallmarks of cancer, depicted as a circle by Hanahan and Weinberg [1], not as the circle but hierarchically, from molecular levels to the organism (Figure 1).

Molecular level: Somatically inheritable molecular alterations.

Genome instability is an enabling hallmark of cancer because it enables the acquisition of molecular alterations, such as DNA mutations, aneuploidy and epigenetic alterations [7]. Vogelstein et al. suggested that a typical human tumor contains relatively few driver gene mutations that each confers a growth advantage of 0.4% and numerous passenger gene mutations that confer no selective advantage [8, 10].

Intracellular signaling pathways: Oncogenic translation of ambivalent signaling

Signal-transduction pathways are ambivalent, causing opposite outcomes depending on cellular context. Oncogenic mutations re-wire signal transduction pathways. For example, MAPK pathways can simultaneously induce cyclin D1 and CDK inhibitors, leading either to cellular proliferation or senescence

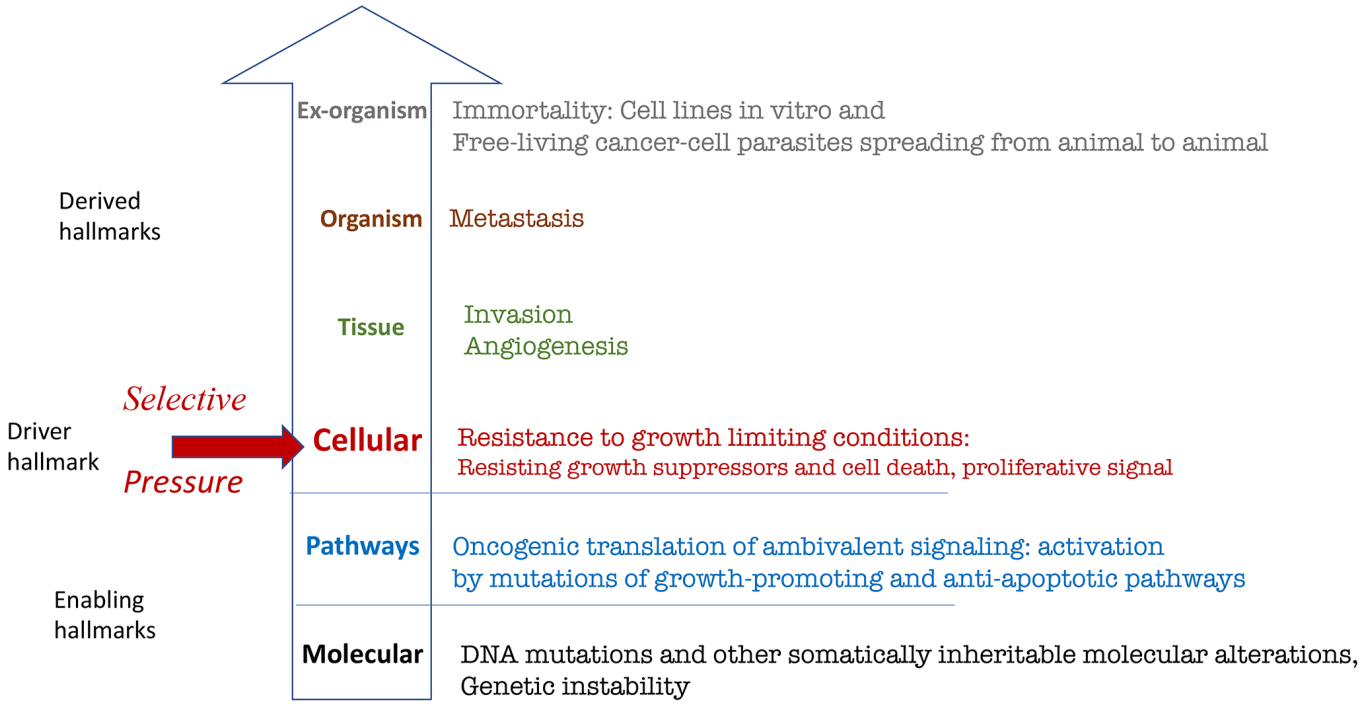

Figure 1. Hierarchical representation (from molecular to organismal levels) of the original hallmarks of cancer based on Hanahan and Weinberg. See text for explanation.

[11]. Inactivation of CDK inhibitors such as p16 may translate this ambivalent signaling into proliferation [3, 12]. TGF-beta inhibits normal cell proliferation, but in cancer it can induce proliferation and invasion [7, 13].

Growth-promoting and mitogen/nutrient-sensing signaling pathways are constantly activated by mutations to promote growth and proliferation as well as self-sufficiency in mitogen signaling. This, in turn, is manifested as three hallmarks of cancer on the next hierarchical level: cellular. This trio can be combined as one super-hallmark of resistance to growth-limiting conditions.

Cellular level: Resistance to growth-limiting conditions

Oncogenic mutations make cancer cells resistant to growth-limiting conditions (a definition of oncogenic-type of resistance was discussed previously [4]). This is the driver hallmark of cancer because it provides a selective advantage to cancer cells. Cells, capable of proliferation, are unicellular organisms in a Darwinian sense [2, 14, 15]. Selection can be "natural" (during carcinogenesis) and "artificial" (during cancer therapy) [14, 16]. For example, selection for therapy resistance increases oncogenic properties of cancer cells because many mutations in oncogenes and tumor suppressors that render cells drug-resistant also make them more oncogenic [5, 17–19]. Simultaneously, the same combination of mutations enables metastasis and other higher-level hallmarks. There is no direct selection for metastatic potential, angiogenesis and immortality. They are derived hallmarks.

Tissue level: Invasion and angiogenesis

Cancer cells produce cytokines and enzymes, which enable the cells to invade and to attract normal cells of different tissues in order to sustain angiogenesis [7].

Organismal level: Metastasis

Metastasis is the deadliest hallmark of cancer. Yet, there is no direct selection for metastatic potential. Direct selection for metastatic potential could take place only if metastases produced new metastases; in other words, if metastases reproduce. Simply, selection for cells resistant to growth-limiting conditions (survival and proliferation) brings about mutations that confer not only resistance, but also metastatic potential. There are no specific "metastasis" genes [8, 20]. They are the same oncogenes and tumor suppressors that act on cellular levels for the "trio" hallmark. Let us consider an analogy. If we select people for their ability to run faster, these selected people will also jump higher than average, although selection was not for jumping ability. The fastest runners are the farthest jumpers.

Extra-organismal level: Cellular immortality

Some cancer cell lines live for more than half of a century in cell culture and for thousands of years in the wild. Originating in one animal, viable cancer cells are directly transmitted into unrelated hosts in a process similar to metastasis [21, 22]. Transmissible cancers have been observed in domestic dogs, the Tasmanian devil, hamsters and six bivalve species such as the soft-shell clam [23]. Canine transmissible venereal tumors (transmitted during sexual intercourse) may have originated thousands of years ago from the cells of a wolf or East Asian breed of dog [21–25]. The Tasmanian devil facial tumor disease [24] spreads through the Tasmanian devil population by transfer of cancer cells through biting [22]. [26]. Derived from a single original clam, leukemia-like cancer spreads among marine bivalves through sea water, leading to massive population loss [23, 27].

Six levels rather than six hallmarks

The number of hallmarks of cancer is arbitrary. Some can be combined, and others can be added. Numerous authors have re-visited the hallmarks of cancer, adding hallmarks or suggesting a new set of hallmarks [28–37].

Some hallmarks of cancer may be pseudo-hallmarks. For example, visiting an oncologist is a "hallmark" of cancer. We can be 99% sure that if someone has 20 appointments in an oncology clinic, then this person has cancer. However, it would be ridiculous to include this pseudo-hallmark in Figure 1. And the hierarchical principle makes this impossible, because there is no level (among the six levels) to include it.

Hallmarks of aging

To start with, let us depict the hallmarks of aging suggested by López-Otín et al. [38] based on the hierarchical principle. This representation renders hallmarks tangible but reveals three shortcomings (Figure 2).

First is the lack of hallmarks on the organismal level. Yet, the main hallmark of organismal aging is age-related diseases in all species from *C. elegans* [39–42] to humans [39, 43]. Aging is the sum of all age-related diseases, which cause death "from aging".

Second, the relationship between hallmarks on different levels are unclear.

Third, the inclusion of genetic instability as a hallmark is based on the theory that aging is caused by accumulation of molecular damage. The molecular damage theory was refuted by key experiments, as discussed in detail [44–51].

Yes, damage accumulates and is harmful and potentially lethal [52–55] but it is not life-limiting because aging caused by hyper-functional signaling terminates life first. The reason why mTOR-driven aging is life-limiting has been discussed [49, 56, 57].

It was also suggested that the levels of DNA repair needed to avoid cancer at a young age greatly exceeds the levels that are needed to prevent damage-induced aging during a normal lifetime [58]. As previously discussed, the role of molecular damage in cancer supports the role of mTOR-driven hyperfunction in aging [59].

Let us depict hallmarks of aging, according to the hyperfunction theory of aging (Figure 3).

Hallmarks of aging and hyperfunction theory

The hyperfunction theory of aging was extensively reviewed previously [44, 45, 49, 56, 57, 60–66], and responses [60, 67] to its critics [68, 69] were also provided.

According to hyperfunction theory, aging is a continuation of developmental and reproductive programs that were not turned off upon their completion. Continuously active signaling pathways that initially drive developmental growth, drive aging later in life. Signaling pathways establish feedback loops, involving also gene expression and epigenetic modifications. These pathways become hyperfunctional, meaning that their activity is higher than optimal for longevity.

How does normal function become a deadly hyperfunction? Consider an analogy. When you pump air into an inflatable balloon, it grows in size. But when it reaches its intended size and you continue to pump air at the same rate, it will not grow further but instead will burst. This event can be compared with a stroke due to hypertension, resulting in brain damage. The brain is not damaged by life-long accumulation of molecular damage, but by hyperfunction, such as hypertension and hypercoagulation, thrombosis.

Hyper-function does not necessarily mean increased function. Even unchanged or slightly decreased activity

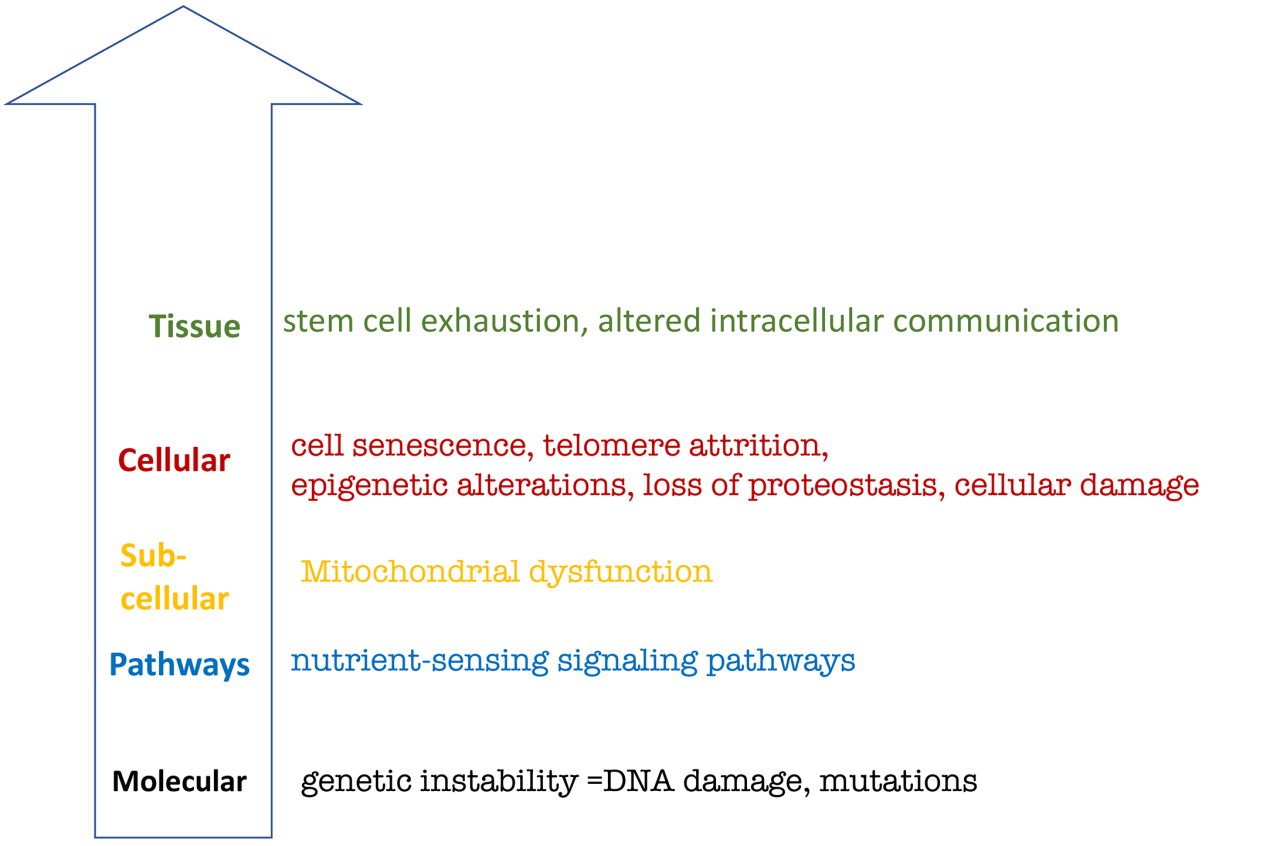

Figure 2. Hierarchical representation of the hallmarks of aging based on López-Otín et al. See text for explanation.

of growth-promoting pathways, such as mTOR, can be hyperfunctional when developmental growth is completed. As an analogy, 55 mph on the highway is not speeding, but even 40 mph on the driveway is too fast.

Hyperfunction causes organ damage and functional decline. The accumulation of molecular damage is associated with decline, but it is hyperfunction that causes decline during a normal lifetime.

Unlike cancer, aging is not a molecular disease. Development is not driven by accumulation of molecular damage or mutations in signaling pathways, and aging is not either. Nutrient-sensing pathways (e.g., mTOR) are not altered by random mutations.

The lowest level of hallmarks of aging is a continuous activation of normal signal transduction pathways. Deactivation of these pathways by knockout of a single gene extends lifespan in animals [70–73]. Rapamycin, a drug that inhibits normal mTOR signaling, extends lifespan [74–77].

Hyperfunctional signaling directly drives age-related diseases. There are no longevity pathways/mechanisms inhibitable by pro-aging pathways such as mTOR. Pro-aging pathways do not drive aging by inhibiting longevity mechanisms. Why would nature create something that inhibits longevity mechanisms? Pro-aging pathways such as mTOR directly drive age-related diseases because they are a continuation of development.

The key to understanding aging: life-limiting vs. non-life-limiting hallmarks

Among numerous harmful processes, only one can be life-limiting in a particular individual. If an animal dies from one cause, it cannot die from another cause even a day later. If quasi-programmed (e.g., mTOR-driven) aging is life-limiting, then accumulation of random damages cannot kill the organism.

López-Otín et al. [38] suggested three criteria for hallmarks of aging but two of them are criteria for both life-limiting and non-life-limiting processes: (1) hallmarks are observed during normal aging and (2) its experimental aggravation should decrease lifespan. However, experimental aggravation can make any process life-limiting. Telomere shortening becomes life-limiting in mice lacking telomerase, but their symptoms are drastically different from normal age-related

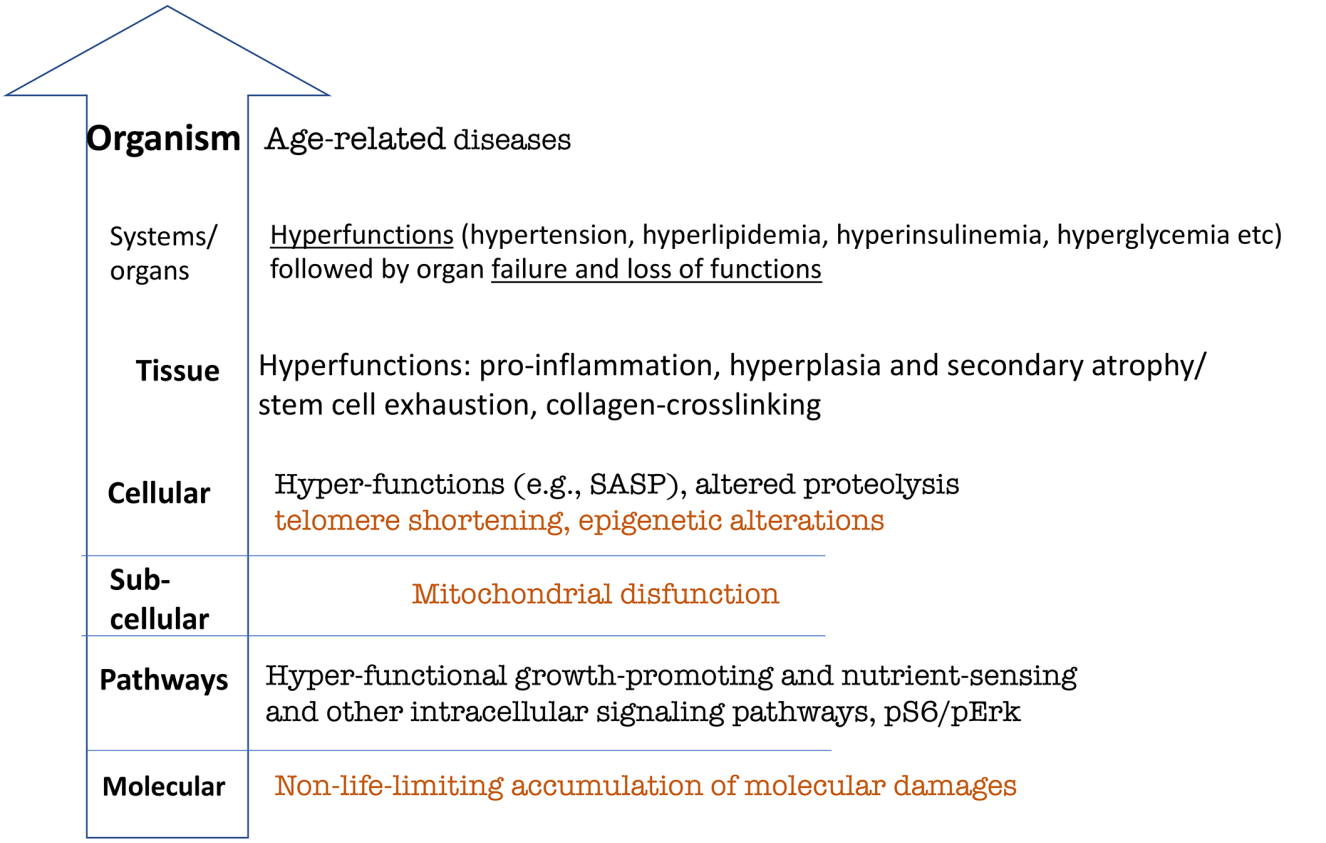

Figure 3. Hierarchical hallmarks of aging based on hyperfunction theory, applicable to humans. Non-life-limiting hallmarks are shown in brown color. See text for explanation.

diseases [78]. Although telomere shortening is associated with cardiovascular disease (CVD) in humans, patients with dyskeratosis congenita (DKC), a condition caused by short telomeres, do not die from CVD but from bone marrow failure (which is not a typical age-related disease) [79]. Hyperfunction theory explains how hyper-functional signaling leads to CVD in humans [80]. But telomere shortening cannot explain it.

Anything can shorten lifespan including starvation and the atomic bomb but they are not causes of aging. Only the third criterion matters: (3) its experimental amelioration should slow down aging and increase healthy lifespan. Not surprisingly, "the last criterion is the most difficult to achieve and not all of the hallmarks are fully supported yet by interventions," as noted by López-Otín et al. [38]. In other words, they are not hallmarks of normal aging.

(Note: Even the third criterion is not sufficient to define a life-limiting hallmark.

Besides interventions may have off-target effects. For example, NAC, an antioxidant, is also a mTOR inhibitor [81]).

In conclusion, numerous deadly processes develop in parallel but only a few (or one) are life-limiting.

Therefore, non-limiting hallmarks are not included in the version of life-limiting hallmarks of aging (Figure 4). This final re-presentation is generic and can be applied to any species, from *C. elegans* to humans.

Aging as a selective force for cancer

Common cancers are age-related diseases. This cannot be explained by simple accumulation of mutations with age. For example, melanoma and lung cancer in smokers have atypically high mutation burden [8] but still develop at old age. Centenarians, who age slower, are protected from cancer. Rapamycin and calorie restriction slow aging in mice and prevent cancer.

As discussed, the selective force driving carcinogenesis is growth-limiting conditions, also named micro-environmental constraints in aging [16]. For example, the aging hematopoietic system selects for robust hematopoietic cells and such a preleukemic clone can originate leukemic clone [82]. Specifically, chronic inflammatory microenvironments in old age may select for cells harboring oncogenic mutations [83].

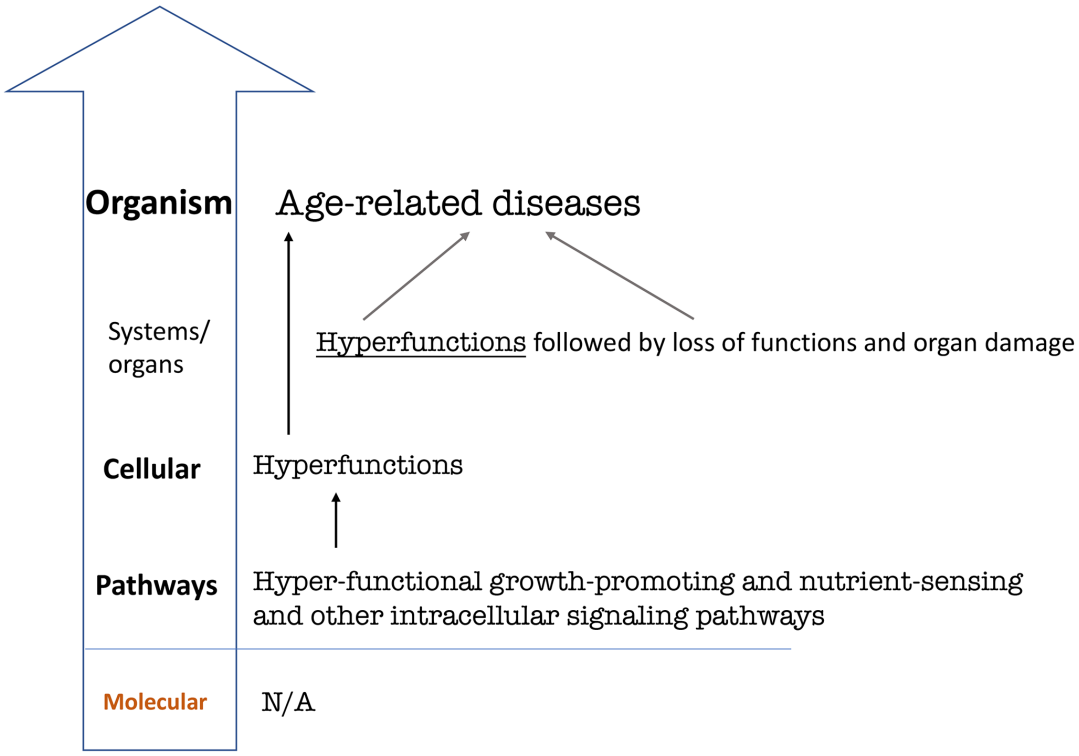

Figure 4. Hierarchical hallmarks of aging based on hyperfunction theory, universal. Hyperfunction of intracellular signaling pathways leads to cellular and systemic hyperfunctions, which in turn lead to age-related diseases on the organismal level [56]. Specific hyperfunctions and diseases may be different in different species and therefore are not shown. For example, human systemic hyperfunctions (e.g., hypertension, hyperlipidemia, hyperglycemia) and diseases (e.g., cardio-vascular diseases) differ from diseases in C elegans [40, 41].

Chronic inflammation is a hyper-function and is in part mTOR-dependent [84–88]. An aging microenvironment puts stem cells on the path of hyper-activation [89] and geroconversion [90–92], leading to their exhaustion [89–92].

Mutations are necessary (with a few exceptions) but not sufficient for inducing cancer. The second requirement is selective force, favoring these mutations. Aging is a leading selective force.

One of the potential mechanisms of growth-limiting conditions that drive cancer progression is mTOR-dependent cellular senescence.

Common hallmarks of cancer, aging and cell senescence

Cellular senescence is a two-step process: cell cycle arrest followed by geroconversion [93]. Like organismal aging, geroconversion is a continuation of growth driven in part by hyperfunctional mTOR. When the cell cycle is blocked by p21/p16, but growth-promoting pathways such as mTOR and MAPK are active, the cells become hypertrophic (large cell morphology) and hyperfunctional: beta-Gal staining (lysosomal hyperfunction) and SASP. A hallmark of cellular senescence is active mTOR pathway in non-proliferating cells. Rapamycin suppresses geroconversion to senescence [93–97]. Figuratively, organismal aging is a quasi-growth after developmental growth is completed.

In cancer, the PI3K/mTOR pathway is almost universally activated by mutations [98–100]. Figuratively, cancer cells are proliferating senescent cells. In organismal aging, cancer and cellular senescence, the same key signaling pathways, such as mTOR, are involved. This is why the same drugs, such as rapamycin, can suppress all of them.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest related to this study.

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Commentary

As predicted by hyperfunction theory, rapamycin treatment during development extends lifespan

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As suggested in 2006, by slowing down the mTOR-driven developmental program, rapamycin must slow down quasi-programmed aging [1]. In other words, targeting development with rapamycin must lead to a longer lifespan. An elegant study by Gladyshev and co-workers has confirmed this prediction [2].

According to hyper-function theory, aging is a quasi-program, a purposeless continuation of the growth program that has not been switched off upon its completion [1]. Aging is not programmed, only development is. Unlike a program, a quasi-program has no aim, although, like a program, it can be modulated [1, 3, 4]. For example, excessive nutrients and calorie restriction can accelerate and decelerate aging, respectively.

Aging is driven by hyperfunctional signal-transduction pathways which, via cellular and systemic hyper-functions, cause age-related diseases, whose sum is called aging [1]. Hyperfunctions cause organ damage (not molecular damage), resulting in loss of functions and secondary functional decline [1, 5].

The nutrient-sensing mTOR pathway promotes cellular growth [6-8] and cellular senescence, which is a continuation of cellular growth, when the cell cycle is blocked [9, 10]. According to hyperfunction theory, age-related diseases are quasi-programmed [1,11] with clear-cut examples in simple organisms such as *C. elegans* [11-15]. Hyperfunction theory was extensively reviewed [1, 5, 11, 16 -20]. Critical comments [21-23] have been addressed [5, 24]. Importantly, hyperfunction theory is mTOR-centric, describing mTOR-driven aging and its diseases [1]. By slowing down aging, rapamycin delays age-related diseases [1, 25, 26].

To maximally extend health and lifespan in humans, it was suggested that the treatment with rapamycin should be started at a young age: "As an anti-aging drug, rapamycin will prevent diseases rather than cure complications of diseases. Rapamycin will prevent [organ] damage but not to reverse damage. It might prevent diabetes and obesity but not diabetic gangrene and stroke. It might prevent macular degeneration but will unlikely cure blindness. Rapamycin will not repair broken bones but might prevent osteoporosis… rapamycin will be most useful as [an] anti-aging drug to slow down senescence and to prevent diseases" [1].

It was suggested in 2006 to take rapamycin immediately to the clinic to suppress human aging [1], even though longevity studies in animals were not yet performed. Starting from 2009, numerous studies demonstrated that rapamycin extends lifespan in mice [27-39].

Hyperfunction theory predicts that rapamycin can slow down aging by two complementary mechanisms:

(a) directly suppressing the quasi-program of aging

b) reprogramming aging by slowing the developmental-growth program

To demonstrate reprogramming, rapamycin should be given for a brief period during development.

Shindyapina et al. showed that treatment with rapamycin for the first 45 days of life extends median lifespan by 10% [2]. Health was improved as measured by gait speed, frailty index, and glucose and insulin tolerance tests [2]. Rapamycin-treated mice were small and did not catch up on growth later [2].

The hyperfunction theory explains why a large-body correlates with longevity between species (for example, elephants live longer than mice, which live longer than flies), but in contrast, within each species, it is a small body size that is associated with longevity [40]. Life-long small body size after a brief treatment is consistent with reprogramming of the growth program.

Notably, life extension by rapamycin was mostly observed in male mice [2]. This is consistent with the finding that mTOR is overactivated in young male mice compared with young female mice, thus explaining robustness of males at young age and their shorter lifespan [41].

Supporting the notion of rapamycin-induced re-programming, previous studies found that (a) even transient treatment with rapamycin can extend lifespan [27, 36, 39] (b) a single rapamycin injection can lower body weight set point in the long run [42] and (c) rapamycin can affect the mTOR pathway activity long term by preventing obesity [43, 44].

Further suggestions

To further study rapamycin-induced reprogramming of aging, pregnant mice should be treated with a single subcutaneous injection of rapamycin and the lifespan of their offspring should be measured. Prenatal (before birth) rapamycin treatment on early postnatal development has been studied [45-47]. For example, prenatally rapamycin-treated neonates are small, and body weight and left ventricular mass remain reduced in adulthood [47]. However, lifespan was not measured. (Note: rapamycin pre-treatment increased mortality immediately after the birth [47] because mTOR is essential early in life. Early-life death is not aging-driven and should be excluded from the age-related mortality curve).

At what age may rapamycin treatment be started in order to maximally extend human lifespan? Based on murine data, treatment with rapamycin can be started at a very old age. Still, in theory, the maximal effect potentially may be achieved before age-related diseases and pre-diseases become apparent in humans [1]. However, it should not be started too early because mTOR is essential for growth and early life fitness. In my opinion, rapamycin treatment (for anti-aging purposes) may only be started when a young adult can make informed decisions and should not be allowed before the age of 21. Doctors should consider that rapamycin may negatively affect reproduction, albeit reversibly. I believe that the initial dose should be very low and gradually increase with older age, when full individual doses are achieved. An anti-aging dose/ schedule is a maximum dose that do not yet cause side effects in a particular person [48]. Self-treatment is unacceptable and doses are highly individual [48, 49].

Disclaimer

This commentary is for information purposes, not medical advice.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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Research Perspective

DNA- and telomere-damage does not limit lifespan: evidence from rapamycin

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ABSTRACT

Failure of rapamycin to extend lifespan in DNA repair mutant and telomerase-knockout mice, while extending lifespan in normal mice, indicates that neither DNA damage nor telomere shortening limits normal lifespan or causes normal aging.

INTRODUCTION

As a provocative title has recently announced, "rapamycin fails to extend lifespan in DNA repair -deficient mice" [1]. The word "fails" implies bad news. Rapamycin tried but failed. Yet, it is expected that the anti-aging drug rapamycin should not restore lifespan of short-lived mice that fail to grow and die young from causes other than normal aging [2]. In such growth-retarded mice, rapamycin, an inhibitor of cell growth, further retards weight gain.

Similarly, rapamycin does not extend but even slightly shortens lifespan in telomerase-deficient mice, which die young from poor growth and intestinal atrophy caused by telomere shortening [3]. (As we will discuss, this is predictable by hyperfunction theory.) While shortening lifespan by 18% in unnatural telomerase-deficient mice, in the same study in natural mice, rapamycin increased lifespan by 39% and healthspan by 58% (measured as tumor-free survival) [3]. In dozens of independent studies, rapamycin has not failed to extend lifespan in normal mice [4]. However, while extending lifespan in normal mice, rapamycin may fail to save animals dying young from cellular growth retardation. But something important should not be overlooked. The failure of rapamycin to extend lifespan in these short-lived mice, dying from DNA damage, rules out the damage theory of aging. To understand this point, we must first discuss what limits animal lifespan.

Quasi-programmed (hyperfunctional) aging

In proliferating cells, growth-promoting pathways such as mTOR (Target of Rapamycin) and MAPK drive cellular growth, which is balanced by cell division. When the cell cycle is arrested, however, growth-promoting pathways drive cellular senescence, which is a continuation of cellular growth in the absence of cell division [5]. During geroconversion to senescence, cells become hypertrophic and hyperfunctional. One example of hyper-function is SASP or Senescence-Associated Secretory Phenotype [6]. Rapamycin can cause reversible cycle arrest but suppresses geroconversion, thus ensuring quiescence instead of senescence. (Note: Rapamycin does not prevent cell cycle arrest, it only prevents geroconversion that makes this arrest permanent [7]. This point is often miscited by others). Rapamycin slows down both growth and geroconversion, figuratively slowing down time [8]. Like cellular senescence is a continuation of growth, organismal aging is a continuation of growth too [9].

According to hyperfunction theory, aging is quasi-programmed, a continuation of developmental growth programs, driven in part by hyper-functional signaling pathways including the mTOR pathway [9]. Hyperfunction is an excessive normal function later in life. It's not necessarily an increase of function; it may even be insufficient decrease of function. For example, protein synthesis is decreased in *C elegans* but

is still too high: its further inhibition extends lifespan [10, 11].

Hyperfunction leads to age-related diseases, secondary organ damage and loss of function. For example, cellular hyperfunctions result in hypertension, culminating in stroke and damage of the brain. Aging is a sum of all age-related diseases [12, 13]. This theory was discussed in detail [9, 14–20] and has gained experimental support [11, 16, 21–26]. I will not discuss it here, just to mention that accumulation of molecular damage is not a driving force of development and therefore of aging. It is hyperfunctional signaling pathways such as mTOR (one of many) that drive both growth and aging, causing age-related diseases that in turn damage organs, leading to secondary loss of function.

Although molecular damage accumulates, this accumulation is not life-limiting because quasi-programmed aging terminates life first (Figure 1A). Quasi-programmed (hyperfunctional) aging is life-limiting, because it is favored by natural selection. Natural selection favors robust development and fitness early in life at the cost of aging. For example, growth hormone receptor-deficient mice (GHR-KO mice), with decreased mTORC1 activity, live longer but are small and weak early in life [27, 28]. In such mice mTORC1-driven aging is inhibited and mice live longer but would not survive in the wild and therefore do not exist in nature. As another example, knockout of PI3K, an activator of mTOR pathways, extends lifespan 10-fold in *C. elegans* [29]. The mutant worm undergoes prolonged developmental arrest, which would be lethal in the wild [29]. Therefore, natural selection favors hyperfunctional mTOR that is optimal for development but drives age-related diseases later in life.

According to damage theories, aging is functional decline caused by molecular damage. According to hyperfunction theory, quasi-programmed aging is not functional decline but a hyperfunction: cellular and systemic functions are higher than optimal for longevity. They are optimal for early life fitness and in part (only in part) mTOR-dependent.

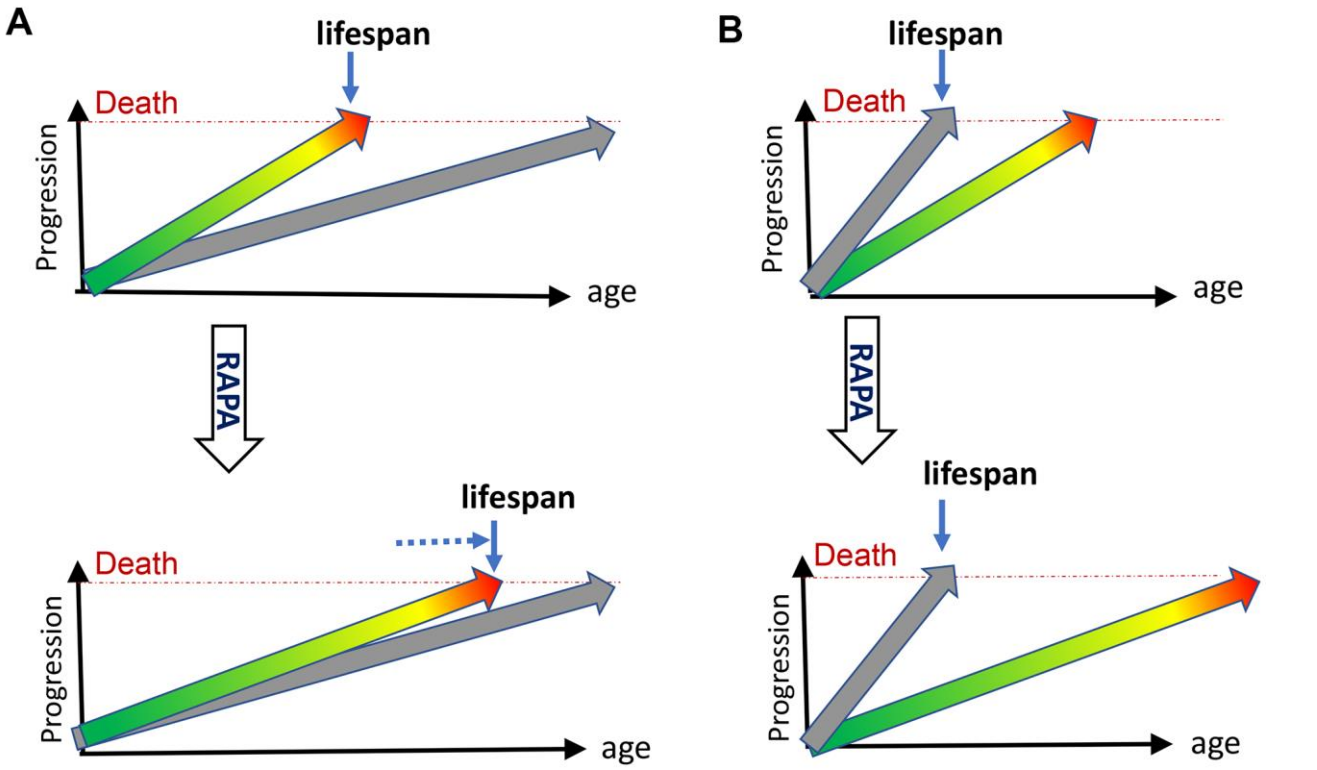

Figure 1. Rapamycin extends lifespan in natural but not progeroid mice. (**A**) Natural mice. Hyperfunctional aging (green/yellow/red arrow) progresses from development (green) to diseases (red), reaching death threshold and limiting lifespan. Accumulation of molecular damage (gray arrow) is slow and does not reach death threshold in animal lifetime. It would take longer to die from molecular damage. Treatment with rapamycin (RAPA) extends lifespan by slowing down mTOR-driven aging (**B**) Progeroid, telomerase- or DNA-repair-deficient mice. Accumulation of molecular damage (gray arrow) is artificially accelerated to become life-limiting. Treatment with rapamycin (RAPA) cannot extend lifespan.

In both molecular damage and hyperfunction theories, aging exists because late-life is shadowed from natural selection. But quasi-programmed aging is not simply shadowed from, it is promoted by natural selection, because accelerated aging is hardwired with fitness early in life. By selecting for fitness, nature indirectly selects for accelerated aging. This makes quasi-programmed aging life-limiting. One of predictions of hyperfunction theory is that rapamycin must extend lifespan in animals [9]. This prediction has been confirmed. In dozens of studies, rapamycin prolongs lifespan and healthspan in mice [3, 30–65]. Rapamycin extends lifespan in *C elegans* [66] and *Drosophila* [67–69]. Furthermore, rapamycin even extends life of the simplest animal, *Hydra*, which is thought to be immortal. Depending on conditions, *Hydra* can be either immortal or undergo aging. Rapamycin slows aging, stem cell exhaustion and extends life span in *Hydra* [70].

mTOR-driven aging is only one component of quasi-programmed (hyperfunction) aging. In addition, MEK/MAPK, NF-kB, p63, HIF-1 and many other signaling pathways are involved, interacting with the mTOR pathway and forming networks. Rapamycin cannot affect all of them. In theory, mTOR-independent quasi-programmed aging can be life-limiting in some conditions and diseases. I suggest that long-lived GHR-KO mice with low mTORC1 activity undergo partially mTORC1-independent quasi-programmed senescence, because rapamycin cannot prolong lifespan in these mice further, while prolonging lifespan in parental normal mice [71]. Discussion of mTOR-independent components of quasi-programmed aging is beyond the focus of this article. Let us return to stochastic accumulation of molecular damage.

How molecular damage can become life-limiting

Molecular damage can become life-limiting in two ways. First, hyper-functional aging should be eliminated or slowed down, so an organism lives long enough to die from accumulation of molecular damage. In this scenario, accumulation of molecular damage causes post-aging. Such examples are unknown, but it is a very intriguing possibility. Could a PI3K-null worm [29] with 10-fold longer lifespan die from molecular damage?

Second, accumulation of molecular damage can be greatly accelerated artificially by knockout of repair/maintenance enzymes (Figure 1B). Such animals do not exist in nature. But artificially created, they may provide a glimpse of how post-aging may look. Their pathology differs drastically from normal aging, for example, telomere shortening. Second-generation telomerase-deficient mice (G2 *Terc*$^{-/-}$) with critically short telomeres fail to grow and die young from unfamiliar diseases such as intestinal atrophy due to failure of cell proliferation [3]. When telomeres reach critical length, it can cause DNA-damage response, leading to aplastic anemia, organ fibrosis, atrophy of the small intestine and the spleen, skin and hair lesions. In humans, diseases of short telomeres cause death from bone marrow failure and pulmonary fibrosis [72]. This does not resemble normal aging.

In humans, mice and *C. elegans*, telomere shortening is not life-limiting [73–75]. In mice lacking telomerase, even accelerated telomere shortening is still not life-limiting in the first generation [76]. It took several generations to achieve critically short telomeres, leading to syndromes strikingly different from normal aging. In humans, telomere length does not reach telomere threshold during life time [75, 77, 78]. Normal telomere shortening would cause telomere-driven pathologies, but normal animals do not live long enough to reach this threshold. Rapamycin prolongs life in normal mice, proving that telomere length does not constrain normal lifespan [3]. When artificially shortened, then telomeres become life-limiting and rapamycin cannot extend lifespan anymore [3].

Ercc1$^{\Delta/-}$ mutant mice are defective in DNA repair, such as transcription-coupled repair, global-genome nucleotide excision and crosslink repair [1, 2]. Therefore, multiple types of DNA damages accumulate. This leads to decreased cell proliferation, arrested development, poor growth, abnormal liver nuclei of liver and kidney, absence of subcutaneous fat, ferritin deposition, kidney malfunction and early death [2]. Unlike natural mice, short-lived *Ercc1*$^{\Delta/-}$ mice do not develop tumors, probably because they do not live long enough to suffer typical age-related diseases [1, 2]. In such mice, dying from molecular damage, rapamycin fails to extend lifespan [1].

CONCLUSIONS

Here I discussed new evidence that normal aging is not caused by accumulation of molecular damage or telomere shortening: while extending normal lifespan in mice, rapamycin failed to do so in mice dying from molecular damage (Figure 1).

Previously, several lines of evidence suggested that molecular damage does not cause normal aging. Their detailed discussion is beyond the focus of this article, so I will just mention some of them, without referencing them (I will reference these points in forthcoming review "When longevity drugs do not increase

longevity: Unifying development-driven and damage-induced theories of aging", In press). First, overexpression of enzymes that decrease damage does not extend lifespan in most studies. Similarly, antioxidants do not extend lifespan in animals and may increase mortality in humans. Furthermore, even data that support damage theory can be explained by other mechanisms. For example, N-Acetyl-L-Cysteine, a commonly used anti-oxidant, can inhibit mTOR. Second, according to calculations, molecular damage, especially mtDNA mutations and telomere shortening, cannot reach deadly threshold during animal lifetime. Third, genetic knockout of signaling pathways can extend lifespan without affecting molecular damage. Similarly, pharmacological interventions can extend life without affecting damage accumulation. Forth, dramatic intra- and inter-species differences in lifespan poorly correlate with the rate of molecular damage. Fifth, nuclear transfer and nuclear reprogramming both rule out DNA damage as a cause of aging. Following adult somatic cell nuclear transfer, cloned animals are healthy and have normal lifespan. Sixth, low levels of molecular damage may increase longevity. This phenomenon is known as hormesis. Regardless of mechanistic explanations, this indicates that molecular damage is not-life-limiting even when moderately increased. Finally, rapamycin increases lifespan in all normal animals tested, indicating that mTORC1-dependent quasi-program is life-limiting. The list can go on and on. Once again, damage accumulates and must cause death eventually, but quasi-programmed (hyperfunctional) aging terminates life first. Molecular damage can become life-limiting, when artificially accelerated or, potentially, when quasi-programmed aging is decelerated. Then interventions to repair molecular damage may increase life further.

CONFLICTS OF INTEREST

The author declares that he has no conflicts of interest.

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Review

From causes of aging to death from COVID-19

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ABSTRACT

COVID-19 is not deadly early in life, but mortality increases exponentially with age, which is the strongest predictor of mortality. Mortality is higher in men than in women, because men age faster, and it is especially high in patients with age-related diseases, such as diabetes and hypertension, because these diseases are manifestations of aging and a measure of biological age. At its deepest level, aging (a program-like continuation of developmental growth) is driven by inappropriately high cellular functioning. The hyperfunction theory of quasi-programmed aging explains why COVID-19 vulnerability (lethality) is an age-dependent syndrome, linking it to other age-related diseases. It also explains inflammaging and immunosenescence, hyperinflammation, hyperthrombosis, and cytokine storms, all of which are associated with COVID-19 vulnerability. Anti-aging interventions, such as rapamycin, may slow aging and age-related diseases, potentially decreasing COVID-19 vulnerability.

COVID-19 vulnerability: age, diseases, gender

COVID-19 is caused by coronavirus SARS-CoV-2. Most cases of COVID-19 are asymptomatic, but some are severe and lethal. Mortality is the simplest marker of COVID-19 vulnerability. COVID-19 vulnerability can be defined as a chance of death from COVID-19, once infected.

Age:
In all studies conducted in all countries, the mortality rate from COVID-19 increases exponentially with age [1–11]. Exact mortality rates varied in hundreds of studies because they depend on testing and therapeutic interventions. But the rule is clear: the mortality rate is increasing exponentially with age.

Age-related diseases:
Mortality is especially high in patients with pre-existing conditions [6, 9, 10, 12–23].

In Italy, 99% of patients, who died, had at least one illness.

https://www.bloomberg.com/news/articles/2020-03-18/99-of-those-who-died-from-virus-had-other-illness-italy-says.

In other words, infected people without pre-existing diseases do not die. This may seem paradoxical because we just discussed that age is sufficient to increase mortality exponentially. This is because pre-existing conditions are manifestations of biological age, whereas aging and diseases are two sides of the same coin [24–26]. These conditions are typical age-related diseases: hypertension, diabetes, obesity, ischemic heart disease (IHD) and chronic obstructive pulmonary disease (COPD) and other diseases [9, 12–23].

Of course, not all (only some) patients with age-related diseases die from COVID-19. In other words, age-related diseases are necessary but not sufficient for mortality from COVID-19.

Age and pre-existing (age-related) diseases are interdependent. A number and severity of diseases correlate with age. An average 60 year old person has

more age-related diseases than an average 50 your old person. Yet, a particular 60 year old person may have no age-related diseases, whereas a particular 50 year old person may have multiple diseases including hypertension, diabetes, obesity and cancer. In this case, it is a chronologically younger person who is biologically older. And it is the biological age that determines the likelihood of death from COVID-19.

Male Gender:
At the same age, the mortality rate is twice higher in men than in women [9, 27, 28], in part, because men age faster than women and, at any chronological age, men are biologically older than women [29].

So, three rules can be combined in one: COVID-19 vulnerability is determined by biological age. Biological age combines chronological age, age-related diseases and gender. A combination of all age-related diseases (and pre-diseases) is a biomarker of biological age. Figuratively, SARS-Cov-2 can "measure" biological age, which is thus the best predictor of mortality from both COVID-19 and other diseases.

Mortality from aging compared with COVID-19 mortality

Aging can be measured as an increase in the probability of death with age. Mortality increases exponentially, starting from age 8-9. Men have a higher "normal" age-related death rate than women because men age faster than women [29].

COVID-19 mortality rate parallels the "expected" aging-related death rate (Supplementary Figure 1) and see second graph in:
https://medium.com/wintoncentre/how-much-normal-risk-does-covid-represent-4539118e1196.

Chances to die from COVID-19 are proportional to chances to die from aging itself at any age. The only discrepancy between natural and COVID-19 mortality is observed below the age of 8 years old. Whereas natural death rate is relatively high, COVID-19 mortality is low (no mortality [11]). This discrepancy will be discussed later. But first how do animals, including humans, die from aging?

Age-related diseases

Humans and other animals (including the worm [30] and the fly [31]) do not die from aging itself but from age-related diseases such as ischemic heart disease (IHD), hypertension, diabetes, cancer, Alzheimer's and Parkinson's diseases, age-related macular degeneration, osteoporosis and sarcopenia (As we will discuss, even seemingly non-deadly diseases such as osteoporosis can lead to deadly complications). The incidence of these diseases increases exponentially with age. Some diseases such as obesity, hypertension and diabetes develop earlier in the course of aging. Other diseases, such as Alzheimer's disease and macular degeneration, are usually diagnosed later [32, 33]. Age-related diseases may also occur in younger people with genetic predisposition and environmental exposure hazards. But even without these factors, diseases develop because they are quasi-programmed (see "Quasi-programmed aging section"). These diseases are not diseases of civilization, as it may seem. Humans simply now live long enough to develop them. Of course, "hazards of civilization" can accelerate them at a younger age.

Aging and its diseases cannot be separated. Healthy aging, or aging without diseases, is merely a slow aging, when biological age is less than chronological age. During a period of seemingly healthy aging, pre-pre-diseases and pre-diseases are progressing until they eventually reach clinical manifestations. Thus, healthy aging progress to unhealthy and pre-diseases become diseases [34].

Age-related diseases and COVID-19 vulnerability are highly intertwined. Patients, who die from COVID-19, otherwise would die from age-related diseases such as heart disease, cancer, diabetes, hypertension, just a year later. COVID-19 approximately doubles a patient's aging-dependent risk of dying during one year. For example, (numbers are very approximate), a sixty year old woman has 1% chance to die from aging before her 61st birthday. At that age, if infected, the death rate from COVID-19 is around 1% for females. If infected, a patient has approximately doubled chances to die compared with usual age-related mortality during one year. As David Spiegelhalter put it: "getting COVID-19 is like packing a year's worth of risk into a week or two". https://medium.com/wintoncentre/how-much-normal-risk-does-covid-represent-4539118e1196.

Children and young adults have a very low risk of death from aging-related diseases, so that risk remains extremely low even when doubled.

Although natural mortality is relatively high in the youngest age group, especially in infants, they do not die from age-related diseases of course. Instead, infants are vulnerable to bacterial infections and candida infections due to underdeveloped immune system [35]. Low COVID-19 mortality in the pediatric age group [11] is consistent with the notion that COVID-19 vulnerability is not due to a "weak" immune system. In contrast, as we will discuss in the next section, it is hyper-functional immune response that leads to death from COVID-19 in the elderly by causing cytokine storm.

Cytokine storm as a hyperfunction

Severe COVID-19 is characterized by hyper-inflammation, cytokine storm, acute respiratory distress syndrome (ARDS), damage to the lung, heart and kidneys [36–39].

In response to viral replication, hyperfunctional monocytes and macrophages infiltrate the lung, causing hyper-inflammation and hyper-secretion of cytokines such as interleukin (IL)-6, IL-2, IL-7, IL-1ra, interferon-γ inducible protein (IP)-10, tumor necrosis factor (TNF)-α, ferritin, monocyte chemo-attractant protein (MCP)-1, macrophage inflammatory protein (MIP) 1-α, granulocyte-colony stimulating factor (G-CSF), C-reactive protein (CRP) and procalcitonin. [22, 36–42].

This leads to leukocyte recruitment, vascular permeability, edema and further pulmonary damage in vicious cycle [37, 38, 41, 43, 44]. Hyper-inflammation becomes systemic, in turn causing hyper-coagulation and thrombosis, disseminated intravascular coagulation [45]. This causes injury of distant organs such as the kidneys. Pre-existing organ damage (late stages of age-related diseases) exacerbates organ damage caused by cytokine storm [42, 43, 46]. In addition, cellular hyper-functions and systemic hyper-inflammation may lead to cellular exhaustion, such as exhaustion of lymphocytes (lymphopenia) [47–49]. Hypercoagulation is associated with hyperactive fibrinolysis and increased D-dimer blood levels [23]. Cytokine storm is a systemic hyperfunctional response (Figure 1).

Of course, age-related hyperfunctional response, such as cytokine storm, is not caused by lifelong accumulation of molecular damage. Aging is not caused by molecular damage after all. Instead it's a continuation of developmental/growth programs that lead to hyper-functions and in turn eventually to dysfunctions.

Hyperfunction theory of quasi-programmed aging

"Quasi" means "resembling" or "seemingly, but not really." Quasi-program of aging is not a program but a continuation of developmental programs that were not switched off upon their completion [24, 50]. They purposelessly unfold, leading to age-related diseases, secondary organ failure and death. Quasi-programmed (program-like) aging is associated with higher than optimal cellular and systemic functions, which eventually, via cellular exhaustion and organ damage, lead to functional decline (Figure 2). For example, starting from birth, blood pressure increases and continues to increase after organismal growth is completed. Therefore, hypertension is the most prevalent age-related disease. In turn, hypertension can cause organ damage: stroke, infarction and renal failure. Similarly, obesity develops in post-development as a continuation of growth (yet, it can be prevented by low caloric diets, illustrating that quasi-program of aging can be decelerated).

Hyperfunction is an excessive normal cellular function: contraction by smooth muscle cells (SMC), adhesion and aggregation by blood platelets, insulin secretion by

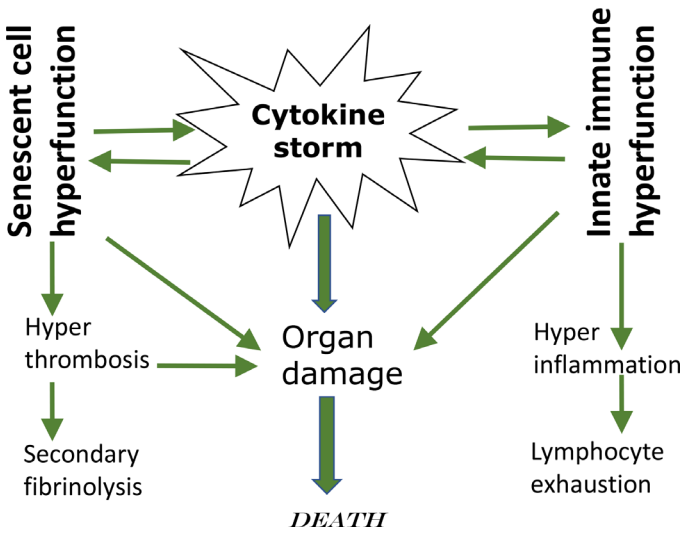

Figure 1. Cytokine storm as a systemic hyperfunction.

beta-cells, lipid accumulation by adipocytes, secretion by stromal and immune cells, oxidative burst by leukocytes, just to name a few. When higher than optimal, they cause vasoconstriction and hypertension, thrombosis, hyperinsulinemia, hypertrophy, hyperplasia, obesity, hyper-secretory phenotype or Senescence-associated secretory phenotype (SASP), hyper-inflammation and so on.

Hyper-function is not necessarily an absolutely increased function. It may be also insufficiently decreased function (relative hyperfunction). Levels of IGF-1 and growth hormone decrease during lifespan. Despite this decrease, IGF-1 levels are still higher than optimal (relative hyper-function) because further genetic decrease in IGF-1 levels (by genetic means) extends health span and lifespan in mammals [51–53].

Cellular hyperfunctions may eventually switch to cellular exhaustion and loss of functions at late stages. During the course of type II diabetes, mTOR overactivation and hyperinsulinemia eventually lead to beta-cell exhaustion and insulin insufficiency, from pre-diabetes to diabetes [54, 55]. As another example, after puberty, hyperstimulation of the ovary eventually leads to oocyte exhaustion and menopause (see Figure 3 in ref. [29]). Depletion of naïve lymphocytes is another example, as reviewed here later. Age-related alterations are mostly noticed when they switch to functional decline, which is a late event.

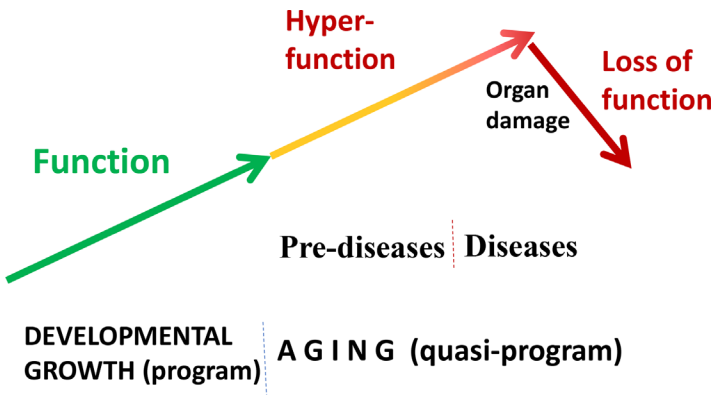

Figure 2. Quasi-programmed hyperfunctional aging. Aging is a continuation of developmental programs that were not switched off upon their completion. An increase in cellular and systemic functions (manifested as pre-diseases and then as diseases) leads to eventual organ damage and secondary loss of function.

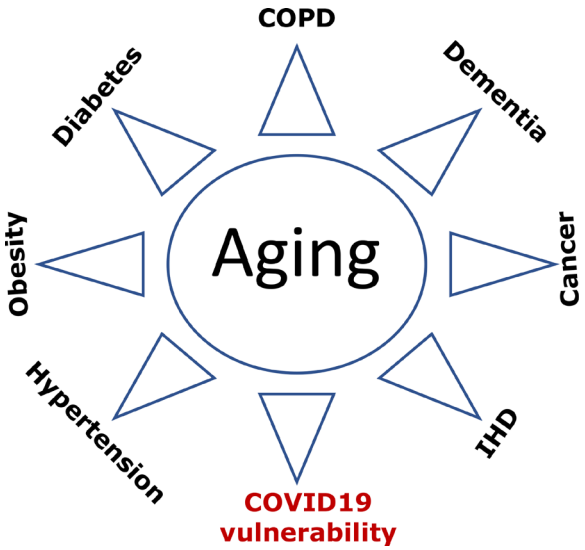

Figure 3. COVID-19 vulnerability as an age-related disease. Age-related diseases, including COVID-19 vulnerability, are manifestations of aging. Abbreviations: Ischemic heart disease (IHD); Chronic obstructive pulmonary disease (COPD).

In some cases, functional decline can be primary and programmed. For example, thymus involution (replacement of T cells by adipocytes) starts early in life, accelerates at puberty and continues later. Still loss of thymocytes and their niches may be in part due to adipocyte hyperplasia and hypertrophy [56]. In fact, obesity accelerates involution, whereas calorie restriction decelerates it [57, 58]. Furthermore, the oblation of sex hormones decelerates or even reverses thymus involution [59]. Thus, involution is triggered by adipocyte hyperplasia and increased production of sex hormones during puberty [56].

Quasi-programmed aging is not driven by molecular damage. It is driven by nutrient/hormone/cytokine-sensing and growth-promoting signaling pathways such as Target of Rapamycin (TOR; mTOR), which are involved in developmental growth and later cause hyperfunctional aging and its diseases [24, 26].

Covid-19 vulnerability as an age-related syndrome

What is the cause-effect relationship between age-related diseases and COVID-19 lethality? Do patients die from age-related diseases, complicated by COVID-19? Or, in contrast, do these various diseases make COVID-19 infection lethal? Both scenarios take place to some extent. However, the relationship is mostly indirect. Both age-related diseases and COVID-vulnerability result from the same underlying cause (Figure 3). This is why they are highly correlated. The cause is aging itself. Aging is manifested by a sum of deadly - and not so deadly - diseases and conditions ranging from cancer to grey hair. Although not all diseases seem to be deadly, they can cause complications such as stroke, ventricular fibrillation, renal failure, lung edema. Even sarcopenia and osteoporosis lead to falls and broken bones culminating in a deadly sequence of events. Cosmetic manifestations such as aging spots and wrinkles, while not deadly by themselves, can be manifestations of other diseases. For example, baldness correlates with prostate enlargement [60], and the later can lead to urinary obstruction and renal failure.

Diseases occur together. For example, chronic obstructive pulmonary disease (COPD) is associated with diabetes, cardiovascular disease and hypertension [61]. If a person has one disease (e.g., diabetes), this patient has higher chances of having other diseases (e.g., hypertension, IHD, cancer) or conditions, including COVID-19 vulnerability, which is revealed only during infection but can be predicted by pre-existing diseases.

Aging is initially driven by an increase in cellular and systemic functions (hyperfunction), leading to age-related conditions. For example, hypertension is a systemic hyperfunction due to hyperfunction of multiple cell types such as arterial smooth muscle cells (aSMC). Similarly, COVID-19-vulnerability is associated with hyperfunction of inflammatory cells that, in response to COVID-19 infection, causes cytokine storm, hyper-coagulation and damage of the lung and distant organs.

The COVID-19 vulnerability syndrome is an aging-related disease, strictly dependent on biological age, associated with other age-related diseases, and exemplified by hyper-functional response to infection**.**

Inflamm-aging and immunosenescence

With hundreds of cell types acting in concert, the immune system is so complex that we cannot discuss age-related alterations without oversimplification. The most noticeable alteration is that memory T and B cells replace naive T and B cells [62]. (This seems natural since life-long exposure to pathogens replaces naïve cells by memory cells). Replacement of naïve immune cells decreases adaptive responses to novel antigens such as SARS-CoV-2. In contrast, immune protection by memory T cells from viral re-infection with known pathogens is usually increased with age [62].

Immune responses are roughly divided into (a) innate responses, carried mostly by neutrophils, macrophages and NK cells, which react to pathogen rapidly and nonspecifically, and (b) adaptive responses, carried by T and B lymphocytes, which are delayed, slower and specific (e.g., antigen-specific clonal expansion of T and B lymphocytes and antibody production by B lymphocytes) [63–65]. In the elderly, immune responses to SARS-CoV-1/2 are "stuck in innate immunity," with insufficient progression to adaptive immunity [37]. However, decline in adaptive response, such as antibody production, plays little role in COVID-19 mortality. It is hyper-functional innate immunity, hyper-inflammation, cytokine storm and hyper-coagulation that lead to organ failure and death. In agreement, hyper inflammatory response rather than high virus numbers leads to death of SARS-CoV-infected old nonhuman primates [66].

Aging is associated with diseases of immune hyper-function such as autoimmune disorders with paradoxical increase in certain signaling pathways and cytokine levels [67–69].

In the elderly, innate immune cells are in a state of sustained activation, producing pro-inflammatory cyto-kines [67, 70–72]. Increased pro-inflammatory activity by the innate immune system, especially by monocytes/ macrophages, is a state of alertness and hyper-reactivity on the cost of potential age-related inflammatory diseases

[67, 70–72]. Whereas some functions are decreased, others are increased. According to the inflamm-aging concept, innate immune system overtakes adaptive immune system in aging. Cause-effect relationships are bi-directional: immunosenescence (namely, a decrease in adaptive response) is a cause and consequence of inflamm-aging [67, 70–72].

We can consider inflamm-aging as an example of hyper-function. While some functions are decreased, others are increased. Hyper-function is damaging. (In analogy, increased electric power, without an adaptor, would damage a laptop). Damaging hyper-functions can lead to loss of function and cellular exhaustion. And vice versa, loss of function may cause compensatory hyper-functions of another components.

Cellular senescence as a continuation of growth

Cellular senescence is a continuation of cellular growth, when actual growth is completed [73, 74]. In proliferating cells, cellular mass growth is balanced by cell division. Cells grow in size and then divide. When the cell cycle is blocked (e.g., p21 and p16), then growth-promoting pathways such as mTOR and MAPK drive conversion to senescence (geroconversion) [24, 74, 75]. During geroconversion, cells become hypertrophic and "fat". Cellular functions increase: hyper-secretion and lysosomal hyper-function are manifested by SASP and beta-Gal staining. Hyper-activated growth-promoting pathways cause compensatory resistance to growth factors/insulin, permanent loss of re-proliferative potential [74]. Rapamycin, everolimus, pan-mTOR and MAPK inhibitors slows down geroconversion, maintaining reversible quiescence instead of senescence [73, 76–88].

Geroconversion is a continuation of cellular growth [73, 74]. Similarly, aging is a continuation of developmental growth (see Figure 1 in ref. [89]). When the developmental program is completed, it becomes a quasi-program of aging. As discussed in detail, chronically activated nutrient-sensing and growth-promoting pathways drive age-related diseases, culminating in organismal death [24, 26].

Age-related diseases are quasi-programmed. Aging is a common cause of age-related diseases, a sum of all age-related diseases. They are diseases of hyper-function, secondary hypo-function and compensation reactions [25]; they are deadly manifestations of aging.

From activation of cellular functions to systemic hyperfunctions, from diseases to organ damage and death, hyperfunction theory of quasi-programmed aging describes the sequence of events [26]. And as discussed in 2006, suppression of aging by gero-suppressants, such as rapamycin, will prevent and treat all age-related diseases [24]. This point of view is becoming widely accepted and, in recent literature, quasi-programmed model of diseases (2006) is called "geroscience hypothesis" [2, 90].

Figuratively, rapamycin rejuvenates immunity [91]

If aging were functional decline due to accumulation of molecular damage, then it would be near to impossible to restore functions and rejuvenate the immune system. In contrast, if functional decline is secondary to hyperfunctions (see Figure 2 in ref. [89]), these hyperfunctions can be suppressed pharmacologically to restore lost functions. Typical drugs are inhibitors of their targets, rather than activators, so they decrease functions of their targets. By decreasing hyper-functions, which otherwise lead to secondary loss of functions, rapamycin may restore "lost" functions (Figure 4).

Rapamycin improves vaccination against viruses such as influenza in old mice, monkeys and humans [92–100]. Importantly, rapamycin increases pathogen-specific but not graft-reactive CD8+ T cell responses [95, 101]. Therefore, rapamycin and everolimus can both be used to prevent donor organ rejection and improve adaptive immunity against new pathogens [96].

Differentiation is an increase of tissue-specific cellular functions. Terminally differentiated B, T, and NK cells can rapidly react to already known pathogens [102]. Decrease in naïve T and B lymphocytes (and thus diminished response to novel antigens) results in part from cellular hyper-differentiation in the immune system [64, 103]. Hyper-functional differentiation can be counteracted by rapamycin [98].

As another example, age-related exhaustion of stem cells is partially due to loss of quiescence caused by growth over-stimulation [92, 104–106]**.** In general, senescent cells characterized by hyper-proliferative drive coupled with cell cycle arrest [77]. In young mice, mTOR hyper-activation causes senescence of hematopoietic stem cells (HSC) and decreases lymphopoiesis [92]. In old mice, rapamycin rejuvenates hematopoiesis, and improves vaccination against influenza virus [92].

Third, production of lymphoid cells may be decreased because of disruption of hypoxic niches due to adipocytes hyperplasia [56, 107]. Hypoxic niches can preserve HSC [108, 109] probably because hypoxia inhibits mTOR and cellular senescence [110]. In agreement, rapamycin preserves HSCs [92, 98, 111, 112] reduces the proportion of memory cells and maintains a pool of naïve T cells [92, 98].

Fourth, growth factor (GF)- and insulin-resistance is loss of function because cells cannot respond to GF/insulin. But it may be caused by over-activated mTOR, which via S6K/IRS feedback loop blocks insulin and GF signaling. Rapamycin abrogates the loop restoring signaling [113–118].

Anti-aging medicine

A high prevalence of age-related diseases, often called "diseases of civilization," is a success story of modern medicine. In the past, most people did not live long enough to develop age-related diseases and those who developed them died soon after. Due to medical advances, people survive to 85 on average, despite suffering from age-related diseases. Standard medicine preferentially extends life span, without necessarily affecting health span (see Figure 3 in ref. [119]). For example, defibrillation and coronary stenting can save life but not cure heart disease. It is anti-aging interventions that extend health span, delaying diseases, thus extending lifespan. Aging is a common cause of all age-related diseases. By suppressing aging, anti-aging interventions may delay all age-related diseases [119].

As a well-known example, low calorie diets such as calorie restriction, intermittent fasting, and low carbohydrate diets extend both health and lifespan. Figuratively, low calorie diets prolong life by improving health. Nutrients and obesity activate growth-promoting pathways (e.g., mTOR), thus accelerating development of quasi-programmed (age-related) diseases. Obesity is associated with all age-related diseases from cancer to Alzheimer's and from diabetes to sarcopenia. COVID-19 vulnerability is also associated with obesity [9, 19, 20, 22]. According to hyperfunction theory, obesity accelerates aging and all age-related conditions including COVID-19 vulnerability.

Diabetes is one of main risk factors of death in COVID-19 [5, 6, 12, 13, 15, 21]. Can type 2 diabetes, an age-related disease, be reversed? In remarkable studies, it was shown that a brief course (6-8 weeks) of very low calorie diets (VLCDs) can reverse type II diabetes. In one study, VLCD reversed diabetes in 46% of patients with up to a 6-year history of diabetes [120]. VLCD is most effective for its prevention and at early stages of diabetes [121]. This anti-aging modality is so simple that remission can be achieved at home by health-motivated individuals [122]. Simultaneously, it treats other age-related diseases such hypertension [123]. Obesity is associated with other diseases of hyper-function from diabetes and sarcopenia to cancer and Alzheimer's' disease. Since age-related diseases are predictors of COVID-19 mortality, VLCD in theory may decrease COVID-19 vulnerability.

Rapamycin and everolimus as anti-aging drugs

In the soil of Easter Island, a complex bacteria produces anti-fungal antibiotic rapamycin to suppress yeast growth but, as a by-product, it also suppresses yeast aging (quasi-programed aging is a continuation of growth). Approved for human use in 1999, Rapamycin

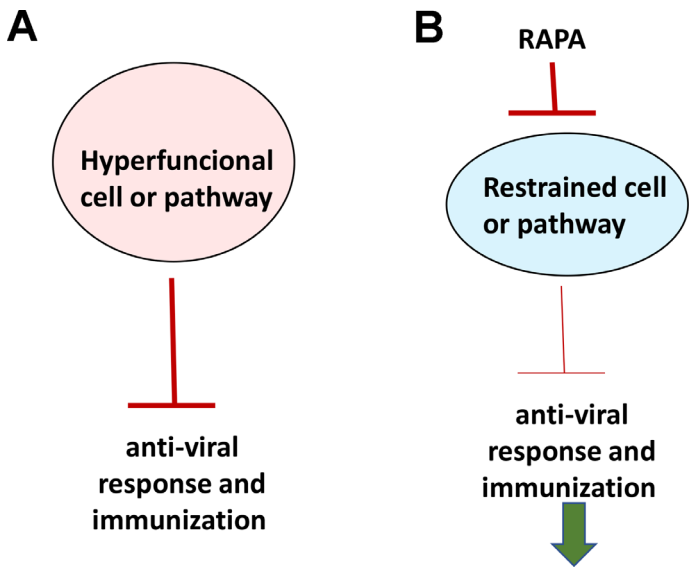

Figure 4. Rejuvenating immunity by inhibiting hyperfunction. (**A**) Specific hyper-functional cells (or signaling pathways) can inhibit some other cell types (or pathways) that are needed for proper anti-viral response and immunization. (**B**) By inhibiting hyper-functional cells or pathways, rapamycin can reactivate "loss-of-function" otherwise suppressed by hyper-functional cells or pathways.

(Sirolimus) and its close analog Everolimus are widely used in several diseases including cancer and organ transplantation. Hundreds of clinical trials (and twenty years of clinical practice) have ensured their safety and good tolerability especially in healthy older adults [119].

Currently, several anti-aging clinics prescribe rapamycin out of label to prevent age-related diseases and slow aging. Hundreds of recent reviews discussed rapamycin and everolimus in detail, so I will just emphasize a few points:

1. Crucial prediction of hyper-function theory of quasi-programmed aging in 2006 was that rapamycin will slow aging, extend healthspan and lifespan and decrease all age-related [124]. It has been confirmed: it extends lifespan in animals from worm to mammals. In some strains of short-lived mutant mice, it extends life span two fold [98, 125].

2. Rapamycin slows geroconversion to cellular senescence in cell culture [74].

3. mTOR is a potential therapeutic target in chronic obstructive pulmonary disease COPD [126], [127]. Rapamycin (sirolimus) is already approved and successfully used in lymphangioleiomyomatosis (LAM), a progressive, cystic lung disease, associated with inappropriate activation of mTOR [128]. Long-term daily use of rapamycin improves lung function without causing serious side effects (and of course no even minor side effects in the lung, given that rapamycin improves lung function) [128].

4. Despite widespread misunderstanding, rapamycin and everolimus do not cause diabetes. In contrast, they prevent diabetic complications in animals with diabetes (see for references [129]). In rodents, in some conditions they may cause symptoms of starvation pseudo-diabetes similar to prolong fasting and ketogenic diet [129]. Although, the *Johnson* study found a slight but significant correlation between Medicare billing for insulin and the use of rapamycin in renal transplant patients, this correlation was mechanistically explained by interaction of rapamycin with two other drugs used in the same patients [130, 131]. In cancer patients, everolimus may cause reversible hyperglycemia as a mild, infrequent and reversible side effect after several weeks of daily high doses of everolimus and rapamycin [132]. Mechanistically, everolimus decrease insulin production, not causing insulin resistance [132]. If anything, everolimus and rapamycin can be considered to treat complications of type II diabetes and prevent hyperinsulinemia and obesity ([129] and references within). What actually contributes to type 2 diabetes is excess of nutrients (and especially carbohydrates), which activate mTOR and cause hyperinsulinemia and insulin resistance.

Potential applications of rapamycin/everolimus to COVID-19

As soon as COVID-19 epidemic started, it become clear that COVID-19 vulnerability is an aging-dependent condition and the use of rapamycin (Sirolimus) was immediately suggested by independent researchers [1, 3, 133–137]. These proposals were based on a mixture of several rationales, which need to be clearly distinguished. In theory, there are at least three independent applications of rapamycin and everolimus for COVID-19. Currently, they all are still hypothetical.

1. Anti-aging effect (Figure 5). By decreasing biological age and preventing age-related diseases, a long-term rapamycin therapy may in theory decrease COVID-19 mortality rate in the elderly. Anti-aging application is especially important because it is beneficial regardless of COVID-19. After all, mortality rate from aging and its diseases is 100%, causing more than 2 million deaths in the USA annually. Continuous use of rapamycin is expected to improve health, decrease age-related diseases and extend healthy lifespan, rendering individuals less vulnerable, when infected with the virus.

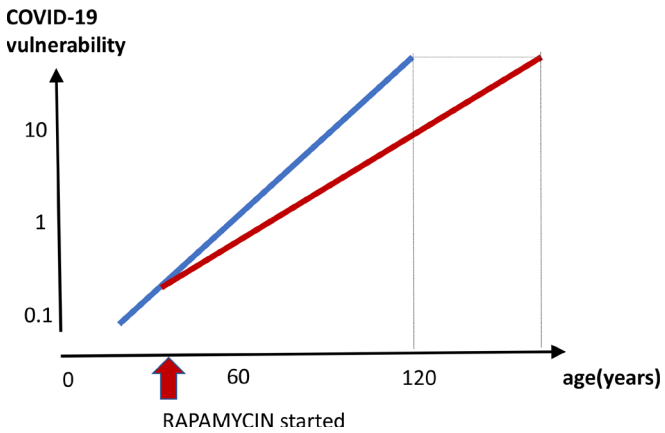

Figure 5. Prevention of COVID-19 vulnerability by staying young. Hypothetical graph in the absence of COVID-19. COVID-19 vulnerability (log scale) increases exponentially with age (blue line). The line ends at age 120, a maximum recorded age for humans. In theory, a continuous rapamycin treatment would slow down an increase of the vulnerability with age (red line). The increase is still logarithmic but at a different slope, because rapamycin slows the aging process. The maximum lifespan, in the absence of COVID-19, is extended because the 100% natural death threshold is achieved later.

2. Rejuvenating immunity. As we discussed in section "Figuratively, rapamycin rejuvenates immunity" [91], mTOR inhibitors can improve immunity to viral infections, improve immunization and vaccination to some viruses such as flu [92–100, 111, 112, 138]. In addition, viruses such as flu [139] and coronavirus (MERS-CoV) [140] depend on mTOR activity for replication. Currently, however, there are no data regarding COVID-19. Although aimed to evaluate safety, Phase 1 clinical trial "Sirolimus in COVID-19 Phase 1 (SirCO-1)" may reveal anti-viral effects too https://clinicaltrials.gov/ct2/show/NCT04371640.

3. Potential suppression of cytokine storm and hyper-inflammation (Figure 1). As we discussed in the section "Cytokine storm is a hyperfunction", cytokine storm and hyper-inflammation is a main cause of death in COVID-19 pneumonia [36–40, 42, 45, 135, 141–143] Rapamycin, an anti-inflammatory agent, inhibits hyper-functions, cellular senescence and decrease secretion of cytokines ([74, 81, 144]. Rapamycin inhibits the Jak2/Stat4 signaling pathway [145] and reduces IF-γ and TNF-α levels [112]. Rapamycin (Sirolimus) treatment improves outcomes in patients with severe H1N1 pneumonia and acute respiratory failure and was associated with improvement in virus clearance, and shortened ventilator days [146]. Clinical trial "Sirolimus Treatment in Hospitalized Patients With COVID-19 Pneumonia (SCOPE)" has been started https://clinicaltrials.gov/ct2/show/NCT04341675.

Disclaimer

This review is intended for a professional audience, to stimulate new ideas and to aid the global efforts to develop effective treatments for COVID-19 disease. This article does not represent medical advice or recommendations to patients. The media should exercise caution and seek expert medical advice for interpretation, when referring to this article.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary Figure

Supplementary Figure 1. The mortality risk with COVID-19 superimposed on background annual risk. Annual risk of death (hazard) for England and Wales, 2016–2018, from Office for National Statistics. https://medium.com/wintoncentre/how-much-normal-risk-does-covid-represent-4539118e1196.

Research Perspective

Rapamycin for longevity: opinion article

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ABSTRACT

From the dawn of civilization, humanity has dreamed of immortality. So why didn't the discovery of the anti-aging properties of mTOR inhibitors change the world forever? I will discuss several reasons, including fear of the actual and fictional side effects of rapamycin, everolimus and other clinically-approved drugs, arguing that no real side effects preclude their use as anti-aging drugs today. Furthermore, the alternative to the reversible (and avoidable) side effects of rapamycin/everolimus are the irreversible (and inevitable) effects of aging: cancer, stroke, infarction, blindness and premature death. I will also discuss why it is more dangerous not to use anti-aging drugs than to use them and how rapamycin-based drug combinations have already been implemented for potential life extension in humans. If you read this article from the very beginning to its end, you may realize that the time is now.

"If you wait until you are ready, it is almost certainly too late." Seth Godin

In one short-lived mutant strain of mice, the mTOR inhibitor rapamycin (known in the clinic as Sirolimus) extends maximum life span nearly three-fold [1]. Albeit less spectacularly, rapamycin also prolongs life in normal mice as well as in yeast, worms and flies, and it prevents age-related conditions in rodents, dogs, nonhuman primates and humans. Rapamycin and its analog, everolimus, are FDA approved for human use and have been used safely for decades. In 2006, it was suggested that rapamycin could be used immediately to slow down aging and all age-related diseases in humans [2], becoming an "anti-aging drug today" [3].

But rapamycin was unlucky

Rapamycin known in the clinic as Rapamune or Sirolimus, was unlucky from the start, however. Twenty years ago, it was labeled an immunosuppressant and used to treat renal transplant patients. If rapamycin had been labeled an immunomodulator and anti-inflammatory drug instead, it would sound much more appealing now. At anti-aging doses, rapamycin "eliminates hyperimmunity rather than suppresses immunity" or, more figuratively, it "rejuvenates immunity" [2]. This enables rapamycin and everolimus, a rapamycin analog, to act as immunostimulators [4-6], improving immunity in cancer patients [7] and the elderly [8, 9]. For example, rapamycin reduces the risk of CMV infection in organ transplant patients [10-12], improves antipathogen and anticancer immunity in mice [13-15], prolongs lifespan in infection-prone mice [16] and protects aged mice against pneumonia [17]. Rapamycin also inhibits viral replication [18, 19]. As a noteworthy example, rapamycin inhibits replication of the 1918 flu virus (the deadliest flu virus in history) by 100-fold [19], and also protects against lethal infection with influenza virus when administered during vaccination [13]. Still, as Dr. Allan Green advises, patients taking rapamycin should be carefully monitored for skin and subcutaneous bacterial infections, which should be treated with antibiotics https://rapamycintherapy.com.

Twenty years ago, it was thought that rapamycin might increase the risk of cancer (see a forthcoming review "Understanding the side effects of rapamycin"). Despite that concern, it was revealed that rapamycin actually prevents lymphoma and some types of cancer in transplant patients [20-27]. Currently, in fact, rapamycin analogs, everolimus and temsirolimus, are widely used in cancer therapy. Furthermore, rapamycin is the most effective known cancer-preventive agent in mice [25, 28-32] extending the lifespan of cancer-prone mice [33-36]. It has even been suggested that rapamycin extends lifespan by preventing cancer [37].

Nevertheless, social media often warn that although rapamycin prevents cancer, its use to prevent cancer may come at the cost of getting cancer. This self-contradiction miscites a twenty-year-old warning by the FDA for all drugs marketed as immunosuppressants (including rapamycin and everolimus): "Increased susceptibility to infection and the possible development of malignancies such as lymphoma and skin cancer may result from immunosuppression." This statement does not say that rapamycin or everolimus cause malignancies. (Just read it again). Although rapamycin and its analogs are now approved by the FDA for treatment of cancer and lymphomas, the rumors that these drugs may cause cancer persist. To my knowledge, no study has shown that mTOR inhibitors cause cancer.

At this point, most scientists agree that rapamycin is not counterindicated because of concerns about immuno-suppressive effects. But a new objection against rapamycin has emerged, namely that rapamycin may cause diabetes. As discussed in detail [38], the new wave of "fear of rapamycin" is groundless. So, what are the metabolic effects of rapamycin?

Metabolic effects or rapamycin and starvation

When it is over-activated by nutrients and insulin, mTOR acts via S6K to inhibit insulin signaling, thereby causing insulin resistance [39-44]. Acute treatment with rapamycin abrogates insulin resistance in cells and animals including humans [45-51]. One study showed that chronic treatment with rapamycin may also prevent insulin resistance [52]. However, in some (but not all) rodent models, chronic treatment with rapamycin can also cause glucose intolerance and even insulin resistance [53-56]. This was interpreted as a deleterious side effect or even type 2 diabetes (T2D). Actually, however, these metabolic changes are features of benevolent starvation pseudo-diabetes (SPD), which was described 170 years ago in fasted animals and later in humans [57, 58]. During prolonged fasting, utilization of glucose by non-brain tissues must be suppressed to ensure an adequate supply to the brain. When a fasted animal or human is then given a meal, glucose appears in the urine (glycosuria), which is a canonical symptom of diabetes. But this is because prolonged fasting (starvation) leads to decreased insulin secretion and to insulin resistance, and subsequent re-feeding then causes transient hyperglycemia and glycosuria. This SPD can be caused not only by prolonged fasting, but also by severe restriction of calorie and carbohydrate intake [38]. For example, severe calorie restriction can cause diabetes-like glucose intolerance [59]. Despite that, very low calorie diets are the most effective treatments for type 2 diabetes [60-62]. Some researchers re-discovered SPD in obese patients on strenuous weight loss program and erroneously warned that low calorie diets cause type 2 diabetes [63].

The obligatory symptom of starvation is ketosis, as ketones substitute for glucose as the main fuel for the brain. The ketogenic diet, a promising treatment for diabetes and obesity in humans, can cause symptoms of SPD in rodents (see for references [64]). Once again, some researchers warned that the ketogenic diet can favor type 2 diabetes [65]. As discussed, such warnings may not be justified [64, 66-68].

Rapamycin can be viewed as a partial starvation-mimetic [69-71]. It is therefore predictable that, under some conditions, prolonged treatment with rapamycin may lead to the emergence of SPD [72]. This has been confirmed in rapamycin-fed mice, which developed insulin resistance, glucose intolerance and mild hyperglycemia [54]. Nevertheless, rapamycin-fed mice lived longer and thus were healthier than mice fed a standard diet [54]. It is not completely clear why SPD was observed in only some studies and was not observed in other studies (see for references [38, 73]).

Importantly, SPD is reversible and does not lead to complications. Furthermore, rapamycin reduces the incidence of diabetic complications such as diabetic nephropathy in rodents [42, 74-85]. In healthy elderly humans, chronic treatment with rapamycin or everolimus did not cause hyperglycemia [8, 9, 86]. On the contrary, the risk of hyperglycemia was lower in the mTOR inhibitor treatment group than the placebo group [9].

In some cancer patients, high doses of rapamycin or everolimus can cause hyperglycemia, which is usually mild (grade 1-2) and reversible, and does not lead to treatment interruption [87-89]. Hyperglycemia is a common side effect of many oncotargeted drugs and is not a manifestation of diabetes. Everolumus, for

example, can cause hyperglycemia by decreasing insulin production [89].

To summarize, chronic treatment with high doses of rapamycin may cause symptoms of reversible SPD. Diet-induced SPD, at least, is beneficial and therapeutic. Rapamycin-induced SPD is a relatively rare side effect and probably can be avoided by administering the drug intermittently or at lower doses, and if SPD does occur, it can be reversed by discontinuation of the drug.

In some studies in transplant patients, rapamycin (sirolimus) and everolimus did not increase the risk of diabetes [90-95, 96]. In one study, no patient, out of 21 patients treated with rapamycin, developed diabetes, while the incidence of diabetes was 7% in patients treated with either cyclosporine or tacrolimus [96]. Most importantly, cyclosporine- or tacrolimus-induced diabetes resolved in 80% of patients after conversion from tacrolimus/cyclosporine to rapamycin (sirolimus) [96].

On the other hand, a large retrospective study reported an association between Medicare billing for diabetes treatment and rapamycin use, implying that rapamycin may increase the risk of diabetes [97]. However, this association was explained by the interaction between rapamycin and calcineurin inhibitors, which increase each other's levels [96, 98, 99]. Consequently, it remains unclear whether rapamycin per se increases the risk of diabetes in transplant patients [96]. Moreover, this is further complicated by the fact that most transplant patients develop type 2 diabetes spontaneously without rapamycin treatment [100].

Rapamycin is not much more dangerous than ordinary drugs

If used properly, rapamycin is not much more dangerous than ordinary aspirin. Aspirin, one of the most widely used nonprescription medications, may cause numerous side effects, including life threatening gastric bleeding. The manufacturer lists as possible side effects: ringing in ears, confusion, hallucinations, seizure, severe nausea, vomiting, bloody stools, coughing up blood, fever and swelling. Still, millions of people take aspirin daily to prevent cardiovascular disease and cancer. It was calculated that the benefits of aspirin are greater than their risks [101, 102]. I believe the benefits of the anti-aging effects of rapamycin/ everolimus may even be greater (Figure 1).

In the case of rapamycin and everolimus, the most worrying side effects have not been confirmed. At low doses [8, 9, 86], or when administered as a single high dose [103], no side effects have been detected so far in the elderly. At high doses, rapamycin and everolimus slow cell proliferation, which decreases blood cell counts. As a result, mild and reversible thrombocytopenia (low platelet count), anemia and leukopenia are their most common side effects. But a mild reduction of platelets may be beneficial. In fact, one of the intended effects of aspirin is a decrease in platelet function.

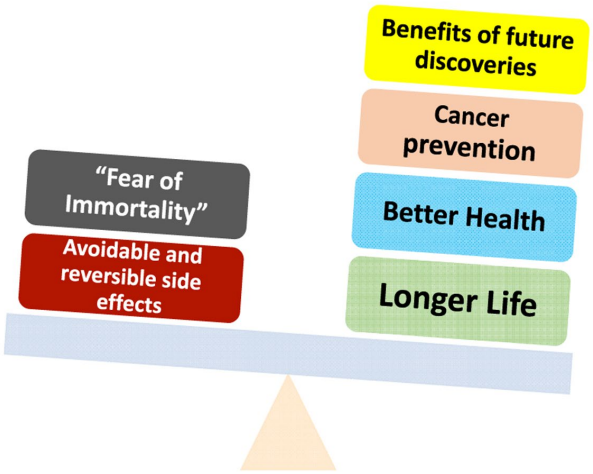

Figure 1. Potential risk vs benefits of rapamycin-based anti-aging therapy. Pros and Cons: Potential benefits of rapamycin may outweigh its risks.

There is one crucial reason why the side effects of rapamycin are exaggerated. The frequency of rapamycin side effects has often been estimated in studies lacking a placebo group. In cancer and transplant patients, numerous effects ascribed to rapamycin, such as fatigue (asthenia), for example, are often caused by the disease itself. In a placebo study of healthy volunteers, the placebo group reported more side effects such as fatigue than did the rapamycin group [104]. In recent placebo-controlled studies in healthy elderly people, no side effects were noticed as compared to placebo [9, 86].

While aspirin may cause gastric ulceration and bleeding, rapamycin may cause stomatitis and mycositis (ulceration of the mucous membranes of the mouth and the digestive tract) when used at high doses or chronically. A rare side effect of rapamycin is non-infectious interstitial pneumonitis [105]. And by inhibiting neutrophil function, rapamycin may increase the severity of bacterial infections [106]. These side effects require rapamycin's discontinuation. For antiaging purposes, however, rapamycin may be used either intermittently (e.g., once a week) or at low daily doses and can be discontinued if any unpleasant effects occur.

From a single dose to intermittent schedules

Although nearly all drugs, including nonprescription drugs such as aspirin, can be fatal at sufficiently high doses, there are no known fatal cases of acute rapamycin (sirolimus) overdose [103]. For example, in a failed suicide attempt, an 18-year-old woman ingested 103 rapamycin tablets (103 mg), and the only detected effect was an elevation in total blood cholesterol [103]. In rats, rapamycin's LD50, a measure of drug lethality, could not be determined because it is higher than 2500 mg/kg. While a single dose of rapamycin is safe, it is sufficient to extend life and decrease obesity in several rodent models [1, 107]. Furthermore, transient treatment with rapamycin can be long lasting, extending the lifespan and preventing obesity long after drug discontinuation [107-112]. The magnitude of life extension by rapamycin depends mostly on reaching a high peak blood level [113]. A similar conclusion was reached by a study of rapamycin use in obesity [112]. It was suggested in 2008 that a pulse (intermittent) schedule of rapamycin administration would improve regeneration of stem cells [114] while avoiding mTORC2 inhibition [54, 115].

Therefore, to avoid side effects and maximize anti-aging effects [110], a feasible approach would be to prolong intervals between rapamycin administrations while keeping the total dose constant. For example, instead of daily administration, a weekly administration of a higher dose can be suggested to achieve a high peak blood level, followed by drug-free period to avoid undesirable effects. Still, everyday treatment of the elderly (1 mg/day for several weeks) was not associated with side effects and has been shown to be safe [86]. Similar results were achieved with low doses of other mTOR inhibitors [9]. Another option is an alternating schedule; for example, a 3- month course of weekly rapamycin alternating with a rapamycin-free month. Finally, anti-aging schedules can be very flexible to fit an individual patient. The optimal anti-aging dose is a personalized maximum dose that does not cause side effects in a particular patient (Figure 2).

In conclusion, the side effects of rapamycin are well-known and reversible. When used on an anti-aging schedule, side effects may be absent but, if not, they may be mitigated by combining rapamycin with other anti-aging drugs (metformin, statins) or by temporarily discontinuing it.

Noteworthy, the alternative to the reversible (and avoidable) side effects of rapamycin/everolimus are the irreversible (and inevitable) effects of aging. And by living longer, our generation will benefit from future anti-aging discoveries (Figure 1).

But the fear of nonexistent side effects is not the only reason the use of mTOR inhibitors for life extension has been questioned. The second reason is that there is rightful skepticism about any claims made about anti-aging drugs because thousands of anti-aging remedies have already failed. What then makes rapamycin different?

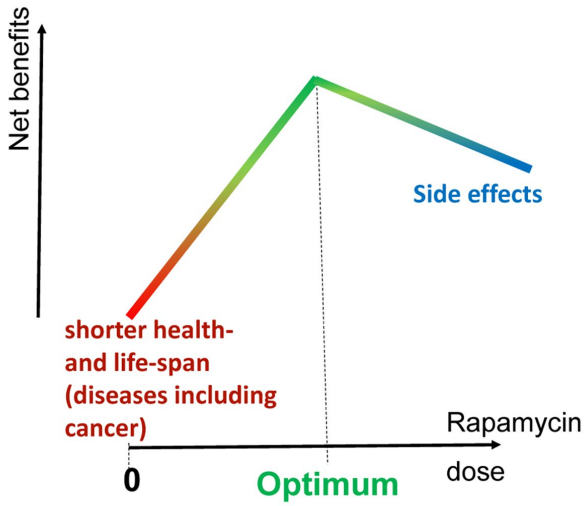

Figure 2. Optimal dose of rapamycin for maximal net benefits. Life extension by rapamycin is dose-dependent in rodents. The higher the dose, the higher the anti-aging benefits, including cancer prevention and life extension. In humans, side effects are dose-dependent and net benefits could potentially decrease at very high doses. This point of the highest net benefit is the optimal dose. The optimal dose varies in different individuals due to the variability of potential side effects. Thus, the optimal dose in a particular individual is determined by the emergence of side effects. The treatment can be viewed as life-long phase I/II clinical trial.

The history of mankind: empty promises of immortality

On the one hand, from the dawn of civilization humans have dreamed of immortality. On the other hand, from the dawn of civilization a myriad of anti-aging remedies turned out to be empty promises. Even worse, they often shorten lifespan. Two notable examples are antioxidants and human growth hormone. The idea that free radicals, or reactive oxygen species (ROS), cause aging was based on a "wild guess," as Harman, a father of the ROS theory, acknowledged when he titled his paper, "I thought, thought, thought for four months in vain and suddenly the idea came" [116]. The idea is simple and intuitive, and it was widely accepted based on circumstantial evidence. In fact, ROS are inevitable products of metabolism, and they do damage bio-

molecules. Moreover, excessive ROS can shorten lifespan. Similarly, the atomic bomb can shorten life span. Yet this does not mean that either atomic bombs or oxidants are the cause of normal aging as we know it.

Numerous experiments support the ROS theory. However, key experiments ruled the ROS theory out (see for references [2, 117-122]. To make a long story short, antioxidants could in theory prolong lifespan if mTOR-driven (quasi-programmed) aging were suppressed and we lived long enough to die from ROS-induced post-aging syndrome (I will discuss the nuances in the forthcoming article "ROS and aging revisited"). Indeed, ROS will kill any organism eventually. However, organisms normally die from mTOR-driven, age-related diseases (aging as we know it) before ROS can kill them (see for discussion [2]). As an analogy, consider most of the passengers on the Titanic. Would antioxidant treatment have been useful to them for life extension? The best way to extend life for members of that group would have been to carry more life boats. Only after their safe rescue could one expect antioxidants to potentially increase their life further. Similarly, only after rescue from the quasi-program of aging may antioxidants potentially have an impact.

Not surprisingly, antioxidants did not extend lifespan in any clinical trials and were detrimental in some [122-133]. As Ristow put it, they were "worse than useless" [119]. For example, in two very large randomized controlled trials, antioxidants increased the incidence of cancer, especially of lung cancer in smokers [131-133]. Antioxidants also increased all-cause mortality. The results were so disturbing that two trials were stopped earlier than planned [131-133]. Also disturbing is the finding that antioxidants accelerate cancer progression and promote metastasis [134-136]. But despite their uselessness, antioxidants continue to be a multibillion-dollar business. They are widely sold as natural products in the forms of nutritional supplements and in foods "rich in antioxidants."

Another example is human growth hormone (HGH), which is widely used for rejuvenation and longevity. Yet, it actually accelerates aging and shortens lifespan [137, 138]. Growth hormone is a pro-aging hormone because it indirectly activates mTOR [139]. Notably, the hype around growth hormone is based on a single publication [140], which misinterpreted its acute effects [141].

Given that all previous anti-aging remedies have failed to meet expectations, it is not surprising that the discovery of the anti-aging effects of rapamycin are being met with skepticism too. But unlike HGH, the effects of rapamycin are not based on one single paper as were HGH, nor is it based on a wild guess as were ROS.

Rapamycin is a proven anti-aging drug

The evidence that rapamycin can function as an anti-aging drug is the product of thousands of scientists working independently all over the world, studying mTOR and its inhibitors for a variety of different reasons in diverse organisms, ranging from yeast to humans. Studies in model organisms, such as yeast, worms and flies, have revealed components of the TOR signaling pathway [142-145]. It was predicted in 2003[146] that conversion from quiescence to senescence (geroconversion) is driven by growth-promoting mediators, such as mTOR, when the cell cycle is blocked [147]. Figuratively, geroconversion is "twisted" growth that occurs when actual growth is completed [2], [147]. In cell culture, mTOR is maximally activated and geroconversion lasts 3-6 days, whereas in the human body it may take decades. mTOR drives geroconversion, rendering cells hypertrophic and hyperfunctional (e.g. senescence-associated secretory phenotype), which eventually leads to the development of age-related pathologies [2]. Working independently, clinical researchers have studied rapamycin for the prevention and treatment of nearly every age-related disease, including cancer, obesity, atherosclerosis and neurodegeneration. If a drug is indicated for all age-related diseases, it must be an anti-aging drug in that it targets a common driver of age-related diseases – that is, aging (see for references [2]). This is because aging is the sum of all age-related diseases, which limit lifespan [148-150]. Does rapamycin suppress aging and extend lifespan by preventing diseases, or does it prevent diseases by slowing aging? Actually, both reflect the same process.

By 2006, an extensive body of work from several independent fields all pointed to rapamycin as an anti-aging drug [2]. According to hyperfunction theory, aging is an unintended (not programmed but quasi-programmed) continuation of the developmental growth program, driven in part by mTOR [2, 120, 121, 151, 152]. Testable predictions have been formulated [2, 153] and confirmed in numerous independent studies (see for references: [150, 154]).

In two dozen studies using different strains of mice, rapamycin extended life span. Starting from a thorough study by Harrison et al. [155] and followed by nearly simultaneous studies by others [33, 108], the anti-aging effects of rapamycin have been confirmed many times (see for references: [113, 150, 156, 157]). Importantly, rapamycin and everolimus are indicated in most, if not

all, age-related diseases, from cancer to neurodegeneration [2, 158].

Conventional drugs as anti-aging agents

Several conventional drugs used to treat age-related diseases (e.g., hypertension, ischemic heart disease, diabetes, cancer, prostate enlargement) can be viewed as somewhat anti-aging drugs [150, 154]. First, these drugs extend lifespan in the same model organisms (see for references: [159]). For example, metformin extends lifespan not only in mice, but also in the worms, which do not suffer from human diseases [160, 161]. ACE inhibitors prolong life not only in hypertensive rats, but also in healthy normotensive rats [162]. If these drugs were not ordinary drugs for human diseases, then gerontologists would call them anti-aging agents.

Second, these drugs prevent or treat more than one disease. For example, metformin is indicated to treat type 2 diabetes as well as pre-diabetes, obesity, metabolic syndrome, cancer, and polycystic ovary syndrome [163-168]. Aspirin not only reduces inflammation (a hallmark of aging), it also reduces the risk of cardiovascular disease, thrombosis and cancer. Low-dose aspirin prevents one-third of colorectal, gastric, and esophageal cancers [169]. PDE5 inhibitors such as Sildenafil and Tadalafil, which are widely used for erectile dysfunction, are also effective against benign prostatic hyperplasia (BPH) and pulmonary arterial hypertension in humans and suppress inflammation-driven colorectal cancer in mice [170]. Aging is the sum of all these age-related diseases. Given that humans and animals die from age-related diseases, life can be extended by treating multiple pre-diseases and diseases. Rapamycin and these drugs may complement each other in an anti-aging formulation by further extending life and/or by mitigating each others possible side effects [159]. For example, metformin may counteract rapamycin-induced hyperglycemia [171].

Not taking rapamycin may be as dangerous as smoking

Strangely, the fear of tobacco smoking is less intense than the fear of rapamycin. But whereas smoking shortens both the healthspan and lifespan, rapamycin extends them. Smoking increases the incidence of cancer and other age-related diseases. Rapamycin prevents cancer in mice and humans. Heavy smoking shortens life expectancy by 6-10 years. In other words, simply *not* smoking prolongs life by 6-10 years. In middle-aged mice, just 3 months of high-dose rapamycin treatment was sufficient to increase life expectancy up to 60% [109]. When taken late in life, rapamycin increases lifespan by 9-14% [155], despite the dosage being suboptimal [111]. This possibly equates to more than 7 years of human life. By comparison, smokers who quit late in life (at age 65 years), gain between 1.4 -3.7 years [172]. Considered in those terms, one could say that in the elderly, *not* taking rapamycin may be even more "dangerous" than smoking. Finally, rapamycin may be especially beneficial to smokers and former smokers. While the carcinogens from tobacco cause lung cancer in mice, rapamycin decreases tobacco-induced lung cancer multiplicity by 90% [28].

Diet and rapamycin

Calorie restriction (CR) and intermittent fasting (IF) extend both the lifespan and healthspan in diverse species. However, CR is of little benefit when started in old age [73, 173-178]. Fasting inhibits the mTOR pathway in young but not old mice [179, 180]. By contrast, rapamycin strongly inhibits mTORC1 at any age. It extends lifespan, whether started late or early in life [108, 155, 181], even if used transiently [109]. So, whereas CR is more beneficial early in life, rapamycin may be indicated later in life. In addition, the beneficial effects of rapamycin and CR may be additive, given that they are exerted through overlapping but distinct mechanisms [182-186]. Intermittent rapamycin and CR (24-48 hours after) can be combined, to avoid potential hyperglycemia. Physical exercise may be most beneficial starting immediately after rapamycin use, to take advantage of rapamycin-induced lipolysis as a fuel for the muscles. By itself, chronic rapamycin treatment does not compromise muscle endurance [187] and even prevents muscle loss [188-190].

Do we need new or safer rapalogs to start aging prevention?

Despite the metabolic side effects seen in some mouse models, mice treated with rapamycin live longer and are healthier. Humans also may want to live longer and healthier lives, regardless of whether one calls the means unsafe. Some basic researchers believe that rapamycin cannot be routinely used to treat aging in humans because of its metabolic effects and call for the development of safer analogs. First, rapamycin and everolimus are FDA-approved drugs, safe for human use. Since 1999, rapamycin has been used by millions of patients with no unexpected problems. One may suggest that rapamycin/everolimus are safe enough for very sick patients, not for healthy people.

First, healthy elderly people chronically treated with rapamycin or other mTOR inhibitors showed no ill effects (e.g. hyperglycemia) [8, 9, 86]. Logically, more

threatening adverse effects could be expected in cancer and transplant patients, who are often heavily pre-treated and terminally ill than in healthy people. Second, there are no truly healthy people among the elderly; otherwise, they would be "immortal", given that all humans die from age-related diseases, not from healthy aging. And the sooner they would be treated with anti-aging drugs, the longer they would remain relatively healthy.

That said, it is, of course, important to develop new rapalogs, but not because current rapalogs are unsafe. It is important because such research will help us to learn more about mTOR and aging and may lead to the discovery of agents capable inhibiting the rapamycin-insensitive functions of mTORC1. These future drugs could potentially complement current rapalogs to further extend lifespan. Non-rapalog rapamycin analogs will also be developed [191]. The limitation of current rapalogs is not that they are unsafe but that their ability to extend life is limited. The goal should be to develop new drugs that extend life span further.

Rapamycin is a natural anti-fungal antibiotic produced by soil bacteria of Eastern Island. The patent on rapamycin has expired, and pharmacological companies have developed other rapalogs such as everolimus. (I use the term rapalogs to encompass both rapamycin, everolimus and any other analogs). At equipotent doses, rapamycin and everolimus exert almost identical therapeutic and adverse effects; although, everolimus is weaker and has a shorter half-life in the organism compared with rapamycin.

All current rapalogs exhibit the same side effects as rapamycin and everolimus. Their real side effects are mTORC1-dependent. Inhibition of mTORC1 decreases cell proliferation and function, which is manifested as lower blood cell counts and insulin levels, especially when rapalogs are chronically administered at high doses. We could develop weaker rapalogs, which would have no side effects if used at the same dose as rapamycin. But then why not just use a lower dose of rapamycin? (I will discuss elsewhere how safer rapalogs are probably weaker rapalogs.) Given to mice at the same doses as rapamycin, weaker analogs would have neither side effects and no therapeutic effects. Consequently, their metabolic effects would be diminished and so would their therapeutic effects. However, the same negative result can be achieved simply by decreasing the dose of rapamycin. While waiting for silver bullets, we need to use the currently available rapalogs, such as rapamycin and everolimus, to live longer. When "safer" rapalogs are clinically available, we may use them too.

The time is now unless it's too late

The overwhelming evidence suggests that rapamycin is a universal anti-aging drug – that is, it extends lifespan in all tested models from yeast to mammals, suppresses cell senescence and delays the onset of age-related diseases, which are manifestations of aging [discussed by me in [148, 149, 158, 192]. Although rapamycin may reverse some manifestations of aging [181, 193], it is more effective at slowing down aging than reversing it. Therefore, rapamycin will be most effective when administered at the pre-disease, or even pre-pre-disease stages of age-related diseases [150]. For example, Carosi et al. suggested that mTOR inhibitors could be useful in Alzheimer disease, but only in the earliest stages [194, 195]. In addition, rapamycin and everolimus are more effective for preventing cancer than treating it. They may also be useful for treating osteoporosis, though not a broken hip after an osteo-porotic fracture. Rapalogs may slow athero-sclerosis, thereby preventing myocardial infarction, but they are unlikely to help reverse an infarction. In other words, anti-aging drugs extend the healthspan (Figure 3) and are most effective before overt diseases cause organ damage and loss of function.

So, is it too late to take rapamycin once aging reaches an unhealthy stage? Actually, it is not too late. Even if one or a few age-related diseases renders aging unhealthy, other potential diseases are still at pre-disease stages, and anti-aging drugs may delay their development. And they may slow down further progression of existing overt diseases.

In addition to rapamycin/everolimus, the anti-aging formula metformin, aspirin, ACE inhibitors, angiotensin receptor blockers and PDE5 inhibitors, each of which can prevent or treat more than one age-related disease [159]. Note that I mention only clinically-approved drugs because they can be used now. Later, perhaps, we may be able to consider further life extension through the use of low doses of pan-mTOR [196, 197], mdm-2 [198, 199] and MEK inhibitors [200, 201], lithium [201, 202], as well as next-generation rapalogs.

There is currently no consensus around the short-term markers of anti-aging effects. Therefore, rapamycin trials should be focused on its potential side effects rather than anti-aging effects. We must be sure that the therapy is safe. In the future, the treatment should be conducted as a life-long phase I/II trial, with dose escalation of rapamycin/everolimus until the side effects are reached in an individual patient. The tailored optimal dose (see Figure 2) should be determined individually for each patient and may vary widely.

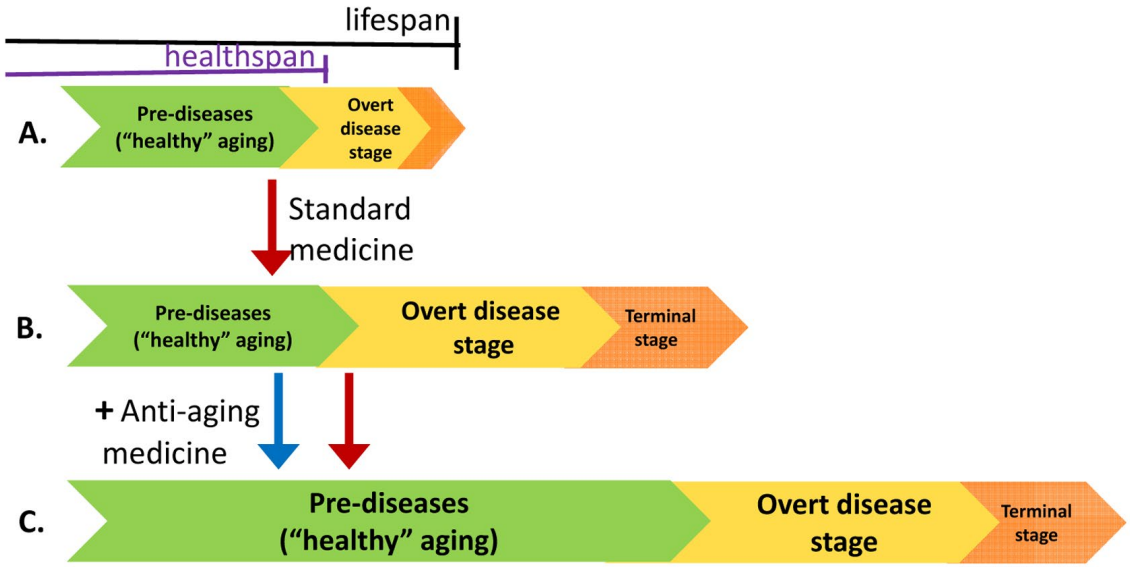

Figure 3. Effects of standard and anti-aging medicine on health- and lifespan. (**A**) The relationship between health- and life-span. Aging is a sum of all age-related diseases, pre-diseases and pre-pre-diseases. Before overt age-related diseases become apparent, there is a seemingly healthy period of aging (so-called healthy aging). Starting from adulthood, pre-pre-diseases progress towards pre-diseases and then towards overt diseases. Unless treated with modern standard medical practice, the diseased stage is relatively brief. From (**A**) to (**B**) Standard medical treatment is usually started when overt diseases are diagnosed. Standard medicine extends life span mostly by preventing death from diseases, thus extending "unhealthy" phase of life, especially terminal stages of diseases, characterized by organ damage, failure and loss of functions. Standard medicine extends lifespan. From (**B**) to (**C**) Anti-aging medicine is most effective at the stage of pre-diseases and initial stages of diseases, characterized by increased functions before complications and organ damage occur. In terminal stages of deadly diseases, anti-aging therapy may not be useful. Thus, anti-aging medicine increases both health span and life span. Anti-aging medicine and standard medicine are additive when aging becomes unhealthy. The schema is simplified because, in reality, age-related diseases start at different ages (presbyopia vs sarcopenia), progress at different paces (atherosclerosis vs cancer), and most are not lethal, and some are well treated (cataract). Therefore, healthspan is an abstraction.

Doses and frequencies should be limited by the side effects: stomatitis/mucositis, anemia, thrombopenia, leukopenia, edema, and pneumonitis. To be safe, even mild hyperglycemia should be avoided or mitigated with metformin. Treatment is intended to be life-long, unless discontinued due to side effects.

Self-medication (even by physicians themselves) should be avoided and strongly discouraged. Instead, we need anti-aging clinics that implement the entire anti-aging recipe, including a complementary low carbohydrate diet and life style changes. Blood levels of rapamycin should be measured, as the rapamycin concentration in blood varies greatly among individuals taking the same dose. Doses of rapamycin should be tailored: personalized dosing and schedules. There is no shortage of potential patients who unfortunately already employ self-medication with rapamycin, but there is a shortage of physicians to treat them. Fortunately, a prototype clinic already functions in the USA, demonstrating that it is feasible from a regulatory standpoint (see Alan Green's practice, Little Neck, NY). We cannot wait for results from others if we want to live longer and healthier ourselves. The time is now.

Disclaimer

This article is addressed to clinical scientists and physicians. It is intended for informational and educational purposes only. Medical doctors interested in this topic may e-mail the author at Blagosklonny@rapalogs.com

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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Commentary

Rapamycin for the aging skin

Mikhail V. Blagosklonny

In 2007, I filed a patent application claiming that topical rapamycin (e.g., in the form of a cream or ointment) https://patents.google.com/patent/WO2008022256A2/en could be used to prevent and treat skin aging. Potential indications include various types of age-related spots, wrinkles, photo-aged skin, and other age-related skin conditions. The patent was not granted, nor were cosmetic companies interested in pursuing this avenue of product development. Cell senescence has traditionally been seen as growth arrest. It seemed weird that rapamycin, a drug that inhibits growth, could inhibit cellular senescence. Nonetheless, it works because, actually, senescence is a continuation of growth when true growth is impossible [1]; in other words, senescence is "twisted" growth [2]. In an exciting 'twist', these claims were recently confirmed in a clinical trial by Chung et al. [3], which I will discuss later.

Even in 2007, the idea of using rapamycin topically was not novel [4, 5]. (What was novel in my application was the idea of using topical rapamycin as an anti-aging drug for the aging skin [1]). By now, there have been dozens of papers describing the therapeutic use of rapamycin (Sirolimus) in patients with such skin diseases as lymphatic malformations, vascular anomalies, Facial Angiofibroma and psoriasis [6-13]. These diseases were treated in children and young adults. In one study, topical rapamycin at low doses (0.003-0.015%) decreased facial angiofibromas in young adults. There was no systemic absorption of rapamycin (blood levels were <1.0 ng/mL) [13].

Returning to cellular senescence, signaling in the mTOR (Target of Rapamycin) pathway drives growth of cellular mass and sustains cell cycle progression. Cells grow and divide, balancing growth. But when the cell cycle is suddenly blocked by p16 or p21, mTOR drives growth-like conversion from reversible arrest (quiescence) to senescence [2, 14]. In short, mTOR drives geroconversion [15]. Rapamycin and its analogs, as well as pan-mTOR inhibitors, suppress geroconversion, thereby maintaining cells in a young healthy state. Moreover, these drugs prevent loss of cells' proliferative potential, which is considered a strict definition of senescence [2, 15]. Geroconversion in stem cells leads to stem cells depletion [16, 17]. mTOR-driven hypertrophy can be followed by atrophy at the end stages. Cellular hyperfunction eventually leads to cellular exhaustion and secondary functional decline [1].

Suppression of cellular senescence by rapamycin was demonstrated in numerous studies both in vivo and in vitro [18-30] and see for references [15]. In vitro, rapamycin slows conversion to senescence by approximately 3-fold [14]; it does not suppress it completely. Notably in that regard, in the most rapamycin-responsive mouse model of mitochondrial disease, rapamycin extends the maximum life span by nearly 3-fold [31].

Just as *in vitro* geroconversion is a continuation of growth, organismal aging is an unintended and harmful continuation of developmental growth post-development [1, 32]. These messy quasi-programs inevitably lead to age-related diseases, which include conditions ranging from obesity, cancer and Alzheimer's disease to skin spots, wrinkles and seborrheic keratoses. mTOR drives geroconversion, increasing cellular functionality (e.g., the senescence-associated secretory phenotype). It is noteworthy that this increase in cellular activity can cause secondary exhaustion, tissue damage and decreased of organ function; for example, hypertrophy may be followed by atrophy at later stages. In other words, age-related diseases and conditions initially caused by mTOR-driven hyperfunction eventually lead to organ damage and functional decline [1, 33]. Similar quasi-programs were described even in the worm [34-36]. In sum, aging is an unintentional and harmful continuation of developmental programs, driven in part by mTOR. To be clear, mTOR activity does not need to increase with age, just keeping it at a level as high as during development is sufficient to cause disease. Despite its simplicity, this model accurately predicts that rapamycin will extend life and delay diseases. Indeed, since initial publications [18, 37, 38, 39], numerous studies have confirmed that rapamycin extends lifespan in mice (see for references [40-44]).

In that context, it is predictable that rapamycin would slow skin aging. However, unless rapamycin reverses skin aging, not merely slow it, the effect would be difficult to document. This is because a patient cannot serve as a self-control (placebo control) unless rapamycin reverses aging, which would be easy to detect. This difficulty can be overcome, however, by comparing an untreated hand with a hand treated with topically applied rapamycin in the same subject. This is the approach taken by Chung et al. in their study, which found that treatment with rapamycin-containing cream

improved skin photoaging and skin tone, decreased fine wrinkles, increased dermal volume, and reduced sagging of the skin [3]. These differences between treated and untreated hands were detectable after 4 months of the treatment [3]. Regrettably, the study excluded patients with diabetes, although the therapeutic effect would probably be more significant in diabetic patients, given that mTOR is overactivated in that disease. In addition, it is unclear whether rapamycin reversed skin aging and improved the skin or merely slowed the progression of skin aging. In the latter scenario, the difference between the treated and untreated hands is due to the progression of aging in the untreated hands. In combination with placebo/treatment, comparisons of specific abnormalities before and after treatment is also needed. Despite these open questions the study is remarkable [3].

As a cosmetic, rapamycin-containing cream may be applied to selected areas, like the hands and face, especially skin affected by age-related spots and pathologies. It should not be applied to the entire skin surface of the body. To affect the entire skin surface, systemic use of rapamycin would likely be a better option, as many manifestations of skin aging are probably due to systemic organismal aging and disease; skin aging is not an exclusively local process. And most importantly, systemic rapamycin use increases lifespan and decreases disease. This by itself is so important that solely topical use of rapamycin may seem insufficient. On the other hand, topical application of any drug is safer than systemic administration. Still, the best strategy in some cases may be simultaneous systemic and topical use of rapamycin in selected areas of the skin, especially areas where there are signs of aging marks. However, given that most doctors are fearful of systemic treatment with rapamycin [45], I expect that it will be topical use of rapamycin that becomes widespread, if regulatory hurdles can be overcome. Whether rapamycin cream should be a prescription treatment or an over-the-counter cosmetic will likely be a matter of debate.

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Theory Paper

Disease or not, aging is easily treatable

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ABSTRACT

Is aging a disease? It does not matter because aging is already treated using a combination of several clinically-available drugs, including rapamycin. Whether aging is a disease depends on arbitrary definitions of both disease and aging. For treatment purposes, aging is a deadly disease (or more generally, pre-disease), despite being a normal continuation of normal organismal growth. It must and, importantly, can be successfully treated, thereby delaying classic age-related diseases such as cancer, cardiovascular and metabolic diseases, and neurodegeneration.

Endless debate on aging and disease

For decades, one of the most debated questions in gerontology was whether aging is a disease or the norm. At present, excellent reasoning suggests aging should be defined as a disease [1-7]. I tend to define aging a disease, even though it is the norm. Vladimir Dilman referred to aging as "normal disease" [8, 9].

As I emphasized in my publications, aging is not programmed. I have explicitly stated as such even in my article titled "Aging is not programmed: genetic pseudo-program a shadow of development growth" (PMID: 24240128). Aging is a normal continuation of the normal develop-mental program, so it is NOT a program but a purposeless, unintended quasi-program [10-16]. Yet, aging is also a deadly disease because it inevitably leads to death.

Indeed, aging is "the sum of all age-related diseases" and this "sum is the best biomarker of aging" [17]. Aging and its diseases are inseparable, as these diseases are manifestations of aging. Of course, any one age-related disease can occur at a young age due to genetic and environmental factors. What is important is that aging is sufficient to cause all age-related diseases, sooner or later, without dependence on genetic or environmental factors [18]: if Alzheimer's disease or type 2 diabetes is not diagnosed during ones life time, it is only because cancer or a stroke terminates life before

Alzheimer's diseases or type 2 diabetes can be diagnosed (and vice versa).

Aging is the sum of pre-diseases and diseases

Aging is an increase in the probability of death due to age-related diseases, which are late manifestations of aging [18]. Diseases are preceded by pre-diseases. For example, diabetes is diagnosed when fasting glucose levels are higher than 125 mg/dl, while levels of 100 to 125 mg/dl are considered pre-diabetes. Remarkably, diabetic complications such as nephropathy and retinopathy often develop before type 2 diabetes itself (see for references [19]). Although not formally a disease, pre-diabetes is currently treated to prevent diabetes [20-23]. Moreover, pre-diabetes is initiated by underlying processes that we will call pre-pre-diabetes, which arise while fasting glucose levels and glucose tolerance are still normal, though insulin levels are increased (hyperinsulinemia), indicating mild insulin resistance [24]. Hyperinsulinemia in healthy adults with normal glucose levels is predictive of type 2 diabetes

over a 24-year follow-up [25, 26]. Normal glucose levels (<100 mg/dl) associated with hyperinsulinemia is pre-pre-diabetes [27]. Hyperinsulinemia may in turn be driven by mTOR signaling [19], which suggests a state of pre-pre-pre-diabetes in which both glucose and insulin levels are normal. The condition that we can call pre-pre-diabetes is associated with future diabetes, cardiovascular disease and the all cause mortality rate [28]. Preventive treatment with metformin has been initiated during these very early disease stages in obese adolescents [29].

Another example is hypertension (a disease), which is defined arbitrarily as blood pressure (BP) above 140/90 mmHg. Pre-hypertension (or borderline hypertension) is defined as BP below 140/90 mmHg but higher than 120/80 mmHg. BP tends to increase with age, and those whose BP has not yet reached 140/90 (disease), or even 120/80 (pre-disease), may still have higher BP than they did when they were younger [30]. Mortality is associated with BP, even if it is lower than 140/90 [31]. Both pre-hypertension and pre-diabetes are age-related pre-diseases. Likewise, the asymptomatic stages of Alzheimer's disease are also pre-disease.

In pre-diseases, abnormalities have not reached the arbitrary diagnostic criteria of the diseases. So, aging consists of progression from (pre)-pre-diseases (early aging) to diseases (late aging associated with functional decline). Aging is NOT a risk factor for these diseases, as aging consists of these diseases: aging and diseases are inseparable (Figure 1).

An aged appearance (e.g., grey hair, wrinkles, cushingoid body types and loss of muscles) are manifestations of pre-diseases. For example, an aged appearance may reflect hypercortisolism, sarcopenia, osteoporosis, skin pre-diseases and so on. And age-related skin lesions may herald pre-cancerous skin conditions [32].

What is "healthy" aging?

What then is aging without diseases, so called "healthy" aging. "Healthy" aging has been called subclinical aging [33], slow aging [18, 34] or decelerated aging [35], during which diseases are at the pre-disease or even pre-pre-disease stage. Diseases will spring up eventually. "Healthy" aging is a pre-disease state in which asymptomatic abnormalities have not yet reached the artificial definitions of diseases such as hypertension or diabetes. Instead of healthy aging, we could use the terms pre-disease aging or decelerated aging. Furthermore, decelerated aging can be achieved pharmacologically. For example, rapamycin decelerates aging, thereby making one healthier [36, 37].

Currently, the term healthspan lacks clarity and precision especially in animals [38]. Although the duration of healthspan depends on arbitrary criteria and subjective self-rating, it is a useful abstraction. In theory, a treatment that slows aging increases both healthspan (subclinical period) and lifespan, whereas a treatment that increases lifespan (e.g., coronary bypass, defibrillation) is not necessarily increase healthspan (Figure 1 in reference [33]). The goal of both anti-aging therapies and preventive medicine is to extend healthspan (by preventing diseases), thus extending total lifespan.

Preventive medicine: a step towards anti-aging medicine

Aging is the sum of diseases and pre-diseases. Treatments are generally more effective at pre-disease stages, associated with hyper-function, than at disease stages, associated with functional decline. As discussed in 2006, "rapamycin will prevent diseases rather than cure complications of diseases. For example, rapamycin

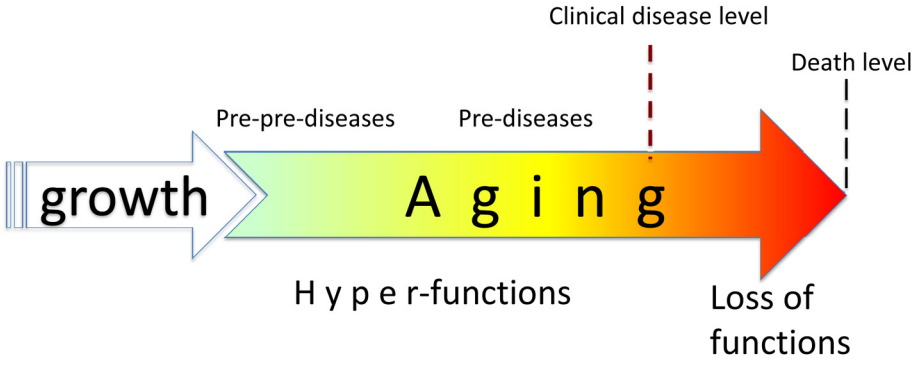

Figure 1. Relationship between aging and diseases. When growth is completed, growth-promoting pathways increase cellular and systemic functions and thus drive aging. This is a pre-pre-disease stage, slowly progressing to a pre-disease stage. Eventually, alterations reach clinical disease definition, associated with organ damage, loss of functions (functional decline), rapid deterioration and death.

will not repair broken bones but might prevent osteoporosis." [10]. In fact, rapamycin prevents osteoporosis [39].

The goal of preventive medicine is to prevent diseases by treating pre-diseases. Thus, preventive medicine is a form of anti-aging therapy. Both preventive medicine and anti-aging therapy should prevent pre-diseases by treating "healthy" individuals. Some of the drugs used in preventive medicine include statins, aspirin, ACE inhibitors (e.g., lisinopril) and metformin, which can be repurposed as anti-aging drugs [40, 41]. And *vice versa*, rapamycin, an anti-aging drug, may become a cornerstone of preventive medicine. As David Gems put it, "anti-aging treatment is any preventative approach to reduce late-life pathology. Its adoption would facilitate translation, since it would shift the emphasis to medical practice, particularly the introduction of preventative approaches." [42].

To treat what is treatable

The fact that aging is an obligatory part of the life of all organisms is not important. What is important is that aging is deadly and, most importantly, treatable. Consider an analogy. Is facial hair (beard) in males a disease? No of course, not. Still most men shave it, effectively "treating" this non-disease, simply because it is easily treatable. Is presbyopia (blurred near vision) a disease? It occurs in everyone by the age of 50 and is a continuation of developmental trends in the eye. It is treated as a disease because it is easily treatable with eye glasses. Unlike presbyopia, menopause in females is not usually treated because it is not easy to treat. Thus, the decision to treat or not to treat is often determined by whether it is possible to treat. It does not matter whether or not the target of treatment is called a disease.

Aging is treatable

As the simplest example, calorie restriction (CR) slows aging in diverse organisms, including primates [43-50]. Similarly, intermittent fasting (IF) and ketogenic diet (severe carbohydrate restriction) extend life span in mammals [48, 51-54]. CR (as well as carbohydrate restriction and IF fasting) improves health in humans [45, 48, 53, 55-62]. However, CR is unpleasant to most humans and its life-extending capacity is limited. Nutrients activate the mTOR (molecular Target of Rapamycin) nutrient-sensing pathway [63-65] and, as we will discuss mTOR drives aging, inhabitable by rapamycin. Rapamycin-based anti-aging therapies have been recently implemented by Dr. Alan Green (https://rapamycintherapy.com).

Rapamycin and other rapalogs

Rapamycin (Rapamune/Sirolimus), an allosteric inhibitor of mTOR complex 1 [63, 66], is a natural rapalog as well as the most potent and best studied rapalog. Rapamycin-analogs such as everolimus, temsirolimus (a rapamycin prodrug) and deforolimus/ Ridaforolimus are also now widely used.

Rapamycin, everolimus and deforolimus slow geroconversion [67-75]. It has been predicted that rapamycin would slow aging in mammals [10, 76]. Starting in 2009, numerous studies have demonstrated that rapamycin prolongs life in mice [75, 77-99], even when started late in life [77, 78, 97-99], or administrated transiently or intermittently [77, 88, 89, 95].

In these studies, rapamycin was most effective at high doses [88, 89, 93-96, 100-103]. Its effect and that of everolimus lingers after their discontinuation [104], even after a single dose [105]. What appears to be important is to reach high peak levels using a single high dose [93, 94].

In non-human primates, chronic and/or intermittent rapamycin improves metabolic functioning [106]. In a randomized controlled trial, middle-aged companion dogs administrated rapamycin exhibited no further side effects as compared to dogs receiving the placebo [107].

Millions of patients with various diseases and conditions (e.g., organ transplant recipients) have been treated with rapamycin (Sirolimus). Typical dose of rapamycin in organ-transplant patients is 2 mg/day. Rapamycin in a single dose of 15 mg was administrated to healthy volunteers without adverse effects [108]. Similarly, a dose of 8 mg/m2 (around 16 mg) was also well tolerated in healthy male volunteers [109]. What is amazing is that the placebo group reported more "side effects" such as astenia than did the rapamycin group [109]. In yet another study, comparison to placebo revealed no real everolimus-induced side effects in the elderly [104]. Moreover, everolimus improves immunity [110] and reduces infections in elderly healthy humans [104]. In placebo-controlled studies, side effects of rapamycin and everolimus are manageable with dose reduction and interruption. Discontinuation due to toxicity was uncommon [111]. In volunteers (aged 70-95 years, mean age of 80 years), treatment with 1mg/daily of rapamycin for 8 weeks was safe [112]. Matt Kaeberlein suggests that conventional doses of rapamycin maybe sub-optimal for maximum life-extension [113]. I agree with this opinion.

Conventional drugs as anti-aging drugs

Metformin is used not only to treat diabetes but also pre-diabetes in order to prevent diabetes [20-23]. Metformin decreases insulin-resistance and body weight and prevents diabetes, cancer and cardio-vascular disease [21, 22, 114-119]. It is expected that metformin would extend life and, in fact, metformin does decrease all-cause mortality [119, 120]. Physicians generally do not think of metformin as an anti-aging drug, simply because it is expected that life will be extended, if diseases are prevented. In mice, metformin extends healthspan and lifespan [117, 121-123]. It also extends the lifespan of *C. elegans* [124-127], which do not suffer from human diseases. Gerontologists think of metformin as an anti-aging drug [121-130], and metformin can be combined with rapamycin [131].

Angiotensin II inhibitors

Angiotensin-converting enzyme (ACE) inhibitors (e.g., Captopril, Lisinopril, Enalapril, Ramipril) and Angiotensin II receptor blockers (ARB) (e.g., Valsartan, Telmisartan, Losartan) are widely used to treat hypertension, which is a typical hyperfunctional disease. Vasoconstriction, cardiomyocyte hypertrophy, beta- and alpha- adrenergic hyperstimulation all lead to high blood pressure (systemic hyperfunction), which, in turn can contribute to stroke, myocardial infarction and renal failure. ACE inhibitors and ARBs decrease vasoconstriction and prevent cardiac hypertrophy. They are life-extending drugs because they treat deadly diseases.

Notably, ACE inhibitors increase the lifespan in rodents with normal blood pressure [132-134], thereby acting as anti-aging drugs.

Combinations of conventional drugs

Combinations of aspirin, statins, beta-blockers and ACE inhibitors are given to aging individuals to prevent cardiovascular diseases [135]. On the other hand, these drugs extend life span in rodents and Drosophila [136].

Typical combinations (polypill) include an antiplatelet agent (aspirin), a statin and two blood pressure-lowering drugs such as lisinopril and a beta-blocker [137,138]. Such combinations are estimated to reduce the 5-year incidence of stroke by 50% [139]. Aspirin, statins, ACE inhibitors, beta-blockers and metformin prevent some types of cancer and pre-cancerous polyps [116-118, 140-146].

Treating aging by preventing diseases or preventing diseases by slowing aging

As discussed, "aging is the sum of all age-related diseases" and this "sum is the best biomarker of aging" [17]. One could say that drugs prevent diseases by slowing aging. Alternatively, it could be said that prevention of diseases slows aging, which is the sum of all diseases and pre-diseases. If a drug prevents diseases, it will extend lifespan (apparently slowing down aging). If a drug slows down aging it will prevent diseases and extend healthspan [17, 147].

As suggested "narrow spectrum anti-aging treatments (e.g. the cardiovascular polypill) could establish a practice that eventually extends to broader spectrum anti-aging treatments (e.g. dietary restriction mimetics)". [42].

CONCLUSION

It is commonly argued that aging should be defined as a disease so as to accelerate development of anti-aging therapies. This attitude is self-defeating because it allows us to postpone development of anti-aging therapies until aging is pronounced a disease by regulatory bodies, which will not happen soon. Aging does not need to be defined as a disease to be treated. Anti-aging drugs such as rapamycin delay age-related diseases. If a drug does not delay progression of at least one age-related disease, it cannot possibly be considered as an anti-aging drug, because it will not extend life-span by definition (animals die from age-related diseases). It has been suggested [17], "in order to extend life span, an anti-aging drug must delay age-related diseases. … Once a drug is used for treatment of any one chronic disease, its effect against other diseases … may be evaluated in the same group of patients." Aging can be treated as a pre-disease to prevent its progression to diseases. Rapamycin-based combinations include conventional life-extending drugs, which are used to treat and prevent age-related diseases. These com-binations could be combined with modestly low-calorie/carbohydrates diet, physical exercise and stress avoidance [40, 41]. And this approach is actually being used now to treat aging at Alan Green's clinic in Little Neck, NY: http://roguehealthandfitness.com/rapamycin-anti-aging-medicine-an-interview-with-alan-s-green-m-d/?print=pdf and https://rapamycintherapy.com

CONFLICTS OF INTEREST

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Commentary

Paradoxes of senolytics

Mikhail V. Blagosklonny

Senolytics are drugs that extend lifespan and delay some age-related diseases by killing senescent cells [1-4]. In fact, drug screens have identified a diverse group of drugs that are preferentially toxic to at least some senescent cells in some cellular models [2-9]. So far, however, their selectivity against senescent cells is modest and cell-type-specific [8-11]. Nevertheless, targeting senescent cells has been shown in animal models to prevent such age-related pathologies as emphysema [12], lung fibrosis [13-15], atherosclerosis [16, 17], osteoporosis [18], osteoarthritis [19-20], renal disease [21], intervertebral disk pathology [2], hepatic steatosis [22] and other age-related conditions [4, 7, 18, 23, 24].

In this editorial commentary, I want to draw your attention to the paradoxes associated with senolytics, which argue against the dogma that says aging is a functional decline caused by molecular damage. This dogma predicts that senolytics should accelerate aging. If aging is caused by loss of function, then killing senescent cells would be expected to accelerate aging, given that dead cells have no functionality at all. Instead, however, senolytics slow aging, which highlights a contradiction in the prevailing dogma.

The theory of hyperfunctional aging [25-32] addresses this paradox. Killing senescent cells is beneficial because senescent cells are hyperfunctional [33]. The hypersecretory phenotype or Senescence-Associated Secretory Phenotype (SASP) is the best-known example of universal hyperfunction [34-36]. Most such hyperfunctions are tissue-specific. For example, senescent beta cells overproduce insulin [37] and thus activate mTOR in hepatocytes, adipocytes and other cells, causing their hyperfunction, which in turn leads to metabolic syndrome (obesity, hypertension, hyperlipidemia and hyperglycemia) and is also a risk factor for cancer [38-40]. SASP, hyperinsulinemia and obesity, hypertension, hyperlipidemia and hyperglycemia are all examples of absolute hyperfunction (an increase in functionality). In comparison, relative hyperfunction is an insufficient decrease of unneeded function. For example, protein synthesis decreases with aging, but that decrease is not sufficient [30]. In analogy, a car moving on the highway at 65 mph is not "hyperfunctional." But if the car were to exit the highway and enter a residential driveway at only 60 mph it would be "hyperfunctional," and stopping that car would likely prevent damage to other objects.

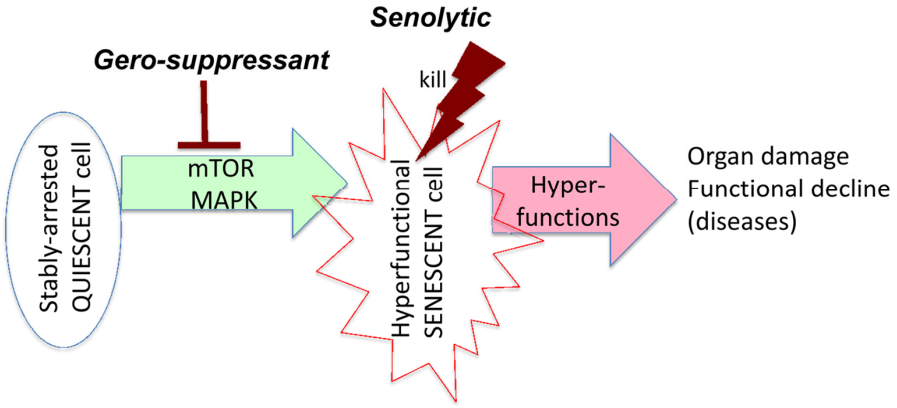

Figure 1. Target of senolytics in the aging quasi-program. In post-mitotic quiescent cells in an organism, growth-promoting effectors such as mTOR drive conversion to senescence. Hyperfunctional senescent cells activate other cells (including cells in distant organs), rendering them also hyperfunctional, which eventually leads to organ damage. This process manifests as functional decline, a terminal event secondary to initial hyperfunction. Senolytics such as ABT263 or 737 kill hyperfunctional senescent cells, preventing damage to organs. Gerosuppressants such as rapamycin suppress geroconversion and may decrease hyperfunction of already senescent cells, thereby slowing disease progression (not shown here in scheme).

Similarly, killing hyperfunctional cells can prevent organismal damage. Senolytics eliminate hyperfunctional cells, which otherwise damage organs (Figure 1).

Senolytics should not be confused with gerosuppressants (Figure 1). Gerosuppressants, such as rapamycin, do not kill cells; they instead prevent cellular conversion to senescence (geroconversion) [33]. Rapamycin also slows disease progression by limiting the hyperfunction of senescent cells. Notably, some senolytics are also gerosuppressants. For example, inhibitors of MEK [41-43] or PI3K [2, 41] are both gerosuppressants [41] and senolytics [2, 42, 43].

It may seem paradoxical that senolytics are anticancer drugs [44] because standard anticancer agents cause molecular damage. According to the hyperfunction theory [45], molecular damage does not cause aging. Although accumulation of molecular damage does happen and would destroy the organism eventually, no organism lives long enough for that to occur because TOR-driven (hyperfunctional) aging kills it first. If TOR-driven aging (i.e., aging as we currently know it) were abolished, then organisms would die from "post-aging syndrome" due to molecular damage (see Figure 8 in ref. [25]). Molecular damage contributes to some age-related diseases. But these diseases would arise even without molecular damage [45]. Molecular damage is essential for most types of cancer, but a senescent microenvironment [46] and overall organism aging (and associated diseases such as diabetes) also play roles [47], as does clonal selection for mTOR activation in cancer cells [48]. Importantly, molecular damage renders cancer cells robust and hyperfunctional. Cancer cells kill an organism not because molecular damage makes them weak; it is because the molecular damage makes them robust and hyperfunctional. If accumulation of molecular damage leads to immortalization and robustness, then aging cannot represent functional decline caused by molecular damage [48].

All senolytics, without exception, were initially investigated or specifically developed as anticancer drugs. But not all anticancer drugs are senolytics. Both senolytics and gerosuppressants belong to a very special subgroup of oncotargeted drugs [49]. Various pathways involving IGF-1, Ras, MEK, AMPK, TSC1/2, FOXO, PI3K, mTOR, S6K, and NFκB comprise a mTOR-related network and are involved in aging [49]. Oncoproteins promote aging, while tumor suppressors are gerosuppressors, which inhibit aging [48, 50]. As depicted a decade ago (see Figure 3 in ref. [51] and Figures 4 and 9 in ref. [25]), oncotargets are gerotargets that are also mTOR activators, while tumor and aging suppressors are mTOR inhibitors. In brief, geroconversion and oncogenic transformation are two sides of the same process [50]. Gerogenic oncogenes activate the mTOR pathway, driving geroconversion of cell cycle-arrested cells. When cell cycle control is disabled, they drive oncogenic transformation [48, 50].

Many puzzles remain. For example, killing senescent adipocytes, macrophages or foam cells will slow diseases such as atherosclerosis and metabolic diseases, and killing senescent glial cells can prevent cognitive decline [23]. On the other hand, killing some senescent cell types may be counterproductive. For example, killing senescent beta cells may lead to diabetes [37], and killing of senescent hyperfunctional neurons in Alzheimer's disease may have unpredictable consequences. Fortunately, senolytics are tissue-specific and only kill some types of senescent cells [8-11], which may make them safer.

To add further complication to the paradoxes associated with senolytics, it was shown that many detected p16/β-gal-positive cells are not senescent cells, but are instead hyperfunctional macrophages, which contribute to aging [52-54]. Notably, β-gal staining is a marker of hyperfunctional lysosomes [55]. A combination of markers, including mTOR targets, is needed to define senescence [33]. Some senolytics that target Bcl2 family proteins may theoretically kill leukemia/lymphoma cells. I hope to discuss these and other issues in a scheduled review "Senolytics, gerosuppressants and conventional life-extending drugs."

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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