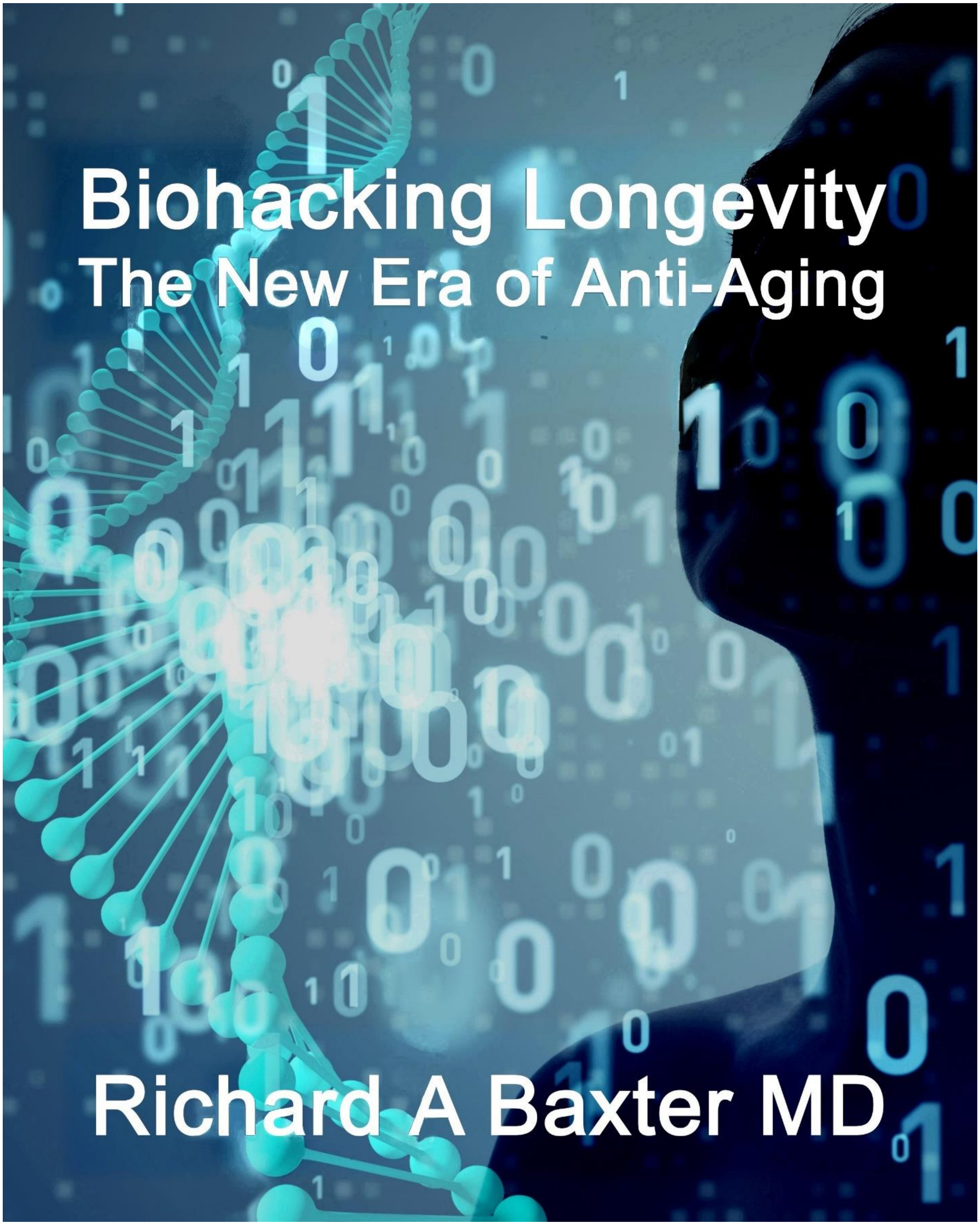
Biohacking Longevity
The New Era of Anti-Aging
Richard A Baxter MD

This book is based on emerging basic and clinical science.

It is not intended to represent specific individual medical advice.

Nature, Sept 2019: "A small clinical study in California has suggested for the first time that it might be possible to reverse the body's epigenetic clock, which measures a person's biological age … The results were a surprise even to the trial organizers. 'I'd expected to see slowing down of the clock, but not a reversal,' says geneticist Steve Horvath at the University of California, Los Angeles, who conducted the epigenetic analysis. 'That felt kind of futuristic.'"

SciTechDaily, December 2020: "Harvard Medical School scientists have successfully restored vision in mice by turning back the clock on aged eye cells in the retina to recapture youthful gene function. 'Our study demonstrates that it's possible to safely reverse the age of complex tissues such as the retina and restore its youthful biological function,' said senior author David Sinclair. If replicated through further studies, the approach could pave the way for therapies to promote tissue repair across various organs and reverse aging and age-related diseases in humans."

Israel Hayom, July 2022: "Israeli researchers discover mechanism for rejuvenating human organs … injecting a special protein into the skin cosmetically and genetically rejuvenates the skin. Throughout the study, symptoms of old age disappeared without trace, and even displayed molecules identified with young skin only. This groundbreaking study … proves that the aging process of human skin can be halted and human organs made younger." Professor Amos Gilhar, head scientist, explains: "In the study, which lasted about two decades, we discovered that the entire skin undergoes a process of renewal"

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Introduction

It is now possible to reverse aging in humans. Not just to look younger or feel younger, but to turn back the biological age clock. After decades of unfulfilled promises, controversies, and sometimes fraud, anti-aging is becoming a legitimate specialty. Prospects for extension of healthy lifespan are engaging some of the brightest minds in science and medicine. This coming of age in anti-aging is happening in research labs around the world and beginning to enter clinical practice. We're biohacking longevity.

The advancements fueling this change are nothing short of mind-boggling. But misinformation about anti-aging and longevity is ubiquitous and persistent; a lot remains to be proven. Finding the true signals amidst this noise is plenty challenging for experienced researchers, and for the consumer nearly impossible. Nostrums and nonsense are everywhere, even as concepts such as gene reprogramming, tissue regeneration, and biologic age clocks are developing swiftly. I have been following longevity science for decades, and I've decided it is time to get the word out. This will be your guide to what works now, what might work, and what doesn't. I'll reveal some powerful discoveries just now emerging from research labs and moving into clinical practice, and introduce what is coming to be known as 21st century medicine.

My personal involvement in the topic started innocently enough with an interest in the health effects of wine. I had no idea that this was only the beginning of a long and fascinating journey. After the "French Paradox" was reported in the 1990's, there was a lot of research looking to explain the association of wine with health and longevity. Wine drinkers were found to have lower rates of *all* of the diseases of aging, so there had to be some essential common denominator that wine drinkers were benefiting from. Few had any idea back then that the seeds of a scientific revolution were just beginning to germinate. We now know that it isn't just wine, or anything in wine alone, that holds the secret.

As these revelations unfolded, my interest morphed from the healthful joys of wine into something of an obsession over longevity science. I began to spend absurd amounts of time looking up the latest studies and signing on to webinars with the world's experts on anti-aging. I became one of the first to complete the recently launched certificate course on Longevity

Medicine. I'm convinced that we are on the threshold of one of the most profound transformations in biomedical science in history.

As I started thinking about how to incorporate this new science into my clinical practice, I recognized the need for a sort of briefing document for patients considering participation in this new version of anti-aging. This book is just that; a plain language overview, a living document, to be updated regularly.

Even the term "anti-aging" is itself becoming passé, in favor of "longevity medicine." The practice of longevity medicine is not intended to replace traditional medical care (yet), but there's no doubt that it will transform the way we think about health and longevity. A fundamental change is underway, framed by the curiosity of science, grounded in massive data known as bioinformatics, and propelled by the lure of solving biology's biggest questions.

Lost in translation

The journey from the research lab to the clinic is known as *translational medicine*, and the process can be long, unpredictable, and expensive. What works in a test tube or cell culture may not do the same in animal studies, let alone humans. Because there are limits on what sort of studies you can do to test your idea in clinical trials, you need to determine not just if it is likely to have the desired result, but also if the doses needed are toxic or have unexpected side-effects. Lab rats make a convenient model for trying out new approaches, but they are not people. More than 9 in 10 cancer treatments that appear promising in animal studies fail in human clinical trials. The odds aren't any better with anti-aging products, though AI computing can accelerate the process and make increasingly reliable predictions.

A brief history of anti-aging

Anti-aging hasn't always had a good reputation. Pressure to make new treatments available has often resulted in shortcutting validation through clinical trials, diluting the impact of genuine breakthroughs. Stem cell therapies, hormone clinics, and a range of supplement products have all gained marketplace traction without clinical corroboration. Similarly, long held beliefs such as antioxidant supplementation persist even as clinical trial evidence points to their futility.

A pivotal breakthrough was the identification of how lifespan extension via caloric restriction (CR) works. It was known for years that experimental restriction of caloric intake triggers a metabolic alteration that prolongs healthy lifespan. The effect requires an impractical degree of CR, so scientists wondered if there could be a way to replicate it without semi-starvation. What they found ushered in what we may now consider to be the modern era of anti-aging. Resveratrol, a molecule concentrated in wine from grape skins, was the catalyst. Resveratrol appeared to explain the CR effect and possibly the whole French Paradox.

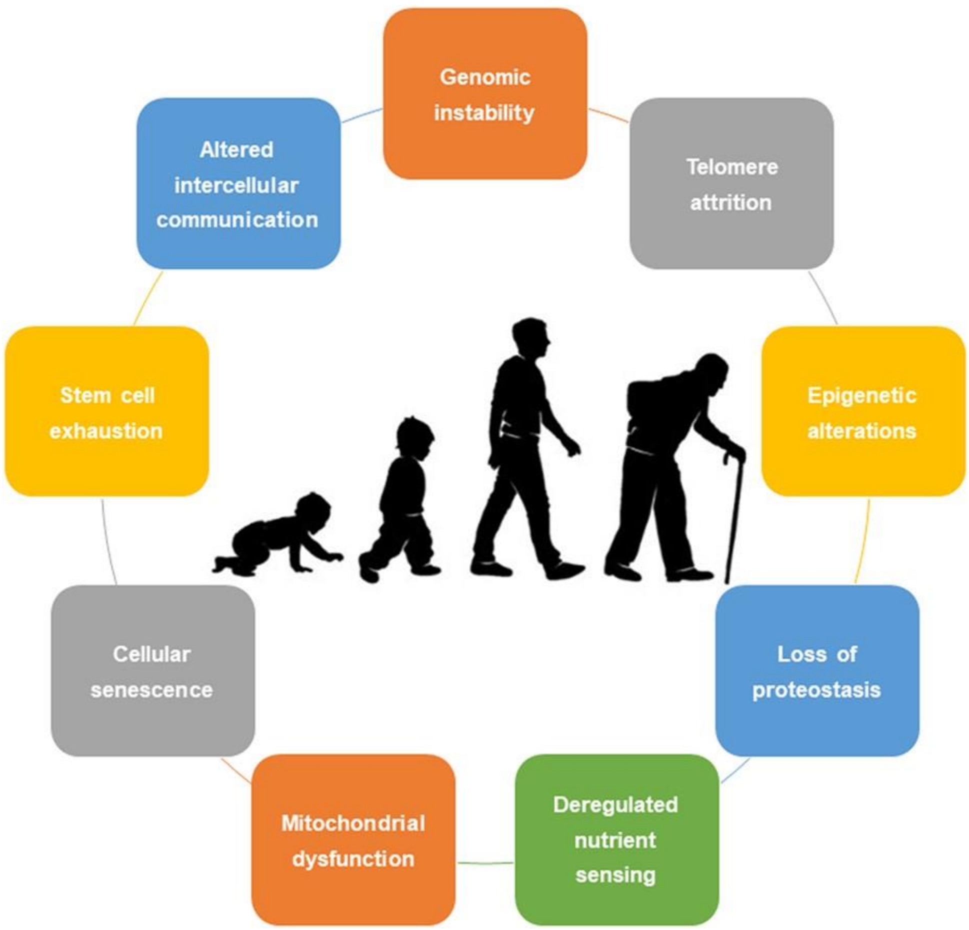
A pioneering advocate of resveratrol's potential was Professor Joseph Vercauteren of Université Montpellier in France, who extracted resveratrol from the lees typically discarded after pressing wine. Few others considered resveratrol to be more than a novel antioxidant until a group at Harvard headed by geneticist David Sinclair, Ph.D. identified it as an activator of a type of gene regulator called *sirtuins.* Sirtuins had earlier been shown to turn on the genes responsible for the CR effect, so now we had the first true anti-aging candidate molecule.

Me, Professor Vercauteren , and David Sinclair

The anti-aging market grew rapidly. Resveratrol supplements proliferated, and vitamin products increasingly focused on an anti-aging message. But while these products referenced progress in science, they often oversimplified it, and anti-aging's credibility problem persisted. That is changing, as several lines of research converge and possibilities unimaginable a generation ago appear within our grasp.

Defining aging

Before we define anti-aging, we need some detail on the aging process itself. The visible and functional aspects are easy enough to see – muscle and joint stiffness, decline in mental sharpness, less energy – but what we really need is to understand the underlying causes. Here's how scientists see it now: At the cellular and subcellular level, interactions among genes and environmental factors result in accumulation of genetic damage, embodied by specific interrelated aging hallmarks:



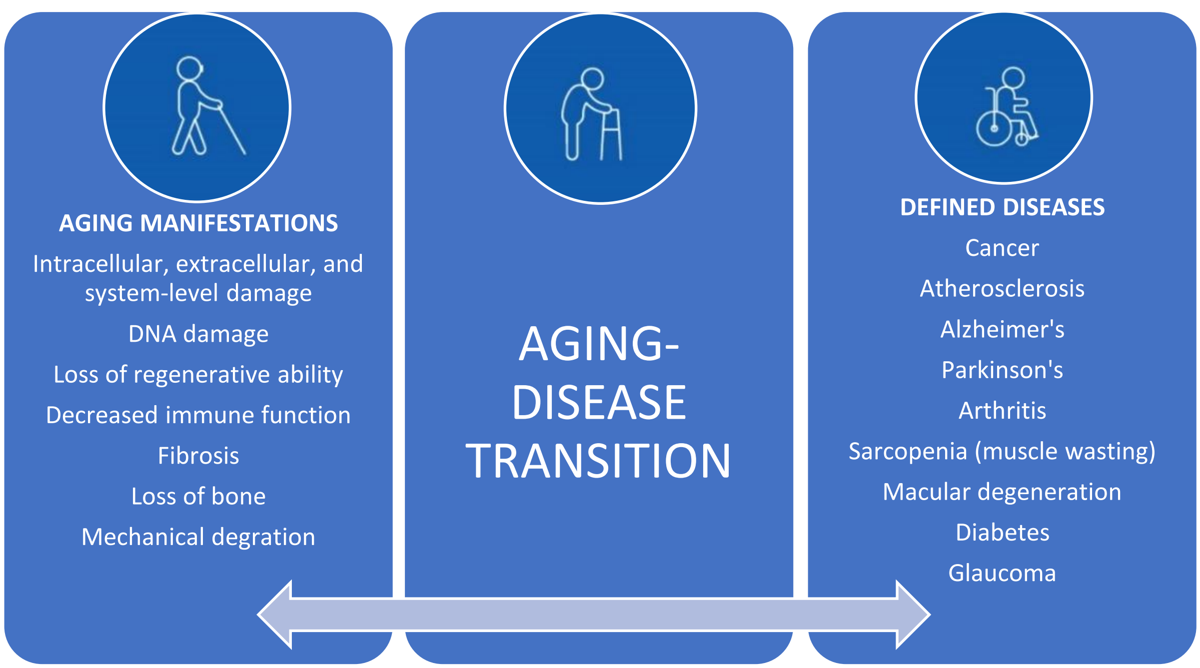
These hallmarks form a framework for identifying targets for anti-aging therapies. Anti-aging aims to slow or reverse the aging process at a molecular level, resulting in physiologic improvements, reduction in disease, and potential lifespan extension.

<u>The new era of anti-aging</u>

This new era of exponential progress in anti-aging science is propelled by three developments:

1. *The recognition that the biological mechanisms of aging at a cellular level are largely the same as those underlying major age-related diseases;*
2. *A substantial influx of capital driving anti-aging research and development; and*
3. *The unprecedented power of applied artificial intelligence computing.*

The understanding that the biology of aging and disease are intertwined points to therapies that might reverse aging while simultaneously addressing cancer, cardiovascular disease, neurodegenerative diseases, diabetes, and many others. Beyond the implications for health care, this has a pragmatic benefit for development of longevity products; given the impracticality of clinical trials for anti-aging as the primary outcome within a realistic timeframe, it informs a strategy for navigation of regulatory constraints on drug and device development. If for example your anti-aging compound also happens to be effective against cancer, you now have a pathway to approvable on-label uses while you continue to test it for anti-aging effects.



This increasing recognition that the factors underlying degenerative diseases are the drivers of aging has led some to propose that aging itself be classified as a disease. Researcher Matt Kaeberlein at the University of Washington went so far as to say that this will be a defining feature of 21st Century medicine. Also here in Seattle is the Institute for Systems Biology founded by Leroy Hood MD, PhD, whose book *The Age of Scientific Wellness* makes a similar case, and I agree. However, realizing the full benefits of next-generation anti-aging will require a dramatically updated model of health care delivery, less constrained by the diagnosis-treatment paradigm that defines it now. It shifts definitively away from sickness care to wellness care. Currently, because aging isn't yet considered a disease entity,[*] unless you are being treated for a specific disease, and given an approved treatment, your insurance company won't cover it. There aren't any diagnosis codes for aging and no billable treatments. That is the reason for the ground rules governing my anti-aging and longevity medicine practice at the present time:

- Both the physician services and prescribed treatments, supplements, or drugs are not billable to a third-party payer.
- Anti-aging practice is not a replacement for traditional medical care. (Perhaps at some point in the future.)

A substantial influx of capital, the second driver of anti-aging research and development, has been ramping up. Dedicated venture capital-funds and endowed research facilities such as the Buck Institute and Google-backed Calico Labs are leveraging the most up-to-date technology to identify and develop anti-aging products. The Switzerland-based Longevity Science Foundation announced plans in October 2021 to devote $1 billion toward the goal of extending human lifespan. In 2022 the Saudi Arabian-based nonprofit Hevolution Foundation announced plans for that amount every year for support of longevity research globally. While the sector remains a high-risk investment category littered with some spectacular failures, many see longevity as "the next trillion-dollar opportunity." There are now dozens of companies working exclusively

[*] The standard reference manual known as the International Classification of Diseases added the qualifier R54 for "age-related physical debility" in its 2019 update.

on anti-aging therapeutics and more than a hundred others with anti-aging products in their pipeline. Investment in longevity companies was estimated at more than US$5.2 billion in 2022 alone, and is increasing.

A legitimate concern is whether the profit motive will have a corrupting influence and reanimate the credibility question that has plagued anti-aging practice. The counterargument points to increasingly open sharing of data, and the fact that it costs a lot of money to do this kind of research properly. The work these labs are doing is being followed with intense interest and scrutiny. If one of these longevity moonshots pans out, we will all benefit.

The third pillar of the anti-aging imperative is the exponential power of *applied artificial intelligence*.* This not the ChatGPT version of AI but more the ability to discern patterns in massive amounts of data, make predictions, and validate them. Analysis of these immense sets of biological data is called "omics," and for all practical purposes was not possible at scale until relatively recently. AI enables detailed insights into central biochemical processes of aging at every level, from the whole body to the cellular, subcellular, and on down to the molecular level. Hidden patterns and connections are being revealed on complex "metro maps" of cellular metabolism and expression of anti-aging genes.

There's a hugely practical application of AI in anti-aging: AI-based analysis is how your biological age, vs. chronological age, can be determined. More on that later, but knowing your biological age means that you can objectively measure the results of anti-aging interventions. Biological age is derived from DNA markers, and tests are now readily available, reliable, and affordable.

AI is also being exploited for discovery of anti-aging compounds. A particularly useful feature of AI is the ability to create virtual 3-D models of biological molecules, called *in silico* modeling. With AI, the specific ways that molecules interact can be understood and predicted with precision, and in silico screening of large numbers of potential therapeutics can now be done rapidly. But identification of candidates is only the beginning, due to a nagging feature of

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* For engaging explanations of AI in anti-aging, there are 3 essential books: *Deep Medicine* by Dr. Eric Topol; Alex Zhavaronkov's *The Ageless Generation: How Advances in Biomedicine Will Transform the Global Economy;* and *Live Longer with AI* by Tina Woods.

biomolecules: They tend to be "promiscuous," meaning that they have a lot of interactions, metabolically speaking. Modified versions or molecules newly imagined in silico may anticipate some of these off-target interactions and minimize unacceptable side-effects. In silico modeling might also help with the issue of *bioavailability*, which refers to the degree to which the compound actually reaches its target. AI can create thousands of possible tweaks to the structure of the candidate molecule and predict the effects, potentially addressing both sets of problems.

A valuable AI resource is the advent of online databases, accessible to researchers around the world. Examples include the UK Biobank, which houses anonymized genetic and health information from more than a half million subjects; the website Geroprotectors.org, a catalog of compounds identified as having anti-aging properties; the Genotype Tissue Expression project (GTEx), an atlas of human gene expression; the U.S. National Genomics Data Center, with database resources for support of research activities in both academia and industry; and my favorite acronym, the "BIG" (Beijing Institute of Genomics) Data Center at the Chinese Academy of Sciences, also available worldwide for researchers. Collectively these "big medical data" warehouses facilitate AI-assisted data mining for advancement of precision medicine and anti-aging research.

Longevity without restrictions

So we know that caloric restriction is a sure-fire if utterly impractical lifespan extension strategy. Cut your calories back by 30% or so, and live 30% longer. It's been proven in everything from yeast to worms to mice. Almost certainly in primates* too, though a controlled experiment in humans with length of life as the endpoint is unlikely to happen. Even if it did, those most interested in the results would no longer be around to see the final report.

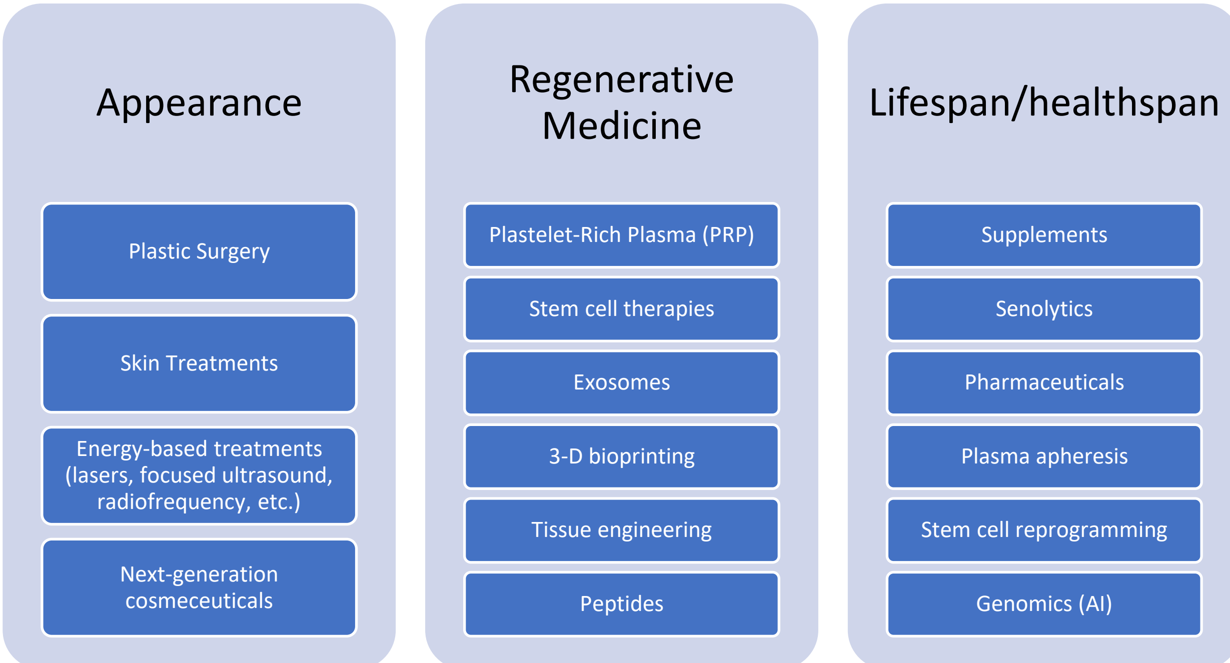
A clinical study released in February 2023 did provide evidence however that caloric restriction can slow the *pace* of aging. The trial, funded by the U.S. National Institute on Aging, included

* A study on rhesus monkeys, whose average life span is 40 years and whose aging patterns are similar to humans, found that the monkeys on a CR diet lived longer. However the results have been questioned because the control group monkeys were apparently given a less healthy diet.

220 healthy adults randomized to either a 25% calorie restriction diet (25% below the participants' estimated energy requirements) or a normal diet (ad libitum) for two years. To measure the pace of aging in participants, a DNA methylation analysis called DunedinPACE (more on that below) was done at baseline, then 12 and 24 months. The CR group saw a 2–3% reduction in the pace of aging compared to the control group. That may not sound like much, but it equates to a 10–15% reduction in mortality risk, comparable to quitting smoking.

Most research has shifted to a focus on means to activate the CR effect pharmacologically or with more realistic diet strategies such as time-restricted eating or intermittent fasting. And this is just one of many anti-aging channels being fleshed out. Leveraged by the three pillars of the aging↔disease linkage, unprecedented investment, and AI, the fruits of this research are beginning to move into clinical practice. I see this manifest by three facets of anti-aging medicine: first, aesthetic treatments and surgery targeting appearances of aging; a second category of regenerative medicine aimed at restoring physiologic function at a macro level; and a third category comprised of strategies to slow or reverse aging at a cellular and genomic level. We shift from disease-specific "whack-a-mole" treatments to a focus on healthy longevity, or healthspan. While longevity medicine integrates AI-based genomics, personalized medicine, and advances in biology, a comprehensive approach to anti-aging includes multiple points of attack. For many, this starts with strategies to look as young as you feel.

The 3 channels of anti-aging



1. If you look good, you feel good – and live longer?

While plastic surgeons and others practicing aesthetic medicine know about the positive impact of their craft, some would debate whether they can accurately be called "anti-aging" since they do not affect biological aging per se. Or do they? Cosmetic surgery does have documented value in terms of quality of life measures, which may translate into biological changes. One review of the psychology of facelift patients found that more than 95% experienced positive changes in their life, increased self-confidence and self-esteem, and overall improvement in quality of life. And a positive outlook has been shown to be associated with longevity.

This connection is supported by a concept known as the "Socioemotional Selectivity Theory" or SSF, developed by Stanford psychologist Laura Carstensen. SST holds that subjective age predicts late life health outcomes. The longer one expects to live (time horizon view) the younger one behaves, and the younger one's self-perception of age. SST evinces that time horizons are pliable, and modifiable with behavioral changes. Subjective age can predict objective health and lifespan.

SST could explain the potential longevity benefit from aesthetic plastic surgery. A study from the Mayo Clinic some years ago suggested that women who have facelifts live up to 10 years longer than women who don't. The authors attributed the lifespan benefit to a boost in self-image and the resulting optimism. There are obvious confounders with studies of this type, but it does support the concept of youthful appearance contributing to longevity. Whether the decision to undergo restorative surgery marks the onset of a personal effort to make lifestyle changes and other anti-aging interventions, or simply resets one's time horizon view, either way it validates the role of plastic surgery in anti-aging.

II. Regenerative medicine: form and function

Think of regenerative medicine as integrating the visual and functional aspects of aging. Healthy skin is beautiful skin, heathy muscles form an athletic and attractive physique, a healthy central nervous system retains mental sharpness, and healthy cells give youthful energy. Regenerative medicine considers aging as it relates to coordinated system-wide signaling, (e.g., hormones) as well as restoration of individual body parts and organs.

Hormone therapies

Declining hormone levels are a hallmark of aging, and hormone replacement improves quality of life for millions. Yet evidence for positive effects on the aging process is often contradictory; in many cases, it may do the opposite. There are questions as to whether making the body perform as it does in youth makes it biologically younger, now that this can be measured. Hormones all operate with elaborate feedback loops and tinkering with them may cause unanticipated side-effects if not managed expertly. For these reasons, hormone replacement therapy (HRT) has long been considered as *age management* medicine rather than anti-aging. That may change as approaches to HRT are updated and re-evaluated.

Testosterone supplementation is a controversial case in point. Studies of testosterone replacement in older men are comparatively few and generally have been of short duration, with small numbers of participants and frequently lacking adequate controls. What studies there are show modest anti-aging effects. Though longevity is a common theme in marketing testosterone to older men, convincing evidence of a healthspan benefit is lacking.

Human growth hormone (GH) has been widely marketed for antiaging, promising to deliver weight loss, improved energy and mood, and better sleep. As with testosterone, support for these claims is mixed. One study in elderly men reported increased muscle mass, reduced body fat, and improved bone density with GH supplementation. Animal studies however suggest that declining levels of GH are associated with greater longevity and are protective against cancer, and that GH supplementation *accelerates* aging. This fits with human epidemiologic studies, which generally associate lower levels of GH with longevity. A GH antagonist (Pegvisomant) has been proposed as an anti-aging drug, supporting the implication that GH by itself is pro-aging, not the other way around.

GH use is further confounded by its effects on glucose metabolism. A primary action of GH is to increase glucose levels, producing a diabetes-like effect. This leads to a compensatory increase in a hormone called IGF-1 (Insulin-like Growth Factor 1), which acts to lower blood sugar. This relationship of growth hormone and IGF-1 is called the GH/IGF-1 axis, and is an extremely important anti-aging intervention target.

Because of these issues, access to GH is heavily restricted. Alternative approaches using peptides to stimulate natural production of GH may offer a safer option (see below).

DHEA (Dehydroepiandrosterone) is a precursor hormone produced in the adrenal gland. DHEA helps produce other hormones such as testosterone and estrogen. Natural DHEA levels peak in early adulthood and then decline with age. Studies on DHEA supplementation are mixed in terms of anti-aging effects, and there is concern that it may promote growth of hormone-sensitive tumors such as breast cancers.

Estrogen replacement has a controversial past but is increasingly considered an indispensable part of a comprehensive anti-aging strategy for postmenopausal women. Thanks in large part to work done at the Buck Institute's Center for Female Reproductive Longevity and Equality, menopause is now known to be associated with accelerated aging. Seen in this context, the use of estrogen replacement becomes more than a quality of life and sexual health issue.

Peptides

Peptides are ubiquitous molecules that serve a variety of functions. Insulin for example is a peptide, and more than 7000 peptides have been identified in the body. Around 60 peptides have been FDA-approved, with more than twice that number being explored in clinical trials. Peptides are sort of mini-proteins, with up to 50 amino acids; around 50-100 is a polypeptide, and above that a protein. They play critical roles in senescence, immunity, and overall aging.

The undisputed guru of peptides is orthopedic surgeon William Seeds, who has many years of experience with them and has unbridled enthusiasm. I've taken his training course and I'm developing an appreciation for the potential of peptides in a variety of applications.

GHRH: Growth-Hormone-Releasing Hormone is a peptide involved in cell function and growth, and as its name implies it signals the body to produce endogenous growth hormone. It is naturally produced in the brain, and is thought of as a safer and less expensive alternative to GH. Peptides that stimulate production of GH are known as GHRH mimetics, and there are several variants available.

Sermorelin is a well-known GHRH mimetic originally FDA-approved to elevate growth hormone levels in children of short stature, but it is considered less predictable in anti-aging protocols. The peptide CJC 1295 (also known as Mod-GRF) is commonly used as an introductory GHRH mimetic, because of its favorable side-effect profile.

Tesamorelin (brand name Egrifta) is a 44 amino acid peptide first FDA-approved to treat a condition known as lipodystrophy that occurs with HIV. Tesamorelin may improve muscle mass, blood lipid profiles, cardiovascular disease risk, and overall body fat.

Ipamorelin headlines the category of what are known as Growth Hormone Releasing Peptides or GHRPs. While GHRH mimetics promote production of natural growth hormone, release of the hormone into the blood stream is under additional feedback control involving the cell receptor known as ghrelin, involved in hunger and satiety. GHRPs act on the ghrelin receptor to facilitate release of GH. Ipamorelin is considered a third-generation GHRP and is preferred because of its high potency and minimal side-effects. It can be used without a GHRH and has a range of potential anti-aging effects. Ipamorelin should be used in cycles as continuous use may lead to receptor desensitization.

All of these are given by subcutaneous injection either once a day before bedtime or twice a day with the morning dose at least a 30-90 minutes before eating. Natural growth hormone release is pulsed and highly linked to a stage of deep sleep. The benefits of GHRH treatments are amplified by syncing to this natural cycle.

Ibutamoren or MK-0677 is the last in our list of GHRP mimetics and possibly the most useful because it can be taken orally. Technically it isn't a true peptide because of modifications needed to prevent it from being broken down by digestive enzymes, but it has a similar safety profile. The primary risk is the potential for irreversible receptor involution if taken continuously for long periods of time, so it is used in 3-month cycles alternating with a different GHRP.

Epithalon: A synthetic version of the peptide *epithalamin*, an endocrine bioregulator naturally produced in the pineal gland. There is evidence that epithalon decelerates aging, suppresses tumor development, enhances antioxidant defenses, and moderates stress response.

BPC-157: Body Protection Compound 157 is a medium-sized cell repair peptide working on the brain-gut axis. BPC-157 promotes wound healing, reduces neuroinflammation, and promotes regeneration.

Thymosins: The thymus is a lymphoid organ that sits just in front of the heart, and is so named because its two lobes resemble leaves of the thyme plant. T-cells, an important aspect of adaptive immunity, are produce in the thymus. After puberty the thymus undergoes gradual shrinkage as senescent cells accumulate and peptides produced by the thymus (thymosins) diminish. Two thymosins are important clinically: alpha-1 (TA1) and beta-4 (TB4).

T**A1** (Zadaxin) is a multifunctional peptide that helps T-cells mature and restores immune system homeostasis. It is used to treat autoimmune disease, viral infections including hepatitis, Lyme disease, and other inflammatory conditions.

TB4 is also involved in immune system functioning and is important in tissue repair and regeneration. It is a potent anti-inflammatory and has been studied for a variety of conditions from traumatic brain injury to dry eye.

Plasma apheresis

Here's a simple idea, and it appears to work: Infuse plasma from a young donor into an older individual, and it measurably turns back the clock on many markers of age. The origin of this remarkable phenomenon is one of science's weirder stories: *Parabiosis,* an experiment in which two animals (mice for example) have their circulatory systems surgically joined. The goal was to determine whether factors in the blood of one "parabiont" have physiological effects on its partner. Uniting an older animal with a younger one is called *heterochronic parabiosis*, and it produces dramatic rejuvenating effects on the older parabiont. Harold Katcher, a Professor at University of Maryland who lectures on the Biology of Aging and Neurobiology, has isolated a plasma fraction called E5. In 2020 he published the results of an experiment in which 2-year-old rats (elderly for a rat) were given E5 from younger rats; the old rats had an average epigenetic age reduction of 54% across a variety of tissues, and more youthful levels of over 20 biomarkers. In addition, the subjects had improved physical strength and cognitive ability. The implication of this, as catcher spells out in his book *The Illusion of Knowledge: The paradigm shift in aging research that shows the way to human rejuvenation,* is that it is the organism that determines the age of the cells not the other way around.

But do they live longer? In February 2023, the final results were released showing that the treated rats indeed outlived their untreated counterparts, with the longest survivor going to the equivalent of 120 human years and 5% longer than the known longest living rat, on caloric restriction. The study has not been published in a peer-reviewed biomedical journal as yet.

We don't know what's in Katcher's E5, and figuring out how plasma apheresis works is complicated. He has formed a company to commercialize it as *Elixir.* It will be a while before it is ready for human clinical trials. Another company, California-based Alkahest, seems to be making progress. Alkahest is working to isolate the active components in the constellation of circulating proteins – the plasma proteome – and has clinical trials underway with a few promising candidates.

These age-reversing proteins are called *chronokines*. Seemingly, identifying them is just a question of connecting the dots, but there are a lot of dots – around 8,000 different proteins and peptides to screen - but the three trends seem to align: age-related diseases to target in addition to anti-aging per se, financial backing, and AI. Alkahest have identified a few highly selected plasma fractions and completed stage 2 clinical trials for Alzheimer's and Parkinson's, and other applications including enhancing recovery after surgery. Alkahest was recently acquired by the Spanish firm Grifols for $147 million, and Katcher's company is reportedly attracting sizable investment interest. Watch this space.

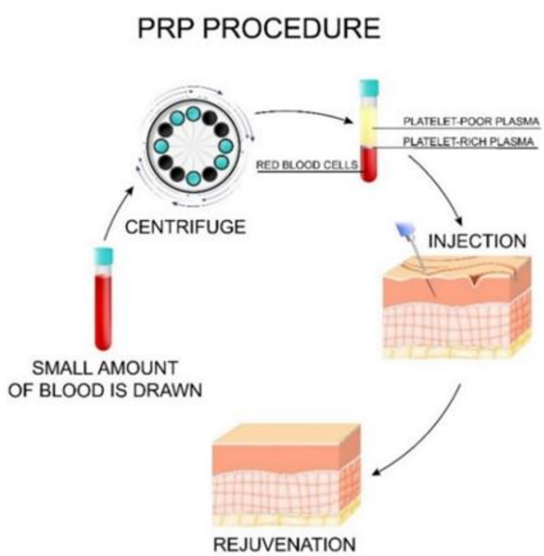
A cautionary note on this approach comes from the experience of another company called AmbrosiaPlasma. They were charging several thousand dollars a pop for plasma infusions from young donors, but were shut down by the FDA in 2019 for lack of clinical proof. Their model was a pay to play clinical trial, which should be a red flag in anyone's book.

Also, recent research suggests the whole thing due to dilution of pro-aging factors rather than exposure to pro-youth proteins from young donors. The smart money seems to be pivoting to strategies for clearing pro-aging factors, and there are some interesting ideas.

Platelet Rich Plasma

Platelets are tiny cells in the bloodstream associated with clotting, and platelet-rich plasma (PRP) is defined as the plasma fraction of blood with a high platelet concentration. PRP is an abundant source of growth factors. Much of the early use of PRP was in orthopedics, and it remains widely used in sports medicine. It is now used for an array of clinical applications from oral surgery to gynecology. Yet despite an abundant medical literature on PRP in regenerative medicine, there are few high-quality studies. One reason for this is the large number of available systems for processing PRP and lack of standardization of preparations and treatment protocols, which makes it challenging to compare effectiveness.

Regulation of PRP treatments has been a thorny issue. In the United States, products such as PRP fall under the purview of the FDA's Center for Biologics Evaluation (CBER), which is responsible for regulating products derived from human cells and tissues. CBER deems products such as PRP to be exempt from FDA oversight, up to a point; while the product itself may be exempt, the devices for their preparation are not. No devices are specifically approved for regenerative or aesthetic medicine, so their use in this setting is considered "off label." This is legitimate as long as certain standards of good practice are met; off-label doesn't necessarily mean off-base.



How PRP works: A small blood sample is drawn. This is then placed in a centrifuge, which separates the blood into its components, and the plasma layer is drawn off. Processing takes less than an hour, and the PRP is then ready for use. For skin rejuvenation, it is either injected or applied topically after microneedling. Downtime is fairly short, but several sessions a few weeks apart are required for maximum effect.

Stem cells

Stem cell therapies are increasingly viewed as being synonymous with regenerative medicine. As it happens, harvesting stem cells is surprisingly easy: Adipose tissue (fat) is an abundant source. A minimally invasive liposuction procedure, a few processing steps, and you've got a vial of stem cells in concentrations 500-fold greater than bone marrow. What's more, adipose-derived stem cells (ASCs) have distinct advantages over other sources of stem cells, not the least of which is their ubiquity in tissue that is often present in excess.

ASCs are multipotent, meaning that they have the potential to differentiate into tissues of several types including bone, cartilage, muscle, and nerve. ASCs are being studied for a range of applications including aberrant wound healing, organ repair, and cartilage regeneration.

In aesthetic surgery, much of the early focus on ASCs was on soft tissue augmentation. Because depletion of facial fat is an important aspect of aging, fat grafting is often done in conjunction with a facelift. Because volume retention of fat grafts is variable however, a concept called cell-assisted lipotransfer (CAL) aims to improve this by adding extra ASCs to the fat. For this procedure, the fat from liposuction is divided, the ASCs are isolated from one portion, then added back to the fat to be used in the graft. The enhanced content of ASCs is believed to contribute to blood vessel growth into the grafted fat and cell renewal.

Despite technical progress, there are still several controversies with the use of ASCs. The so-called "stem cell facelift" (really just cell-assisted fat grafting) has been earnestly promoted, and just as vigorously condemned as a misleading exaggeration. As stem cell procedures became popular, regulatory agencies began to take a critical look in the U.S. and elsewhere. A well-publicized closure of stem cell clinics after 3 cases of blindness from stem cell injections for macular degeneration furthered these efforts. Stricter FDA guidelines went into effect in 2021.

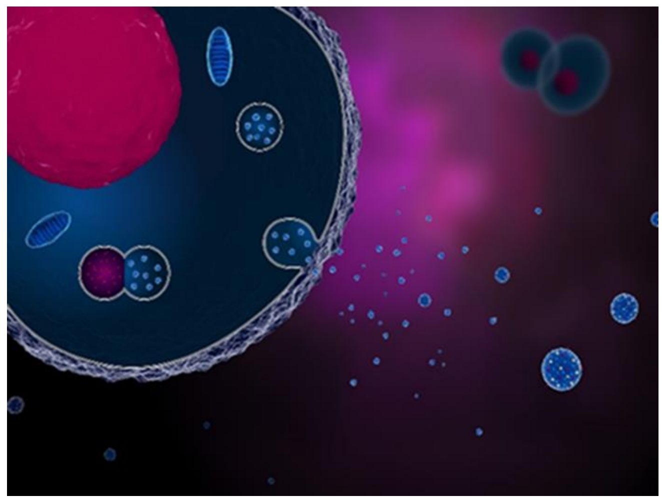
Nevertheless, the regenerative potential of ASCs has become progressively more recognized. In order to take leverage these benefits with less reliance on costly and time-consuming devices, simpler processing methods have been developed. Using low-tech filtration techniques, specific types of grafts can be prepared, tailored to intended effect and placement site. These are classified as millifat, microfat, and nanofat, the latter largely consisting of stromal vascular fraction. Nanofat is used as a superficial subdermal injection for skin rejuvenation rather than volumization, while millifat and microfat are placed deeper.

Realizing the full potential of ASCs likely requires more elaborate processing however. A study by plastic surgeons in Brazil evaluated the effects of subdermal injection of concentrated ASCs prepared by using an enzyme to separate the stem cells and then culturing them in a lab, a procedure requiring 3 weeks. They then placed injections into facial skin of 20 patients who were scheduled for facelift, so that the skin could be evaluated in the sections that were removed 3-4 months later. Impressively, they found full regeneration of damage from aging and the effects of lifetime sun exposure. The elastin fiber network (the type of collagen that gives youthful skin its elasticity) was restored, and the deeper layers of the skin were reconstituted after a single albeit elaborate adipose-derived stem cell treatment.

I am excited about the potential for stem cell treatments, but do not want to get ahead of what regulations allow or clinical science proves. Stem cell treatments remain experimental. Fortunately there is a less risky way to leverage the benefits of stem cells, by understanding how they communicate with neighboring cells. They do this with little bubbles of biomolecules called exosomes.

Exosomes

Evidence suggests that the local tissue effects of stem cell therapies are mediated largely by what are called “paracrine” actions – effects on other cells in the immediate area – rather than by differentiating into resident cells of a particular type. This phenomenon is termed the *paracrine hypothesis*, and it is important because it suggests a sort of shortcut. Paracrine communication between neighbor cells occurs by exchange of tiny packets containing cargoes of proteins, peptides, nucleic acids (DNA and RNA), and other biomolecules. These are categorized by size, the smallest of which are called *exosomes.* Stem cell-derived exosomes could possibly be used as a surrogate for stem cell therapies, by imparting the function of the parent stem cell directly into the tissue. Exosome treatments have a further advantage in that they can be purchased as an off-the-shelf solution, though regulations limit their use to topical applications as with PRP. These commercially produced exosomes are derived from stem cell cultures, and the effects of exosomes are related to the specific type of parent stem cell. I expect to hear a lot more about exosomes in the next few years, as a number of clinical trials are underway for a wide range of applications. For now their primary clinical use is skin rejuvenation, typically combined with microneedling to enhance penetration.



Artists rendition of exosomes

III. Epigenetics: The new era

As our cells age and go through multiple replicative cycles, our DNA degrades, like a photocopy of a photocopy. Traditionally, models based on this cumulative DNA damage have been generally classified into two broad categories: The ***error hypothesis*** and the ***programmed***

hypothesis. The error hypothesis attributes aging to the accumulation of mutations in genes. In this scenario, the primary mechanism is oxidative damage, mediated by reactive molecules called free radicals. While it is well-documented that oxidation leads to DNA mutations, clinical studies consistently find no correlations to longer lifespan or healthspan with antioxidant vitamins or supplements despite decades of study. The fact that antioxidants are ineffective for anti-aging is still not a widely appreciated fact, but the evidence is comprehensive and irrefutable. There's clearly more to it.

What could explain this apparent contradiction? In terms of cellular metabolism, *some amount of free radicals is beneficial*. Here's why:

- Free radicals are key information mediators in cellular response to stress
- Most antioxidants are easily transformed into pro-oxidants
- Antioxidants can actually *increase* tumor cell viability in some cases

So paradoxically you need free radicals, and even under the most optimistic circumstances antioxidants can turn against you.

The *programmed hypothesis* of aging holds that it is caused by evolved biological mechanisms. In this paradigm, a predetermined genetic program tamps down hormone levels as we age, suppresses the immune system, and expends fewer resources on DNA repair as the organism (or person) gets older. Genes associated with aging are either activated or suppressed depending upon their function at various stages of life. Numerous genes involved in aging have been identified.

After decades of research, neither hypothesis has produced proven longevity therapies. The **information hypothesis** of aging, developed by David Sinclair, unifies these concepts and points to new strategies. As our DNA degrades, cellular repair mechanisms deteriorate as well, due to important genes being silenced or rendered dysfunctional. This is the machinery of epigenetics, the processes that regulate how specific genes are turned on (expression) or turned off (silencing). If genes are the pages in a reference manual, epigenetics is the bookmarks, dogeared corners, and highlighter markups. Epigenetic changes are heritable through cell replication cycles but do not involve mutations in the DNA sequence itself.

To take the analogy a step further, if the genome is our biological hardware, the epigenome is software; aging can then be seen as a software problem that could be restored by rebooting from a backup copy! In February 2023, Sinclair released results of a study supporting this concept. The researchers induced accelerated aging in mice by causing DNA breaks that in turn triggered a repair process involving changes to the organization of DNA. They showed that the increased burden on DNA reorganization resulted in loss in epigenetic information, rather than changes in the DNA sequence itself, caused the mice to age faster.

Here's the really cool part: They then used gene therapy technology that partially restored the integrity of the epigenome and showed that it restored the organs and tissues to a youthful state, demonstrating that aging can be driven "forward and backward at will." This "epigenetic reboot" led to improved biomarkers of aging in multiple tissues.

Which brings us back to sirtuins, the epigenetic regulators of genes involved in aging. Basically, sirtuins flip genetic switches on and off. These "switches" are found on proteins called histones, which organize and package DNA. Each time a gene activation switch is accessed, it is altered with a "tag" called methylation, leaving a record of gene expression and/or silencing. This lifetime record of methylations can be counted with AI-derived algorithms. Biological age is determined by focusing on genes associated with aging, and this can now be done with a high degree of accuracy. These epigenetic clocks (also called methylation clocks) have been validated and continue to be refined. Like they say, it isn't the years, it's the mileage; where aging is concerned, methylation clocks are the odometer.

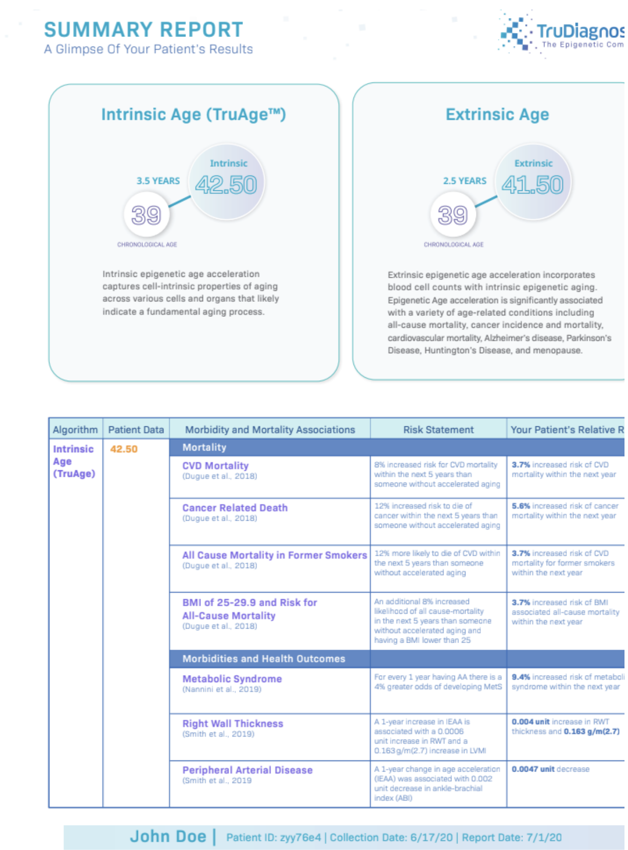
This is also where aging and disease intersect. For example, epigenetic methylation signatures are increasingly used for estimation of disease susceptibility. As these tests become more sophisticated, they can be used to point to specific interventions (lifestyle changes, specific supplements or medications, etc.) This is the basis of a growing specialty called precision medicine. For longevity medicine, epigenetic clocks provide an objective standard for measuring efficacy of anti-aging interventions. The epigenetic methylation signature is increasingly used for estimation of disease susceptibility.

The first generation of these clocks attempted to predict chronological age. What we really want to do is measure the aging process itself, to learn why some people are more fit or more frail, or appear younger or older than their actual age. (These features of aging are known as the aging *phenotype*.) Second-generation clocks began to appear around 2017, trained to predict disease and lifespan. For example, it has been shown that for a middle aged individual, each one year increase in epigenetic age over chronological and there is a 6% increased risk of developing cancer within 3 years and a 17% increased risk of dying of cancer within 5years.

Other second-generation clocks include a gut microbiome clock, a metabolomics clock (developed to track metabolic changes with weight loss surgery), and phenotype age, (DNAm PhenoAge or Levine clock), which combines routine blood tests with DNA methylation patterns.

Facial aging phenotypes. Each of these images were generated by digitally combining actual photos of 10 individuals, with the left column the slowest agers, middle is average agers, right the fastest agers, based on DunedinPACE. All of the subjects are the same chronological age! Source: TruDiagnostic

Third-generation clocks are designed to measure *rate of aging*. In order to do this, the data set used to train the AI algorithm requires measurements taken over a long period of time. There is really only one today, called DunedinPACE, which derived from a decades-long study in Dunedin, New Zealand. Based on analysis of banked samples from childhood through adulthood, the DunedinPACE test yields a rate of aging prediction as well as biological age.



SUMMARY REPORT
A Glimpse Of Your Patient's Results

TruDiagnos
The Epigenetic Com

Intrinsic Age (TruAge™)

Intrinsic 42.50 — 3.5 YEARS — 39 CHRONOLOGICAL AGE

Intrinsic epigenetic age acceleration captures cell-intrinsic properties of aging across various cells and organs that likely indicate a fundamental aging process.

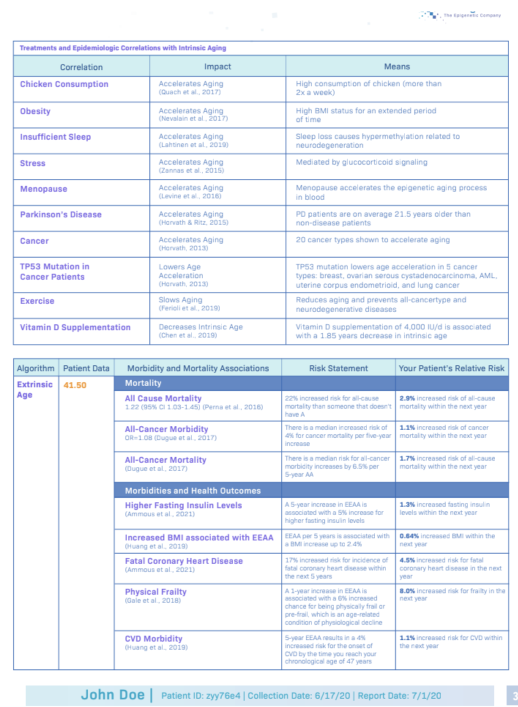
Extrinsic Age

Extrinsic 41.50 — 2.5 YEARS — 39 CHRONOLOGICAL AGE

Extrinsic epigenetic age acceleration incorporates blood cell counts with intrinsic epigenetic aging. Epigenetic Age acceleration is significantly associated with a variety of age-related conditions including all-cause mortality, cancer incidence and mortality, cardiovascular mortality, Alzheimer's disease, Parkinson's Disease, Huntington's Disease, and menopause.

Algorithm	Patient Data	Morbidity and Mortality Associations	Risk Statement	Your Patient's Relative R
Intrinsic Age (TruAge)	42.50	**Mortality**		
		CVD Mortality (Dugue et al., 2018)	8% increased risk for CVD mortality within the next 5 years than someone without accelerated aging	**3.7%** increased risk of CVD mortality within the next year
		Cancer Related Death (Dugue et al., 2018)	12% increased risk to die of cancer within the next 5 years than someone without accelerated aging	**5.6%** increased risk of cancer mortality within the next year
		All Cause Mortality in Former Smokers (Dugue et al., 2018)	12% more likely to die of CVD within the next 5 years than someone without accelerated aging	**3.7%** increased risk of CVD mortality for former smokers within the next year
		BMI of 25-29.9 and Risk for All-Cause Mortality (Dugue et al., 2018)	An additional 8% increased likelihood of all cause-mortality in the next 5 years than someone without accelerated aging and having a BMI lower than 25	**3.7%** increased risk of BMI associated all-cause mortality within the next year
		Morbidities and Health Outcomes		
		Metabolic Syndrome (Nannini et al., 2019)	For every 1 year having AA there is a 4% greater odds of developing MetS	**9.4%** increased risk of metaboli syndrome within the next year
		Right Wall Thickness (Smith et al., 2019)	A 1-year increase in IEAA is associated with a 0.0006 unit increase in RWT and a 0.163 g/m(2.7) increase in LVMI	**0.004 unit** increase in RWT thickness and **0.163 g/m(2.7)**
		Peripheral Arterial Disease (Smith et al., 2019	A 1-year change in age acceleration (IEAA) was associated with 0.002 unit decrease in ankle-brachial index (ABI)	**0.0047 unit** decrease

John Doe | Patient ID: zyy76e4 | Collection Date: 6/17/20 | Report Date: 7/1/20



The Epigenetic Company

Treatments and Epidemiologic Correlations with Intrinsic Aging		
Correlation	Impact	Means
Chicken Consumption	Accelerates Aging (Quach et al., 2017)	High consumption of chicken (more than 2x a week)
Obesity	Accelerates Aging (Nevalain et al., 2017)	High BMI status for an extended period of time
Insufficient Sleep	Accelerates Aging (Lahtinen et al., 2019)	Sleep loss causes hypermethylation related to neurodegeneration
Stress	Accelerates Aging (Zannas et al., 2015)	Mediated by glucocorticoid signaling
Menopause	Accelerates Aging (Levine et al., 2016)	Menopause accelerates the epigenetic aging process in blood
Parkinson's Disease	Accelerates Aging (Horvath & Ritz, 2015)	PD patients are on average 21.5 years older than non-disease patients
Cancer	Accelerates Aging (Horvath, 2013)	20 cancer types shown to accelerate aging
TP53 Mutation in Cancer Patients	Lowers Age Acceleration (Horvath, 2013)	TP53 mutation lowers age acceleration in 5 cancer types: breast, ovarian serous cystadenocarcinoma, AML, uterine corpus endometrioid, and lung cancer
Exercise	Slows Aging (Ferioli et al., 2019)	Reduces aging and prevents all-cancertype and neurodegenerative diseases
Vitamin D Supplementation	Decreases Intrinsic Age (Chen et al., 2019)	Vitamin D supplementation of 4,000 IU/d is associated with a 1.85 years decrease in intrinsic age

Algorithm	Patient Data	Morbidity and Mortality Associations	Risk Statement	Your Patient's Relative Risk
Extrinsic Age	41.50	**Mortality**		
		All Cause Mortality 1.22 (95% CI 1.03-1.45) (Perna et al., 2016)	22% increased risk for all-cause mortality than someone that doesn't have A	**2.9%** increased risk of all-cause mortality within the next year
		All-Cancer Morbidity OR=1.08 (Dugue et al., 2017)	There is a median increased risk of 4% for cancer mortality per five-year increase	**1.1%** increased risk of cancer mortality within the next year
		All-Cancer Mortality (Dugue et al., 2017)	There is a median risk for all-cancer morbidity increases by 6.5% per 5-year AA	**1.7%** increased risk of all-cause mortality within the next year
		Morbidities and Health Outcomes		
		Higher Fasting Insulin Levels (Ammous et al., 2021)	A 5-year increase in EEAA is associated with a 5% increase for higher fasting insulin levels	**1.3%** increased fasting insulin levels within the next year
		Increased BMI associated with EEAA (Huang et al., 2019)	EEAA per 5 years is associated with a BMI increase up to 2.4%	**0.64%** increased BMI within the next year
		Fatal Coronary Heart Disease (Ammous et al., 2021)	17% increased risk for incidence of fatal coronary heart disease within the next 5 years	**4.5%** increased risk for fatal coronary heart disease in the next year
		Physical Frailty (Gale et al., 2018)	A 1-year increase in EEAA is associated with a 6% increased chance for being physically frail or pre-frail, which is an age-related condition of physiological decline	**8.0%** increased risk for frailty in the next year
		CVD Morbidity (Huang et al., 2019)	5-year EEAA results in a 4% increased risk for the onset of CVD by the time you reach your chronological age of 47 years	**1.1%** increased risk for CVD within the next year

John Doe | Patient ID: zyy76e4 | Collection Date: 6/17/20 | Report Date: 7/1/20 3

Example TruDiagnostic summary report (full report is much more detailed)

Because longevity medicine practice centers on sophisticated genomics analysis, selecting the right test is important. My minimum criteria are:

1. The clock algorithm has been published and shares data on its relationship to disease outcomes. As of March 2023, companies like Elysium, Tally Health (founded by Sinclair), DoNotAge, Mudho, and EpiAge have no published data on their algorithms and whether they predict disease. TruDiagnostic with DunedinPACE is the only one.
2. The test should be comprehensive and produce actionable data, supported by clinical studies.

3. The lab should be independent and not tied to supplement marketing or lifestyle apps. Be skeptical of a company that is selling you an unproven solution.
4. They must have strong policies around data privacy. TruDiagnostic does their testing in house.

Mitochondria

So we know that cumulative genetic alterations of aging are mediated by activation or silencing of specific genes in response to circumstances such as caloric restriction. Much of this occurs in structures within cells called mitochondria. These are the energy processors of the cell, and a lot of aging and anti-aging action involves mitochondria. They're sort of a cell within the cell, having their own DNA and their own sirtuins. Mitochondrial sirtuins reflect the metabolic state of the cell, positioning them as stress sensors (nutritional stress, oxidative stress, etc.) If you are interested in healthy longevity, you need to be mindful of your mitochondria.

Caloric restriction mimetics

Because CR-mediated longevity occurs with remarkable consistency across species, whatever drives it is biologically fundamental. Understanding how it works remains a central question in anti-aging. A primary aim of the new era of anti-aging is to replicate the CR effect without the requisite semi-starvation. CR triggers a metabolic change which likely evolved as an adaptation to disruptions in food supply, mediated by sirtuins, which actuate the genes responsible for the metabolic changes of CR. The effect can be reproduced without nutrient restriction (at least experimentally) by sirtuin activators such as resveratrol. I'll confess that I was eager to connect this discovery about resveratrol to the French Paradox and longevity, as did many others. There turned out to be many problems with this hypothesis, and some high-level recent re-examinations of the original studies cast doubt about the results. The discovery did however provide an opening to probe cellular aging at a molecular level.

Substances such as resveratrol that directly or indirectly activate sirtuins are called caloric restriction mimetics (CRMs). With its numerous potential clinical actions, resveratrol was the flagship CRM. A wide range of potential health and anti-aging benefits have been investigated, including cancer prevention and treatment across multiple tumor types; diabetes;

cardiovascular health; viral, bacterial, and fungal infections; senile dementia/Alzheimer's Disease; osteoporosis; arthritis; immune dysfunction; hormone imbalance; and others. These are all mediated at least in part by sirtuin activation, highlighting the commonality between the underlying drivers of disease and aging.

Yet despite the availability of numerous supplement formulations based on resveratrol, validation from clinical trials has been elusive, with only a handful of studies showing measurable benefits in human subjects. Reasons for this include low bioavailability, first pass metabolism (things absorbed from the gastro-intestinal tract are processed through the liver before circulating), and *hormesis*, a phenomenon characterized by differential and sometimes opposite properties at lower vs higher levels of exposure. Bioavailability is limited by resveratrol's poor aqueous solubility and variable absorption. For these reasons, we can't assume that in vitro resveratrol studies extrapolate to in vivo clinical effects. We need to look for other options.

But what if we could find already available compounds and drugs that can be repurposed for anti-aging? This idea is being exploited with some notable successes using in silico screening, resulting in identification of candidates. This process hopes to find more drugs like the cheap and well-tolerated anti-diabetic drug *metformin,* a CR mimetic that has been available for years.

Metformin

Originally derived from the French lilac plant in 1922, Metformin, was developed as an antidiabetic treatment in the 1950's and remains the most widely prescribed medication for type 2 diabetes. As with resveratrol, metformin mimics aspects of CR by activation of SIRT1 in mitochondria.

In addition to optimizing mitochondrial metabolism, metformin has several other beneficial effects. It inhibits expression of cytokines associated with diseases related to cell senescence, immunity, and inflammation. Interestingly, other evidence suggests that metformin also restructures the gut microbiome, promoting the growth of beneficial bacterial species. It's an all-around good team player, especially where anti-aging is concerned.

Epidemiologic evidence strongly suggests that metformin users have a lower incidence of cancer and better survival rates, despite having diabetes. Metformin's potential as an anti-aging drug came to light in a study comparing type 2 diabetics taking metformin with matched non-diabetic controls; one would expect diabetics to have higher rates of mortality, but the opposite was found for those on metformin. This implies that metformin has anti-aging properties beyond of its anti-diabetes effects, and powerful enough to overcome its damaging effects.

Proving this in a prospective trial is another matter. A leading advocate for this is Nir Barzilai, MD, founding director of The Institute for Aging Research at the Albert Einstein College of Medicine. (Check out his book *Age Later: Health Span, Life Span, and the New Science of Longevity*.) In 2015 he conducted a small clinical trial called Metformin in Longevity Study (MILES), a placebo-controlled randomized trial in 15 subjects of average age 70. All subjects had metabolic improvements, and gene expression analysis in muscle and fat tissue from biopsies demonstrated significant shift to youthful patterns. This set the foundation for a much larger trial dubbed TAME (Targeting Aging with Metformin), with the explicitly stated goal of demonstrating that aging can be targeted in a FDA-sanctioned clinical trial. Success of TAME would also show the feasibility of clinical trials for aging as a target for intervention.

Funding issues stalled the launch of TAME, likely to due lack of sponsorship for an inexpensive, off-patent drug. Word is that TAME is finally underway now. It's an important study because it's not clear that metformin is entirely without adverse effects. One concern is that it may suppress mitochondrial respiration in response to exercise, negating the anti-aging benefits of exercise and antagonizing exercise-induced improvements in cardiorespiratory fitness.

An oft-cited study on adults in their early 60's looked at changes in insulin sensitivity and cardiac fitness after aerobic exercise training (AET), and its effects on mitochondrial respiration and protein synthesis in muscle. AET decreased fat mass and improved blood sugar control in both groups, but metformin attenuated the increases in overall insulin sensitivity and exercise capacity. Interestingly, the effect seemed to appear in only half of the subjects. It is not yet known whether this effect impacts the anti-aging properties of metformin. Notably, it was not an adverse effect per se, but a lack of the expected result of exercise. Nevertheless, the study has created some uncertainty about the wisdom of routine metformin use in nondiabetics.

There is some evidence suggesting that *berberine*, a botanical compound in a Chinese longevity medicine, may have similar properties to metformin without the adverse effects on exercise adaptation. It hasn't been tested in clinical trials, though there is evidence that berberine increases insulin sensitivity and alleviates a condition called metabolic syndrome - a cluster of conditions including increased blood pressure, high blood sugar, excess central body fat, and elevated cholesterol – that jointly increase risk of cardiovascular disease and related problems.

Take home message: The potential benefits of metformin probably outweigh its possible adverse effects for most people. Metformin is a prescription medication. If you decide to use metformin and you are not diabetic, you shouldn't have it filled at a regular pharmacy, because the on-label indication is diabetes. You don't want your insurance company categorizing you as a diabetic, and shouldn't expect your prescriber to fudge the diagnosis. Fortunately metformin is inexpensive. (We have made arrangements to dispense metformin through our office.)

<u>NAD+</u>

Mitochondrial sirtuin activity is dependent on the molecule *nicotinamide adenine dinucleotide*, usually expressed as its oxidized form NAD+. a co-factor that is central to metabolism and energy processing. NAD+ has a fundamental role in nutrient sensing, linking directly with the energy-processing enzyme AMPK in mitochondria. Importantly, NAD+ is a requisite substrate for sirtuin activity, and systemic NAD+ depletion correlates with aging and age-related pathologies. An experimental method whereby NAD+-generating enzymes can be "over-expressed" shows that lifespan and healthspan can be prolonged in mice, and restoration of NAD+ levels ameliorates diet- and age-induced pathologies associated with diabetes. Caloric restriction, resveratrol, and resveralogs activate sirtuins, which need NAD+; so why not just try to boost levels of NAD+? You can, and easily.

For these reasons, NAD+ has become a hot topic anti-aging research. The NAD+ precursors nicotinamide mononucleotide (NMR) and nicotinamide riboside (NR), a form of vitamin B3, have been shown to raise levels of NAD+ when taken orally in both human and animal models.

NR supplementation has been shown to extend the lifespan of mice even when administered late in life, while enhancing stem cell and mitochondrial function.

Because the NAD+ precursors NR and NMR occur in the human diet naturally (albeit in very small amounts), they are generally regarded as safe, and several clinical studies line up in support. A 2018 trial of NR plus pterostilbene – a polyphenol related to resveratrol but with higher bioavailability – assessed safety and efficacy in a population of 120 healthy adults ages 60 to 80 over 8 weeks. NAD+ blood levels showed sustained increases with this supplement, with no significant adverse effects. In December 2022 the results of a randomized, placebo-controlled clinical trial of NMN supplementation for 2 months on 80 middle-aged healthy adults evaluated physical performance (six-minute walking test), a blood biological age test (Aging.AI 3.0 calculator), NAD+ blood levels, and a standardized 36-item general health assessment. All subjects in the NMN group showed increased NAD+ levels, improved physical performance, and better general health compared to placebo. Biological age remained unchanged with NMN but increased in the placebo group.

Bottom line: Use of supplements containing the NAD+ precursors NR and/or NMR is almost certainly safe and might have longevity-promoting properties. Brand names to look for include Basis, Chromodex, and newcomers Elevant and Amelior.

<u>Rapalogs: Making sense of senescence</u>

The phenomenon of cellular senescence was first described by the renowned biologist Leonard Hayflick as a condition of cellular dysfunction occurring when cells reach their replicative limit but don’t die. This dysfunctional state can be triggered by telomere shortening, oxidative stress, and accumulated DNA damage. Senescent cells can either die off (using an “auto-destruct” program called apoptosis), enter a zombie-like state, or devolve into cancer. Senescent cells are an important feature of aging because they release pro-inflammatory cytokines and other harmful molecules, a state called the senescence-associated secretory phenotype (SASP). Like the bad apples in the barrel, SASP zombie cells affect not only senescent cells themselves but also nearby cells and the tissue environment, resulting in a state of what is called *inflammaging*. One presumed explanation for SASP is that cellular senescence suppresses tumor genesis, because precancerous cells share many characteristics with senescent cells. The inflammation caused by SASP factors incites destruction of precancerous cells by eliciting an immune system response.

SASP is a blunt instrument though, and may actually provoke adjacent premalignant cells, so on balance it's believed to be better to eliminate senescent cells if it can be done selectively. Substances targeting clearance of senescent cells are termed *senolytics*, and are a major focus of research and product development. A promising approach is the combination of the flavonoid quercetin and the anti-cancer drug Dasatinib. (Quercetin is available in supplement form and is nontoxic in usual doses, but Dasatinib must be administered under close medical supervision. Subjects in the study were in hospital.)

Klotho

In Greek mythology, there were three sisters known as the *fates*: Klotho spun the thread of life, Lachesis measured its length, and Atropos determined when it would end. Hence the name of a protein termed klotho, known to be associated with lifespan. Senescent cells produce less klotho, and a recent study of quercetin and dasatinib for treatment of pulmonary fibrosis implicated klotho as an important mediator. This is another example of the search for an on-label indication of a therapy that may have broader usefulness in anti-aging; pulmonary fibrosis is a specific disease, but fibrosis increasingly affects all tissues as we age. It's the reason why our muscles and joints stiffen. Therapeutics targeting fibrosis are a big deal right now.

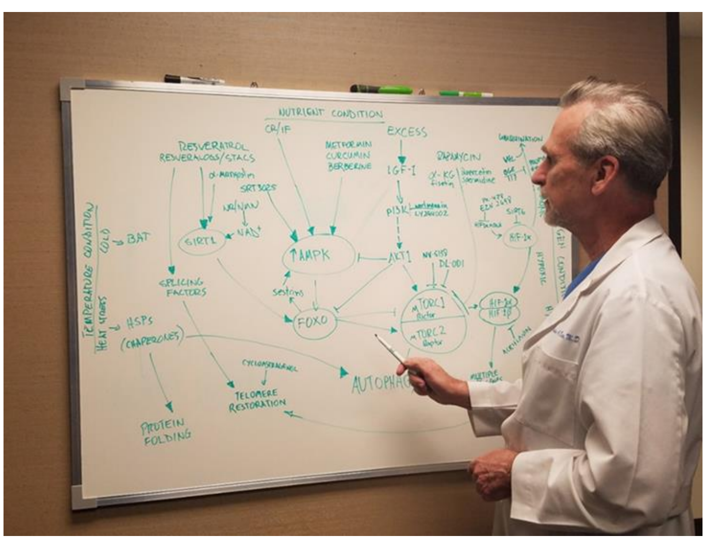
Autophagy

Autophagy (literally "self-eating") is an essential means of suppressing cellular senescence. It is such an important aspect of cellular function that the 2016 Nobel Prize for physiology and medicine was awarded to Yoshinori Ohsumi for the discovery of how it works. Ohsumi's lab and others identified autophagy-related genes (Atg), which code for proteins that package cellular waste. These are controlled by an enzyme complex called *Target of Rapamycin (TOR) kinase.*

TOR got its name when it was identified as the mechanism of action for rapamycin, a naturally occurring compound known for many years (like metformin.) Rapamycin was first isolated from a bacterium found in the soil of Easter Island (Rapa Nui) in the 1960's. Originally developed as an antifungal agent, it was found to have immunosuppressant properties and it is mostly used now to suppress rejection with kidney transplantation. Researchers today feel that it can be better described as an immune modulator and anti-inflammatory drug, which blocks hyper-immunity rather than suppressing immunity, or even that it "rejuvenates immunity."

Exactly how rapamycin worked remained unclear until the 1990's, when it was recognized as an inhibitor of the enzyme complex mTOR (originally called mammalian Target of Rapamycin, now referred to as mechanistic Target of Rapamycin). TOR is a principal orchestrator of cell growth and functions as a central coordinator of metabolism in response to both environmental and hormonal signals such as caloric restriction.

Experimentally, rapamycin has been shown to extend lifespan in mice, even with treatment initiated late in life. In fact, *rapamycin is currently the only known pharmacological treatment that increases lifespan in all model organisms studied.* Conversely, aberrant mTOR signaling is linked to a variety of diseases, ranging from epilepsy to cancer. For this reason, rapamycin and derivatives (rapalogs) may find a path to approval as disease therapies but with potential use in anti-aging. For example, impaired autophagy is a known pathogenic mechanism in neurodegenerative disorders, due to the suppressed removal of neurotoxic misfolded proteins (proteins have to be folded just right). Rapamycin provided the first proof that pharmacological promotion of autophagy can protect brain tissue. For now, rapamycin is off-label for anti-aging.



My metabolic metro map

Our metabolic metro map finds mTOR straddling a major intersection. Here we find that autophagy is regulated two subtypes of mTOR complexes, conveniently called TORC1 and TORC2. Inhibition of TORC1 prolongs lifespan, whereas inhibition of TORC2 does the opposite. TORC1 inhibition therefore represents an enticing objective for anti-aging therapies, ideally without inhibiting TORC2. Rapamycin is a relatively nonspecific inhibitor of mTORC1, and long-term administration results in impaired glucose tolerance related to mTORC2. And as with other nutrient sensing regulators, TORC pathways exhibit significant hormesis (opposite effects at different doses), and the effects of intermittent vs regular dosing differ. So, it's complicated.

A study at the University of Washington is evaluating potential anti-aging effects of non-immunosuppressive doses of rapamycin in middle-aged dogs, based on the rationale that dogs share the human environment, have similar risk factors, receive comparable medical care, and develop many of the same age-related diseases. Plus they live longer than mice, so their aging characteristics are more similar to humans. After 10 weeks, the study found no side effects in the rapamycin-treated dogs compared to those receiving placebo, and possible improvement in age-related measures of heart function. Based on these findings, the study has been expanded.

There aren't many human trials on rapamycin for aging as yet though. One, a pilot study in human subjects aged 70-93, showed that low-dose rapamycin was well-tolerated. A trial called Participatory Evaluation of Aging with Rapamycin for Longevity (PEARL) specifically intended to investigate efficacy and safety of rapamycin to promote longevity is reportedly in the works.

Spermidine

Caloric restriction mimetics also trigger autophagy. Like most biological processes, autophagy can be promoted by either increasing its activators or decreasing its repressors, which is what CRMs do. One increasingly studied inhibitor of autophagy repressors is spermidine, a naturally occurring molecule which is involved in regulation of cell proliferation. (It is particularly abundant in sperm, hence the name.) Spermidine is produced in various cell types, the gut microbiome, citrus fruits, animal proteins, and especially in fermented foods. Dietary spermidine intake has been found to correlate with longevity even after adjustment for lifestyle factors. Spermidine is readily absorbed after oral intake and has high bioavailability.

Spermidine may have anti-cancer effects, and spermidine-based cancer prevention and therapeutics are being actively investigated. Further evidence suggests favorable effects on brain aging, immune senescence, and cardiovascular health. Clinical trials of spermidine supplements are mostly preliminary, but with generally positive results.

Another potentially useful mTOR inhibitor (autophagy promoter) is alpha-ketoglutarate (AKG), a supplement widely used to improve athletic performance (though with debatable evidence.) AKG levels are known to change with fasting, exercise, and aging. In mice, adding AKG to the diet decreases systemic levels of inflammatory cytokines and prolongs healthspan and lifespan.

A product called Rejuvant, developed by Ponce de Leon Health in conjunction with the Buck Institute, is backed by some impressive science. To prove their case, the initial order comes with a DNA methylation age test, which customers are encouraged to repeat after 6 months of use. As supplements go, it's not inexpensive especially considering that the primary ingredient is widely available in other formulations. The manufacturer claims that combining AKG with vitamin D for women and vitamin A for men enhances effectiveness, so there is a different formulation for each. In 2021 they published a study demonstrating an average 8-year reduction in biological age after an average of 7 months of use, measured by the TruAge DNA methylation test. This test is based on an unpublished algorithm, and it isn't known how it compares to the more widely used ones. In February 2022, Ponce de Leon promised that results of a larger, placebo-controlled trial would be out soon. The company's founder, Tom Weldon, reports that his own biological age is slowing faster than his chronological age is advancing.

Several mTORC1-specific rapalogs have been developed and are in various stages of clinical testing. The search has been facilitated by recent technical advances, resulting in identification of a number of available mTORC1 and mTORC2 regulators. The first of these were *everolimus* and *temsirolimus*, both approved for use in treating kidney cancer. San Francisco-based Aeovian Pharmaceuticals has developed a rapamycin analog, DL001, reportedly 40 times more selective for mTORC1 than rapamycin. It appears that Aeovian's objective is for FDA approval to treat a condition called tuberous sclerosis complex, but investor statements clearly identify the large opportunity to also target age-related diseases and anti-aging.

The challenge of proving clinical efficacy for rapalogs is enormous however. Several attempts have stalled or been scrubbed from clinical development following disappointing early results, including samotolisib (Lilly), and gedatolisib (Pfizer). A company called Unity Biotechnology has a compound called UBX0101 which targets senescence through separate pathway intended to eliminate senescent cells outright, but it failed in a phase 2 trial for osteoarthritis. Again, a promising candidate treatment lost in translation.

Urolithin

An important type of autophagy targets worn out mitochondria, a process called *mitophagy.* It is important because dysfunctional mitochondria are a hallmark of aging, manifest for example by age-related loss of muscle mass. For that reason, mitophagy-activating compounds hold promise for maintaining and restoring muscle strength. In 2022, clinical trial results were reported for a mitophagy promoting compound called Urolithin A, a gut-microbiome-derived metabolite of a substance in foods such as pomegranate, berries, and walnuts. After 4 months of supplemental Urolithin A, the data revealed significant gains in muscle strength, aerobic endurance, and physical performance compared to placebo in adults 40-64 years of age. The supplement (brand name *Mitopure*) was shown to be safe and is available online.

What can telomeres tell us?

Telomeres are caps that prevent unraveling on the ends of chromosomes, like the stop at the end of a zipper. Telomeres shorten during each cell replication, eventually depleting, resulting in senescence by disabling cell replication. Telomere length was one of the earliest hallmarks of aging to be identified, but recent studies paint a conflicting picture. *Telomerase* is an enzyme capable of re-elongating telomeres, but factors that regulate it are complex and differ within cell lines and between individuals. Activation of telomerase is a tantalizing prospect in anti-aging, but as a target for intervention it has yielded inconsistent results.

It isn’t even clear that telomerase is a central mediator of aging. Consider for example the Baltimore Longitudinal Study of Aging, which prospectively measured changes in telomere length over 13 years. The study did find that average telomere length shortens with aging, but the direction and scope of change varied considerable in different cell types and even more across individual subjects. Another longitudinal study of older adults in Spain similarly found that baseline telomere length failed to predict what is called “frailty phenotype” or mortality.

Studies on identical twins reveal some interesting insights on the relationship between telomere length and the effects of environmental stressors. A unique opportunity to evaluate the effects of long duration space flight on telomere length and other aging biomarkers was provided by the NASA twin study, when astronaut Scott Kelly had a "ground control" twin Mark. After a year in the International Space Station, assessments on Scott identified spaceflight-specific changes, including genome instability, DNA methylation alterations in immune and oxidative stress-related genes, and unexpected telomere *elongation.* Average telomere length and global gene expression returned to near preflight levels within 6 months after return to Earth, though increased numbers of short telomeres were observed and expression of some genes was still disrupted. This paradox remains unexplained.

Nevertheless, the conceptual simplicity of telomeres restoration by activating telomerase remains compelling. And it might not be especially difficult, if unpredictable; traditional Chinese medicines, a regularly dredged source of anti-aging medicaments, has identified and at least one telomerase activator in the herb Austragalus. There is some clinical evidence to support the claim of telomere lengthening, and it appears to have a favorable toxicity profile. The active compound is a small molecule called cycloastragenol, patented under the name TA-65.

Undoubtedly the most controversial approach to telomere restoration involves gene therapy. A company called Libella Gene Therapeutics is pushing hard to get this accomplished. The idea is to transplant an extra copy of the telomerase gene, and it has been shown to have potential in studies on mice. This type of treatment carries significant risk however, not the least of which is activation of dormant cancers or premalignant cells. Because cancer is the prime example of cellular immortality, this illustrates a fundamental challenge in all anti-aging interventions. How do we selectively extend the life of healthy cells but not precancerous ones? And as if the idea of a clinical trial of telomerase gene therapy at this stage of development wasn't provocative enough, Libella announced that subjects would have to pay a $1 million fee to participate, and travel to South America for the procedure. No results have been posted that I could find, though the study was announced in 2019.

Bottom line on telomerase activating treatments? Be skeptical. Nonspecific telomerase activation as an anti-aging target remains a work in progress.

Oxygen sensing

Nutrient sensing has received the most attention in aging biology, but oxygen sensing may play a vital role as well. Both hyperbaric oxygen treatments (high oxygen) and hypoxia (low oxygen) point to possible longevity-promoting interventions. Hypoxic conditions activate what is called the Hypoxia-Inducible Factor (HIF) pathway, which facilitates adaptation to low oxygen. HIF is a key driver of regeneration involving sirtuins, mTORC1, and mitochondrial activity.

HIF signaling has also been identified as a target longevity pathway and an opportunity to use AI-based “omics” screening for compounds that can be repurposed for anti-aging. Leveraging a multi-omics database of human aging, the California-based biotech company BioAge showed that HIF activation levels were linked to multiple functional improvements as well as healthspan and lifespan. In 2020 they licensed a drug in development for kidney disease by the Japanese pharma company Taiko to commercialize it to treat diseases of aging.

Conversely, under certain conditions repeated *hyper*baric exposure can induce physiological effects which normally result from *hypo*xia, which is known as the hyperoxic-hypoxic paradox. It has recently been shown that hyperbaric oxygen treatment (HBOT) can induce HIF and sirtuin expression as well as promote stem cell proliferation, mitochondrial biogenesis, and telomere elongation. The clinical study involved 35 healthy adults aged 64 or older given 60 daily HBOT sessions. Telomere length increased significantly, and the number of senescent white blood cells declined after the final treatment. That’s a lot of time in hyperbaric chambers though, and these are the “hard” chambers of the type used for decompression for deep sea divers.

Temperature stress

Temperature stress responses yield meaningful insights into aging pathways, but until recently they have received less research interest because comparatively few targets for intervention have been identified. However, an understanding of cold and heat stress response is important, and potentially druggable targets are appearing.

Benefits of cold exposure likely relate primarily to brown adipose tissue (BAT), the function of which is thermogenesis in response to cold, and importantly also to modulate energy balance and insulin sensitivity. BAT develops in the embryonic stage, but was believed to diminish by adulthood. BAT is hard to detect, but a recent series of positron emission tomography (PET) scans – which create images based on metabolic activity – identified a cohort with metabolically active BAT. The presence of BAT correlated with lower odds of type 2 diabetes, dyslipidemia, coronary artery disease, cerebrovascular disease, congestive heart failure, and hypertension.

“Normal” fat or white adipose tissue (WAT) can be induced by cold exposure to undergo partial browning, a phenomenon termed beiging (as in turning beige). Beiging can be produced by daily application of ice packs to the thigh, with systemic effects mediated by mitochondrial respiration. Despite this increased understanding of brown and beige adipose metabolism, and devoted advocates of ritual ice water plunges and cryotherapy, clinical evidence remains scant.

Heat stress has been more thoroughly evaluated, and may play a more central role in longevity. A family of molecules known as heat shock proteins (HSPs) are called “chaperones,” whose function is to refold proteins that have acquired faulty conformations, and to prevent the aggregation of misfolded proteins. HSPs facilitate the development of functionally diverse “client proteins” including enzymes, transcription factors, and hormone receptors. Because these various proteins are involved in multiple cellular signaling pathways, HSPs have been implicated in a range of diseases and are considered a promising target for drug discovery.

HSPs have a dual role, operating in concert with systems that surveille and eliminate damaged proteins, as well as facilitating new protein assembly. For this reason, HSP inhibitors have been recognized as potential anti-cancer and antiviral therapeutics, including coronaviruses. Several tumor types overexpress HSP105, which has led to its possible use in designing RNA-based anti-cancer vaccines. In contrast, the citrus-derived flavonoid *nobiletin* extends lifespan in laboratory models, mediated in part by promoting expression of HSPs. A clinical study on the effects of exercise and protein supplementation in healthy subjects in their 60’s found that improvements in lean body mass were linked to increased expression of HSPs.

Autophagy and HSP activity decline with age along with a concomitant trend for protein aggregation, a hallmark of aging. Evidence indicates that beyond their role in proteostasis – folding new proteins, refolding misconfigured ones, and clearing those too damaged to salvage - HSPs operate as central lifespan determinants. When they malfunction, it manifests in neurological disorders, cardiovascular disease, cancer, and other degenerative disease. These multiple facets also create a challenge to identify specific HSP modulators for anti-aging applications without off-target effects.

Skin and anti-aging

Skin health is of obvious interest in aesthetic medicine and plastic surgery, but its role in systemic health and aging is often overlooked. The accumulation of senescent cells in response to environmental damage has implications beyond the visible manifestations of aging skin. Further, because the skin is the largest organ in the body and its continuous interface between the internal and external environment, it reflects overall biological age. It is now possible to determine biological age with a high degree of accuracy using AI-based algorithms based only on photographs. One AI platform called PhotoAgeClock outperformed the Horvath DNAm clock in predicting chronological age using only photos of the eye area! At the same time, because tissues age at different rates, DNA methylation age estimators trained using internal tissues are less likely to be accurate predictors of skin age. Truly anti-aging skin treatments require validated measures specific to skin to precisely quantify the effects of various treatments.

Topical senolytics are an exciting new strategy for skin rejuvenation. Rapamycin has been tested as a topical senolytic, resulting in reduction of senescence markers. A small placebo-controlled trial was conducted in subjects greater than 40 years of age with evidence of age-related photoaging (sun damage) and thinning of the skin. Subjects showed progressive reduction in levels of a cell senescence marker called p16INK4A protein, and an increase in collagen. Improvement in skin appearance was noted in multiple participants as well. A California-based company called OneSkin has developed a skin care product (called OS-01) with a proprietary senolytic peptide. Haut.ai validated their results. I am convinced that senolytics for skin care is a major advance and I recommend it frequently. Anecdotally, we are seeing faster healing after facial peels and on surgical scars with OS-01.

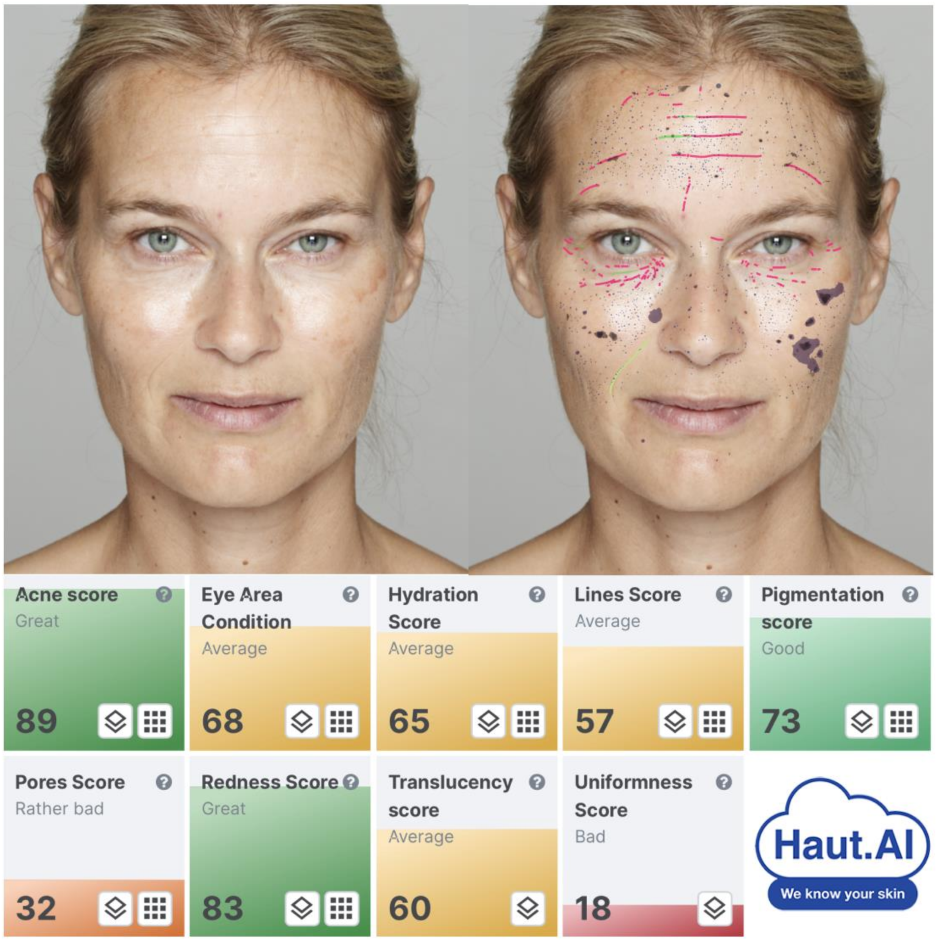


Illustration of the Haut.AI® Skin Metrics Report. Input image (left) is analyzed with AI-based algorithms and scores more than 15 skin conditions. The system produces "detection masks" of skin concerns, e.g. wrinkles/lines, sagging, pigmentation etc. that are overlaid on the original image (right). In this example, we present the detection of fine lines (pink); deep lines (green), pigmentation spots and uniformity (brown) and pores (blue).

Source: Artificial Intelligence approaches for skin anti-aging and skin resilience research. Anastasia Georgievskaya, Daniil Danko, Richard A. Baxter, Hugo Corstjens, Timur Tlyachev

Surprisingly, botulinum toxin A (Botox© and other brands) has also been shown to have anti-photo-aging effects via a senolytic process. One test used human skin cells in vitro, which were induced to undergo premature senescence using Ultraviolet B exposure, similar to photo-aging in living skin. The cells treated with botulinum toxin demonstrated a decrease in a senescence marker called SA-beta-gal, an increase in collagen production, and other restorative effects.

There’s clinical evidence of skin rejuvenation beyond botulinum toxin’s role as a wrinkle relaxer as well. A technique called microbotox, in which tiny doses of diluted botulinum toxin are injected into the facial skin (rather than the standard practice of placement into the muscle under the surface) found improvements in skin texture, tone, and pore size.

The basis for using nanofat (page 20) in skin rejuvenation has also been shown to involve senescence pathways. A study on cultured human skin cells pretreated with nanofat 24 hours prior to inducing senescence by exposure to UVB demonstrated significantly increased cell proliferation, reduced production of free radicals, increased collagen, and fewer cells expressing SA-beta-gal compared to non-treated samples.

For this reason, the use of nanofat in facial rejuvenation surgery is becoming more common. In facelifts it has become common to use fat grafting for volume restoration (volume loss is a feature of facial aging), and nanofat injected into the skin, or at least very superficially, enhances the result. This adds a biological anti-aging benefit to facial rejuvenation surgery.

Post-menopausal estrogen depletion is both a significant challenge and an opportunity in anti-aging skin care. Declining estrogen levels have multiple impacts on both the visible and structural qualities of aging in skin, including accelerated collagen breakdown, decreased elastin, and impaired moisture retention. These translate into thinning, loss of elastic recoil, dryness, and wrinkling. One answer to this is skin care products that selectively target the beta type of estrogen receptors (Erβ), which are abundant in facial skin. Estrogen taken orally does not seem to get into the skin, but topical application risks absorbing too much. Something that stays in the skin without a systemic effect would be the optimal solution.

A topical formulation with an estrogen analog called methyl estradiolpropanoate (MEP) might be just that. MEP exerts strong estrogen-like effects while being metabolized in the skin to an inactive compound, thereby avoiding systemic side-effects. A clinical study found 93% of participants reporting that MEP helped improve wrinkles, texture, and color after 20 weeks, with younger subjects responding after shorter treatment durations. I am happy to say that I was one of the first practices in the northwest to make this product (Emepelle) available through my office and our patients have been very impressed with it.

The use of resveratrol in skin care deserves a mention here too, in that its effects on skin health may relate to its phytoestrogen (estrogen-like plant-derived compounds) properties. It shares a similar chemical structure to estradiol and like MEP has affinity for Erβ. Resveratrol's small molecular size and lipophilic properties (dissolves in lipids better than water) facilitate permeation into the deeper layers of the skin. Here resveratrol also activates sirtuins in skin cells, modulating the effects of oxidative stress from UV radiation.

A company called Jeune is taking a more direct approach to skin rejuvenation by delivering the gene that makes collagen directly into skin cells. Like microbotox, it is delivered with multiple tiny injections. Preclinical studies appear very promising so this is one to watch.

VEGF: A very good factor for skin rejuvenation?

In the introduction to this book, I quoted from three news releases reporting on what I see as amazing breakthroughs in anti-aging. The third on was the study from scientists in Israel and Germany that found a way to experimentally restore aged human to a genetically and functionally youthful state. The protein that was identified as the mediator of this is called Vascular Endothelial Growth Factor-A, or VEGF-A. VEGF is found in PRP and exosomes, and is expressed in stem cells. If VEGF-A passes muster in clinical trials, skin rejuvenation will be only the beginning; the researchers believe that the model can be extrapolated to just about any organ or tissue. You would be correct to surmise that a lot remains to be worked out before we start squirting VEGF into people however; for example, HIF (page 35) seems to be involved, though exactly how is unknown.

Glycation: Targeting a fundamental aspect of aging

Age-related changes in skin have one thing in common with aging in tissues throughout the body: degradation of the extracellular matrix (ECM), the material between cells. This is comprised of proteins such as collagen, hydration molecules such as hyaluronic acid (the stuff that injectable fillers are most commonly based on), and others. A prominent feature of aging in the ECM is the result of sugar residues attaching to proteins, a process called glycation. A familiar example is hemoglobin A1c, which reflects the level of blood glucose levels over time. Glycated proteins are dysfunctional and accelerate tissue deterioration as they accumulate. In the skin this is manifest with thinning, loss of elasticity, inability to retain moisture. In muscles and joints the result is stiffness, loss of strength, and inflammation. Ultimately, these glycated proteins form what are called Advanced Glycation End products. A defective cell surface receptor for AGEs (called RAGE) is involved in several disease conditions such as pulmonary fibrosis, so glycation is a big deal in anti-aging across the board.

The good folks at SkinCeuticals have been working for some time on products to restore the ECM in skin. They have developed a cream based on proxylane, a sugar-protein hybrid molecule that helps repair the ECM. The flagship product in the category is cleverly called “anti-A.G.E.” Other anti-glycation compounds include curcumin, resveratrol, and metformin.

<u>The aging brain</u>

Ultimately no aspect of longevity medicine is more urgent than the aging brain. The baseball hall of famer Satchel Paige is credited with saying that “age is a case of mind over matter; if you don’t mind, it doesn’t matter.” I won’t argue with that, but when it comes to the gray matter of the brain It’s going to take more than a good attitude. Demographic projections foretell an impending crisis as age-related cognitive disorders crest over the coming decades. There is encouraging progress however, if recent findings can be validated clinically. In fact, there has never been more cause for optimism.

There are several big challenges to development of treatments for age-related cognitive decline, whether from effects of aging or specific conditions such as Alzheimer’s disease. For one, the brain is protected by the blood-brain barrier, a layer that restricts what molecules are allowed to enter the central nervous system. Many promising compounds are simply undeliverable to the target tissue. Another is the impracticality of sampling the tissue for analysis of the disease process or the effect of treatments; brain biopsies aren’t exactly kosher. And finally, there is zero margin for error or toxic side-effects where the brain is concerned.

Stem cell treatments are potentially able to overcome all of these obstacles, because they are your own cells, and they know how to deliver messenger molecules where they are needed. Adipose-derived stem cells are particularly attractive because they can promote regeneration of nerve tissue and are generally safe. Numerous clinical trials of stem cell treatments for a range of conditions, from Alzheimer's and Parkinson's diseases to brain injury from stroke.

I still try to keep up with research on wine and health, and this brought me to an interesting finding on brain function. It has to do with what is called the *glymphatic system*, which is the lymphatic system of the brain. As in the rest of the body, metabolic waste products in the central nervous system are cleared by the glymphatic system. Glymphatic channels open up during sleep, explaining a big part of why sleep is so important in anti-aging. What caught my attention was the finding that alcohol enhances glymphatic flow, at least up to a point. Since every major study of risk factors for Alzheimer's disease finds it lower in wine drinkers, that seemed to fit. At least a cause to raise a glass and offer a toast to the researchers.

In the big scheme of things though, the benefit doesn't go nearly far enough. Delving deeper into the biology of the aging brain, we find declining protein synthesis that in turn correlates with defects in protein folding. As misfolded proteins accumulate, they activate what is called the *Integrated Stress Response (ISR),* which impairs the protein synthesis required for memory formation. The ISR is a blunt instrument however - it may also promote inflammation - but there are limited other options to prevent toxic proteins from building up.

A less disruptive way to clear these proteins may a found in the drug-like molecule ISR inhibitor ISRIB, which has been shown in laboratory studies from Calico labs to restore memory function months after traumatic brain injury and to enhance cognition in healthy animals. A subsequent study found rapid and lasting restoration of youthful spatial learning and memory abilities in aged mice within a week after a series of 3 injections of ISRIB. The implications of this potentially long-lasting, simple, and effective way to reverse age-related cognitive decline cannot be overestimated. ISRIB has yet to be tested in humans though.

<u>Putting it all together: what we can do now</u>

Despite the momentum propelling the anti-aging field, completed prospective clinical studies are few in number but informative. Now that biological age can be measured, there are many in progress and we can expect results from these studies to validate anti-aging in ways not previously possible. Here are a few highlights of what we know now:

Time-restricted eating: Also called intermittent fasting, the idea here is to mimic the effects of caloric restriction by eating only during a limited time frame each day rather than reducing calories. It is a popular practice among the anti-aging cognoscenti these days. One crossover study (each group did both parts) compared gene expression patterns for a 6-hour eating schedule (8:00 am to 2:00 pm) to a 12-hour (8:00 am to 8:00 pm) on overweight adults. The time-restricted subjects showed stabilized glucose levels, increased expression of sirtuins, the autophagy gene LC3A, and mTOR. I try to practice time-restricted eating (with an 8-9 hour eating interval) and have not found it too difficult.

TRIIM trial: The "Thymus Regeneration, Immunorestoration, and Insulin Mitigation" trial investigated the use of human growth hormone to prevent or reverse signs of immunosenescence in middle-aged healthy men. GH was used based on prior evidence that it has thymus and immune reconstituting effects in animals, but because of the undesirable diabetes-like effects, it was combined with metformin and DHEA. After one year of treatment, the mean epigenetic age approximately decreased to 1.5 years less than baseline, a −2.5-year change compared to no treatment at the end of the study. The GrimAge predictor of human morbidity and mortality showed a 2-year decrease in epigenetic vs. chronological age that persisted six months after discontinuing treatment.

Diet & Lifestyle study: This randomized controlled clinical trial involved 43 healthy adult men aged 50-72. The 8-week treatment program included a plant-centered, low carb diet plus a special fruit & vegetable powder, a specific probiotic, at least 7 hours/night sleep, exercise 30 minutes/day 5 days/week and twice daily relaxation exercises. DNA methylation analysis found a more than 3 years decrease in DNAmAge compared with controls.

Rejuvant® supplement: Retrospective study of α-ketoglutarate supplement (AKG) showing an 8-year reversal in biological age as measured by the TruAge DNA methylation analysis, after an average of 7 months of use.

CALERIE™ (Comprehensive Assessment of Long term Effects of Reducing Intake of Energy) Designed to determine the biological effects of two years of caloric restriction in humans, this study has produced several findings of interest. Reduction of caloric intake by only 14%

produced improvements in immune function, systemic inflammation, and metabolism. The researchers subsequently identified the specific gene responsible for the effect (Pla2g7).

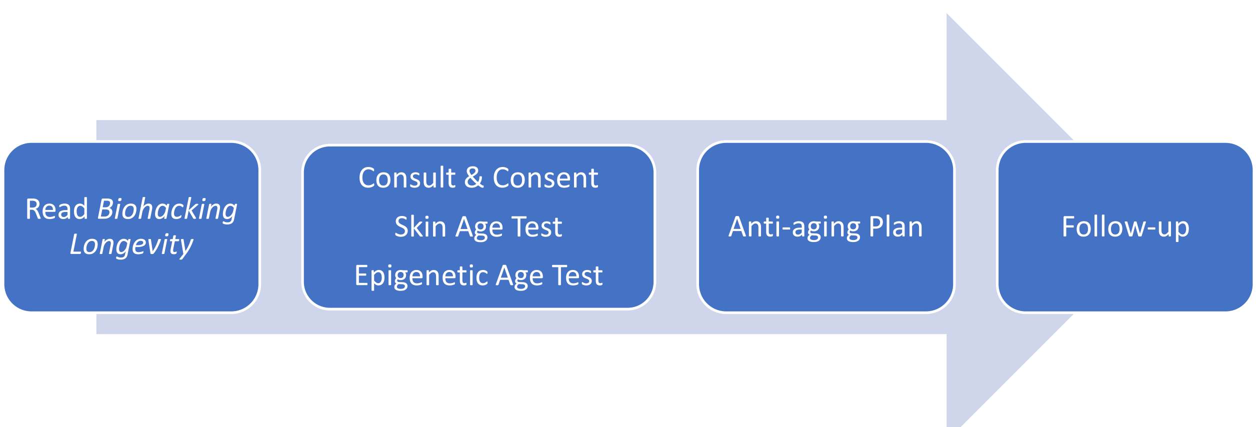
Summary and Future directions

It is now possible to slow and even reverse aging in humans. Anti-aging treatments can be quantified and validated, so the term should be used with precision. Anti-aging in the new era integrates basic and clinical science across multiple disciplines, bridges boundaries between academia and private enterprise, applies artificial intelligence, and reconfigures health care models from disease-based to healthspan-based.

Biohacking longevity is big business already, and getting bigger. Competition to capitalize on this 21st century gold rush is intense. No matter how savvy the consumer, it is ever more challenging to navigate the many options available now. Privacy of genomic data is a vital issue in this enterprise. As longevity science advances, so too does the sophistication of its scammers.

This is where the practice of longevity medicine finds the value proposition. My goal in my anti-aging practice is to provide independent and unbiased guidance. I will make recommendations according to the best information available. I don't want to get ahead of the science, but I don't intend to lag behind either.

Phase Longevity Practice Concept



The reason why I want potential patients for the anti-aging practice to start by reading this is because it represents a fundamental shift from the way most doctor-patient relationships are built. I want you to feel like an insider and empower you to know when you are being sold a bill of goods. We will objectively track outcomes, whether it is skin rejuvenation, prescriptions such as metformin, or aesthetic treatments. The anti-aging plan will be collaborative and personalized. This is only the beginning of what is looking like a long and fascinating journey.

Glossary

Aging biomarkers Measurable parameters that reproducibly, qualitatively and quantitatively reflect the rate of human aging.

Artificial Intelligence (AI) The general definition of AI is a form of computing science which enables the computer to process information in the way that the human mind does. There are several types of AI relevant to biomedical science and anti-aging:

- Neural networks – Software programs modeled after the way that adaptable nerve cells in the human brain are understood to work.
- Machine learning – the computer's ability to learn from examples and experiences. Methods include Random Forest, Bayesian Networks, and Support Vector.
- Deep Learning – a subset of machine learning composed of algorithms that enable software to train itself and process multiple layers of data.
- Other – Convolutional Neural Networks, especially useful for analysis of large datasets of images; Generational Adversarial Networks, where one program generates false images and the other tries to distinguish them from real images; Reinforcement Learning, and many others.

Autophagy Literally meaning "self-devouring," autophagy is the mechanism by which cells remove damaged or dysfunctional components. It facilitates the orderly recycling of cellular debris. Although autophagy was initially identified as a primordial response to starvation, it is now known that it also plays an important role in cell metabolism under normal conditions.

Epigenetics The study of changes to the genome that affect gene activity. Mechanisms of epigenetic function include DNA methylation and modification of histones, leaving a permanent record of gene activation and/or silencing.

Exosome A bubble formed of cell membrane containing a cargo of molecules to be delivered from one cell to another.

Genomics The study of all of an individual's genes, how they interact with each other and the environment, and the resulting impact on physiology and health.

Histones Protein structures forming spools around which DNA wraps, forming units called nucleosomes. Histones prevent DNA from tangling, and regulate gene expression.

Inflammaging Low-grade, chronic, systemic inflammation leading to more rapid aging.

In silico A term that means "done on a computer". In biology it usually refers to the computational modeling of biological processes. Examples include docking simulations, which model how biomolecules fit together and interact, and AI-based predictions of clinical effects.

In vivo In the living organism.

Longevity medicine AI-driven practice incorporating precision medicine concepts and interventions intended to prolong healthspan and lifespan.

Methylation One of the mechanisms of epigenetics. DNA consists of four bases, called cytosine, guanine, adenine, and thymine (the 4-letter "alphabet"). A chemical unit called a methyl group can be added to cytosine, resulting in methylation of that area of the DNA, suppressing activation of the gene. This leaves a record of genetic activity.

mRNA Messenger RNA (ribonucleic acid) is essentially a template transcribed from a gene. The mRNA strand then moves from the nucleus of the cell into the cytoplasm where it directs the assembly of proteins. Other forms of RNA include tRNA, which transfers amino acids onto the protein chain as it is being assembled; RNAi, or interference RNA, whose function is to block protein assembly or gene expression; and many others with a variety of functions.

Nanofat A type of fat graft in which the fat cells are removed by filtration, leaving the platelets, stem cells, and other factors to promote regeneration. It is typically used in conjunction with a facelift where it is injected at a superficial layer under the skin.

Omics The study of collective sets of data within biological systems, and how they translate into structure and function**.** Examples include genomics, transcriptomics, proteomics, and metabolomics. The ending "-ome" is used to address the respective categories of study, such as the genome, proteome, etc. The objective of omics is to identify, characterize, and quantify biological molecules that are involved in the dynamics of a cell, tissue, or organism.

Phenotype A term used in genetics for the observable characteristics or traits of an organism. This includes physical form and structure, how it develops, and its physiological properties. (In body contouring surgery for example we see specific phenotypes of fat distribution: the "apple body" and the "pear body.") An organism's phenotype results from the expression of its genetic code, (genotype) and the influence of environmental factors.

Precision medicine (PM) Also called personalized medicine, PM is a model that customizes healthcare decisions, treatments, practices, or products based on the individual's genome. PM has been widely applied in cancer therapeutics, and increasingly relevant in longevity medicine.

Protein folding The process by which strands of amino acids are formed into a 3-dimensional shape to make a functional protein. For example enzymes have "docking sites" to connect with their substrates. Misfolded proteins are associated with several specific diseases and many of the manifestations of aging. Enzymes that assist in protein folding are called *chaperones.*

Senescence In terms of anti-aging, senescence refers to cellular senescence, which is a zombie-like state cells can enter into when they reach the end of their replicative cycle but don't die. Senescent cells typically have accumulated DNA damage and faulty "self-destruct" signaling that normally eliminates them. Senescent cells secrete inflammatory molecules which contribute to both localized and systemic degradation (inflammaging.)

Senolytic Senolytics are drugs or other substances that selectively clear senescent cells. These include the drugs Dasatinib and Navitoclax, and the flavonoids quercetin and fisetin. Because senescent cells accumulate with aging and are associated with inflammaging, they are a prime target for anti-aging therapies. In contrast, **senomorphics** restore senescent cells rather than kill them off.

Sirtuins A class of signaling proteins that modulate the activity of genes involved in cellular metabolism, stress response, and aging.

Telomere Telomeres are bits of non-coding DNA and protein that cap the ends of chromosomes to prevent unraveling. Typically, they shorten with each cell replication cycle. Since short telomeres are associated with aging, methods to maintain and repair them is a major topic of interest in longevity medicine. The enzyme that restores telomeres is called telomerase.

Transcription factor (TF) A protein that controls the transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence. TFs regulate genes so as to ensure that they are expressed in the right cell at the right time and in the right amount.

Cell Biology 101

If it's been a while since your biology classes, here is a refresher on the basics:

- Cells are the primary building blocks of life forms. Bacteria are single cells, everything else is a compilation of many. Every cells has a complete copy of the organism's DNA.
- Chromosomes have DNA sequences called genes. The process of reading a gene is called transcription, which occurs in response to certain molecular signals. For example UV exposure signals skin cells to activate the gene for making melanin and give you a tan.
- Gene transcription occurs by the formation of sequences of RNA, which then leave the nucleus. The most common form of RNA is messenger RNA, abbreviated mRNA.
- mRNA is used as a template for stringing amino acids together to form proteins. Short sequences of amino acids are called peptides. Think of amino acids as words, peptides as sentences, proteins as paragraphs.
- Proteins then manifest the gene's purpose. Types of proteins include enzymes, which catalyze biochemical reactions; structural components such as collagen; and signal molecules such as hormones.
- Proteins have a specific 3-dimensional structure determined by the sequence of their constituent amino acids. Enzymes that help proteins fold properly are called chaperones.

- In addition to the nucleus, cells contain other structures called organelles. These include mitochondria (pronounce the “ch” like “k”), which process energy; and ribosomes, where mRNA assembles amino acids into proteins.