THE CRISPR Whisperer

PICTURE Series

for Ages 11 to 111

By Dorothy Semenow, PhD

Episode 03

Age Reversal

Illustrations by
Jane Burns &
Dave Wheeler

Graphic Design by
Eric Hansen

THE CRISPR Whisperer

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DEDICATION

If/When ❓
To All Who Make It Happen Safely!

You Don't Have To Be a Scientist
To Think Like One!

Episode 03 Age Reversal
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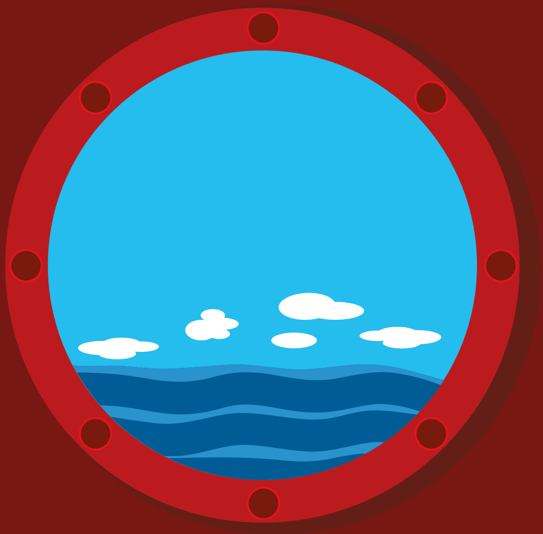
AGE REVERSAL



Age Reversal

Introduction

Since ancient times, people have dreamt of turning back the clock, reclaiming the looks and energy of their younger selves while keeping death at bay. In 2022 alone, they spent over $60 billion on products promising to do just that.

Since Ages Ago: Dreaming Away His Deepest Fear!

Do you share this dream of reversing the signs of aging and becoming young again?

If so, what different choices, if any, might (the now wiser) you make in your life "redo"?

Scientific innovations have already given us cleaner air, better healthcare, and vaccines that have extended our lives. Now, though, we're on the verge of something bigger. With genetic engineering advancing faster than ever, reversing aging is starting to look less like science fiction and more like a real possibility.

By 2024, scientists use genetic engineering to help mice live longer and healthier, and even restore vision in nonhuman primates. The next step? Bringing these breakthroughs to humans. We'll zero in on the science behind these advances and what they mean for the future.

Since Ages Ago: Dreaming Away His Deepest Fear!

What Causes Aging?

What's really behind aging? Getting to the root of that question is the first big step toward reversal. For years, scientists believed DNA mutations—small changes in the order of bases that make up our genes—are the main cause. But then, a surprising realization: some mice and humans with many mutations don't age faster, and many aging cells don't show the expected mutations.

This discovery led researchers to broaden their focus to include 'epigenetic' factors—changes that affect how genes behave without changing the DNA base sequence. One example: methyl (CH3) tags that attach to DNA bases and act like switches to turn certain genes off; their removal turns those genes on.

George Church's Overview

Overall, scientists still disagree about the main cause(s) of aging. In a March 2023 interview with lifespan.io's Arkadi Mazin, leading geneticist George Church share his take on the debate.

"There are two main theories. One says aging happens because of DNA damage, and the cell needs us to repair it. The other says it's all about epigenetics—convince the cell it's young, and it will repair itself. But some damage is beyond repair." Church supports a mix of both points of view, leaning toward the epigenetic side.

Progeria, a genetic condition that causes rapid aging, serves as a good case study, showcasing both theories in action.

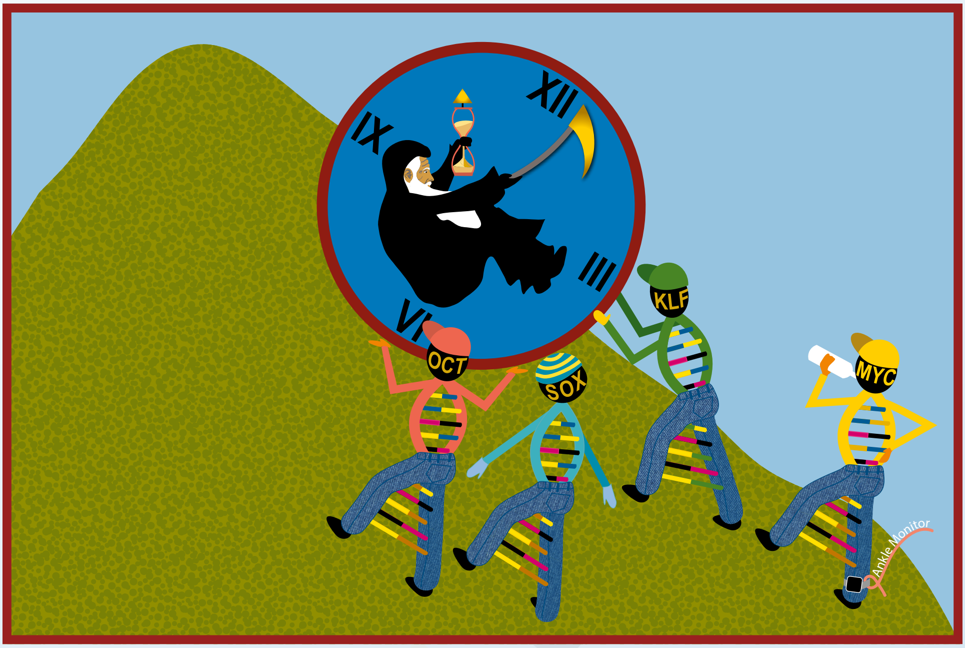
Key Tools in Age Reversal Experiments

David Liu

In 2021, David Liu's team used a CRISPR Base Editor to repair the LMNA DNA mutation that causes progeria, supporting the mutation theory of aging. I'll recap an epigenomic cure after I introduce the cool tool originally used for that breakthrough. That's next.

Shinya Yamanaka

One of the biggest game-changers in aging research comes from the Nobel Prize winning discovery of the Yamanaka factors—a set of 4 proteins identified in 2006 by Shinya Yamanaka's team. These proteins—Oct4, Sox2, Klf4, and c-Myc, a quartet also known as OSKM—can rewind adult cells all the way back to an early, versatile state called induced pluripotent stem cells (iPSCs). In this state, iPSCs are like blank slates, able to morph into various cell types, such as blood, muscle, skin, or liver cells.

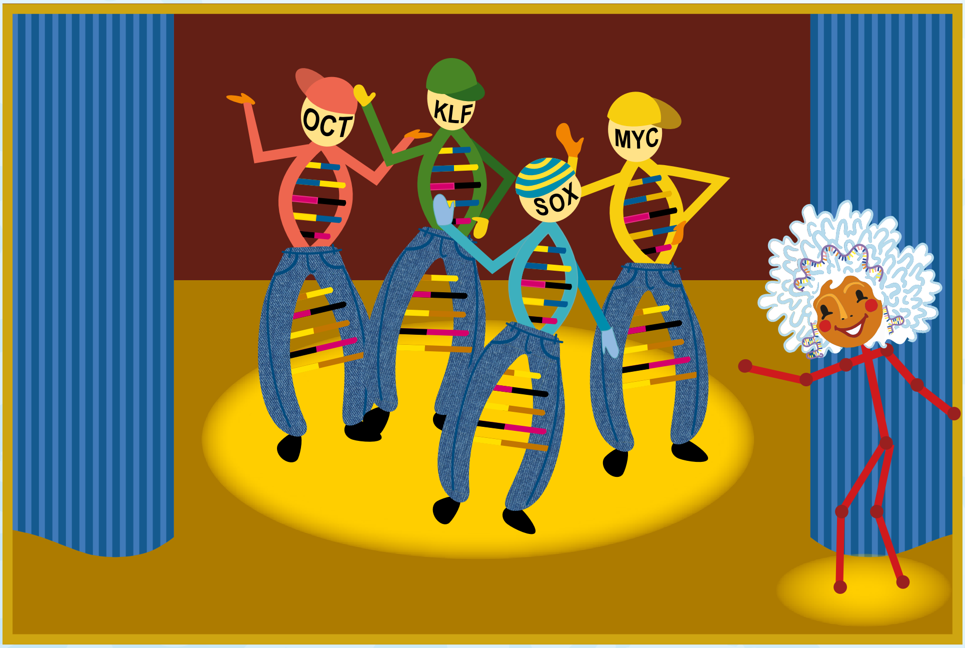


Yamanaka genes rolling back Father Time's downhill blitz!

Still, OSKM isn't a magic fix for aging—there are serious hurdles. If cells are wound back too far, they can lose their very identity, "forgetting" their jobs and failing to function properly in the body. There's also the ever-present risk of cancer. Reprogramming cells can cause them to grow and divide wildly, spinning out of control. And the process can also introduce mutations, turning healthy cells defective.

So, from the start, researchers took steps to minimize these risks. Both Yamanaka's team and Izpisua Belmonte's team successfully reversed cell aging without erasing the cells' "memories" of their specialized jobs. Their trick was to control how long the cells were exposed to the genes for the Yamanaka factors: 2 days on and 5 days off. Best of all, this method produced cancer-free cells. Others went a step further, cutting out the most cancer-prone gene, c-Myc, and relying on the safer OSK-producing trio to do the work.

Reversing aging has long been called biology's holy grail, a goal that continues to drive discoveries. And while CRISPR wasn't part of the first breakthroughs, she is quickly entering the spotlight in the quest to truly reverse aging.



Spotlight is on the Yamanaka Crew,
But CRISPR will soon add her talents too.

The progression: In 2016, researchers showed that Yamanaka factors can reverse aging in mice. By 2021, scientists uncovered that these same factors boost muscle regeneration. And in 2024, new tools like CRISPR began to reshape the possibilities of age reversal.

Medical Treatments: Turning Back Time

Izpisua Belmonte Lab

In 2016, Izpisua Belmonte's team showed that the 4 Yamanaka factors (OSKM) can reverse aging and extend lifespan in mice with progeria—a disease that speeds up aging

Treated mice lived 30% longer, looked younger, had stronger hearts, and were healthier overall. Even in normal, healthy mice, the treatment sped up tissue healing after injury.

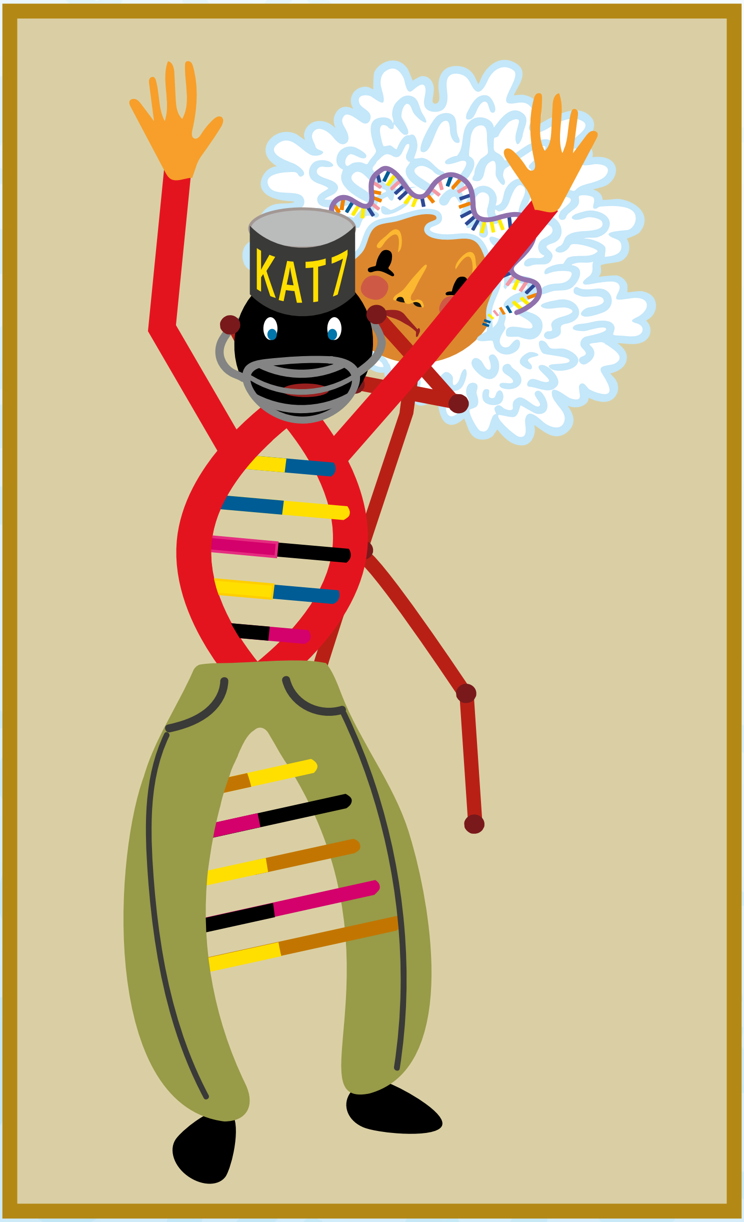
And 5 years later, the team found that the same OSKM factors boosted muscle regeneration in normal, aging mice. This discovery opened new possibilities for repairing tissues—not just for the elderly, but potentially for athletes, too.

Forget protein shakes,
he's all into that gene shakeup!

That raises the provocative question: Could treatments like this be used as performance enhancers in sports?

If age-reversal therapy becomes a reality, do you think the World Anti-Doping Agency (WADA) should ban it in sports?

Increasingly, Belmonte's influence reaches far beyond his own lab. He was co-author of a 2021 study from China, where scientists used CRISPR to turn off the KAT7 gene and reverse aging. His involvement underscores the global teamwork driving the race to understand and control aging.



CRISPR can muzzle genes!

The next year, Belmonte's team collaborated with Genentech to extend their Yamanaka factor research to normal mice across a broader age range—the equivalent of human ages 35 to 80. Their findings were striking: organs grew healthier, epigenetic markers began to resemble those of younger mice, and—perhaps most surprising—skin cells healed faster, with less scarring after injury.

The treatments proved safe: no signs of cancer, weight issues, or behavioral changes emerged. This longer-term study showed that the OSKM crew may hold more promise for slowing human aging than previously thought.

George Church Lab

George Church's lab stands at the cutting edge of age reversal research for humans and other mammals. In his previously cited 2023 interview with Arkadi Mazin for Lifespan.io, he discussed a key 2022 experiment.

His team engineered a gene therapy in mice to boost 2 key proteins: telomerase reverse transcriptase (TERT), which lengthens telomeres and, in turn, delays aging; and follistatin, which supports muscle growth and repair. The results: median lifespans of the treated mice increased by 41.4% and 32.5%, respectively.

One of the most promising breakthroughs was their solution to a major CRISPR challenge: how to deliver larger gene cassettes. By using cytomegalovirus (CMV) to carry these big genetic payloads—through inhalation or injection—they're getting closer to unlocking CRISPR's full power for complex genome editing.

As Church advances his quest to reverse aging, he champions an overarching vision: addressing age-related diseases as a whole rather than tackling them separately. He argues that by targeting the root cause—the aging process itself—science can create therapies to simultaneously combat multiple conditions, such as heart disease, Alzheimer's, and diabetes. For Church, age reversal isn't just a medical breakthrough—it's a transformative strategy to revolutionize how we can deal with all the effects of aging.

Rejuvenate Bio Lab

In January 2023, Rejuvenate Bio, a startup co-founded by George Church and his former postdoc Noah Davidsohn, released findings from a study on elderly mice (roughly equivalent to 77-year-old humans). The team injected the mice with adeno-associated viruses (AAVs) carrying genes for 3 Yamanaka factors (OSK), while deliberately excluding the 4th factor, c-myc, to reduce cancer risk.

The results delivered solid proof of concept: OSK-treated mice lived 18 weeks longer than their untreated counterparts, a 109% increase in their remaining median lifespan. They were healthier overall, with lower frailty scores, younger DNA features—such as fewer DNA mutations, more methyl groups, longer telomeres—and showed no signs of adverse effects. The bottom line: the study found no negative effects from the gene therapy, despite concerns about cancer risks.

Rejuvenate Bio next applied its age-reversal gene therapy to dogs—a smart strategy that paves the way for human treatments while tapping into the lucrative pet care market.

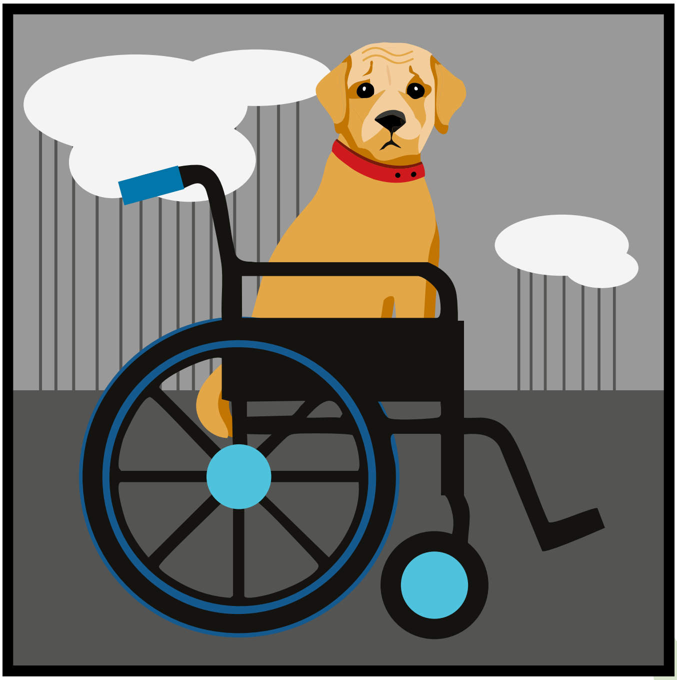
The first canine target, mitral valve disease (MVD), is a leading cause of heart failure. Rejuvenate Bio shared promising results at the May 2023 ASGCT meeting. Cavalier King Charles Spaniels (CKCS), a breed where nearly 80% are affected by MVD, took center stage in the pilot study.

Over nearly 3 years, 17 dogs received Rejuvenate's MVD therapy (RJB-01). Among 12 CKCSs treated with "the combo"—a combination of RJB-01 and the current standard of care, pimobendan—disease progression was delayed by more than 1.5 years compared to the reported progression time in all breeds treated with pimobendan alone. When compared to historical control data—again for pimobendan alone in all breeds—the results got even better: CKCSs treated with the combo gained ~600 extra days before disease progression and more than 800 days compared to placebo.

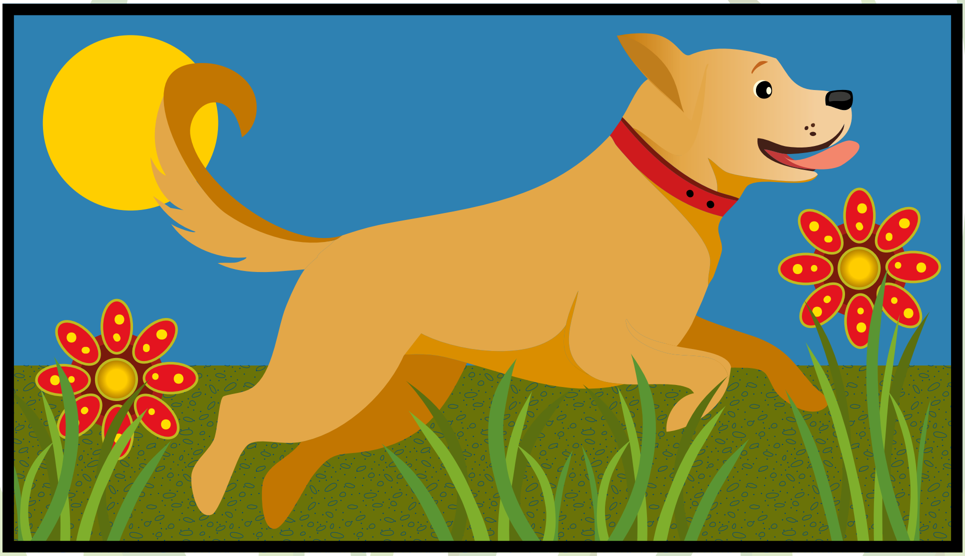
Rejuvenate Bio partnered with Phibro Animal Health in 2022 to commercialize its RJB-01 treatment, which doesn't rely on CRISPR or gene editing. The added genes remained outside the dogs' DNA, ensuring that no genetic changes were passed on to current or future generations—regardless of the dog owner's preferences for a clone-like replacement.

Rejuvenate is also targeting canine osteoarthritis (OA)—which causes intense pain and limited mobility, often leaving owners with the heartbreaking decision to put their pets down. Despite affecting 40% of dogs, the condition remains under-recognized and under-treated.

Rejuvenate Bio aims to change that. In 2022, the company partnered with Phibro Animal Health and 2 years later, added an unnamed world partner, to bring their novel gene therapy treatment to market.



From Rolling Sad,



To Bounding Glad!

George Church teamed up with his former postdoc Denitsa Milanova—an expert in mechanical engineering and genetics—to co-found Marble Therapeutics in 2021. Their mission: to develop a gene therapy that reverses wrinkles at the genetic level. Could this be the royal road to a Botox alternative?

Milanova believes the aging field has relied too much on approaches based on theories that require specific molecular pathways to be involved in aging. Her remedy? "Search the entire genome for those genes and proteins responsible for large, global shifts in the biology of cells as they age." Let the data dictate the way—patterns that turn up there will be her guide.

David Sinclair Lab: Yamanaka Trio Strategy

Do epigenetic changes cause aging, or are they merely a side effect? Scientists have grappled with this question for years.

Then, in January 2023, David Sinclair's lab delivered an answer, based on an impressive 13-year study conducted by an international team of 66 researchers.

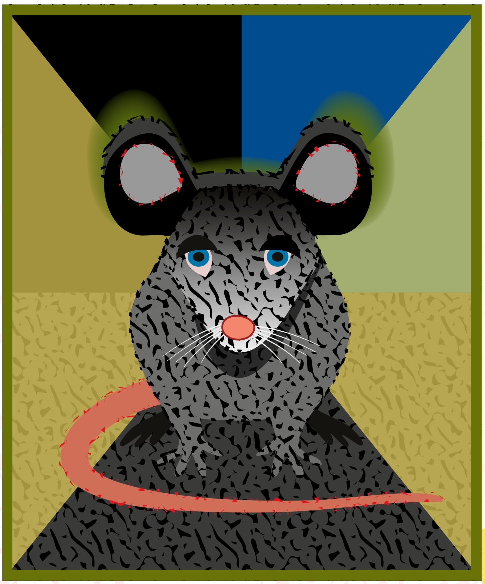
In their experiments, the team mimicked the daily damage to human DNA—from sunlight or chemicals—by creating precise, quick-healing cuts in the DNA of mice. These "ICE" mice (short for Induced Changes to the Epigenome) served as a model for speeded-up aging.

To track biological age, they developed a method to monitor the loss of methyl groups (CH3) from the epigenome. Using Yamanaka's OSK trio, they reversed signs of aging, restoring youthfulness to tissues and organs.

The results provide experimental support for Sinclair's "Information Theory of Aging," first proposed in 1997. According to the theory, aging isn't primarily driven by DNA mutations but by the gradual loss of epigenetic signals as we age—e.g., switches that turn genes on and off such as methyl (CH3) groups attached to DNA bases.

“This research shows how tweaks to the epigenome can directly impact aging,” Sinclair explained, likening it to “rebooting a malfunctioning computer…the therapy triggers an epigenetic program that makes cells remember their youthful state—it’s a permanent reset."

Jae-Hyun Yang, co-author of the study, summed it up: “Manipulating the epigenome allows us to fast forward or rewind the aging process.”



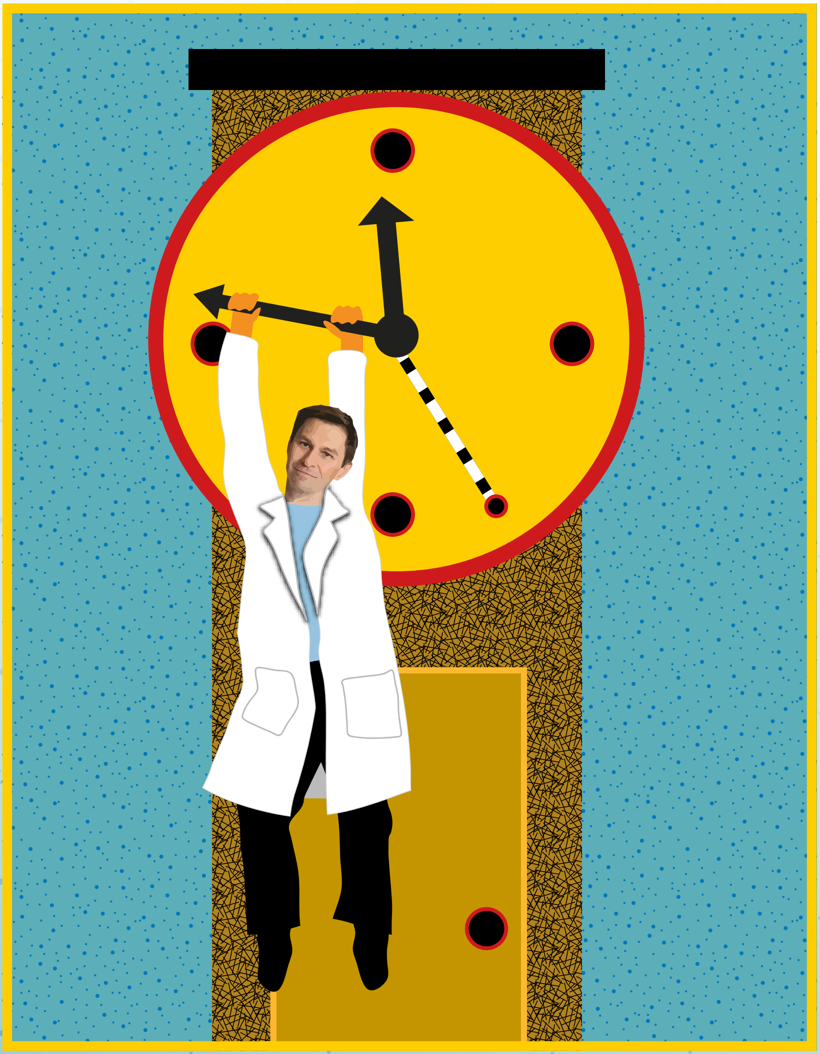
From Life in Decline

To Feeling Fine

Shortly after the ICE mice study, Sinclair's lab teamed up with Life Biosciences, which he co-founded, to reverse vision loss in monkeys afflicted with laser-induced NAION, an eye stroke-like condition. They treated 6 monkeys with OSK therapy, left 4 on a placebo, and used the antibiotic doxycycline to switch the OSK gene trio on and off as needed.

Vision tests over 5 weeks showed a clear outcome: treated monkeys regained their sight, while the placebo group did not.

"This showed mammalian cells have a backup of their epigenetic software," Sinclair explained. "We can reboot them to a youthful, healthy state."



Turning Back Time: David Sinclair Weighs In!

David Sinclair Lab: Small Molecule Strategy

The OSK Yamanaka factors reverse aging effectively, but their large size hampers delivery. So, Sinclair shifted his focus to small molecules (SMOs).

To measure the effectiveness of SMOs in reversing cell aging, Sinclair's team developed a clever, precise test to distinguish between young, old, and senescent cells—cells that no longer divide but haven't died. Their strategy involved tagging proteins with fluorescent molecules to track their movement across the border between a cell's nucleus and cytoplasm, which becomes leakier as cells age.

In young cells, the nuclear border remains strong, blocking movement across it. In old cells, the border weakens, allowing some proteins through. In senescent cells, the border breaks down completely, permitting much more crossing. By tracking the color-tagged protein movements, the team determined a cell’s age without the messy work of taking the cell apart.

Of the 80 small-molecule combinations they tested, 6 reversed aging in vitro. One of these cocktails reduced cellular age by 3 years in just 4 days!

David Sinclair: Charismatic Lightning Rod

David Sinclair: Detractor Attractor

David Sinclair's bullish claim in November 2022—that reversing aging in the elderly could be possible within 10–15 years—sparked intense debate. His statements captivated the public but also raised ethical questions and stirred controversy.

Key issues:

- Moving from animal models to human applications is fraught with risk. While Sinclair's studies showed success in mice and primates, these results may not carry over to humans—only about 5% of animal-tested medical procedures ever gain approval for human use.
- Sinclair's endorsement of compounds like resveratrol has raised concern for promoting treatments before their efficacy and safety are fully validated.
- Sinclair's frequent media presence has drawn criticism in academia, where public engagement is encouraged but not always rewarded, especially for tenure-track faculty who make controversial declarations!
- Despite doubters who question his findings, Sinclair's work has faced no major retractions, and critics often voice their concerns anonymously.

For all the controversy, Sinclair's lab and others have pushed our understanding of aging to a new level. Their work is making both scientists and the public rethink whether aging is truly inevitable. With every advance, however, comes the challenge of ensuring these discoveries are safe, effective, and ethical—a balance that's often hard to strike.

Before any of these treatments can pass regulatory muster, key technical hurdles must be cleared: ensuring safety, achieving precise dosing, and using the 12 hallmarks of aging to guide age reversal efforts. Next up: what these hallmarks are and why they matter.

Hallmarks of Aging: The Science of Getting Older

The hallmarks of aging—the key biological drivers first proposed by Carlos Lopez-Otín and Guido Kroemer's team in a January 2023 *Cell* article—offer a fresh way to think about why we age.

Aging is a series of biological changes where damage piles up in the hallmarks as the years pass. The more damage there is, the faster aging unfolds.

But it's even more complex than that—the hallmarks interact, creating a tangled web for researchers to unravel. Any attempt to fix one hallmark can trigger damaging changes in the others, making the work even trickier.

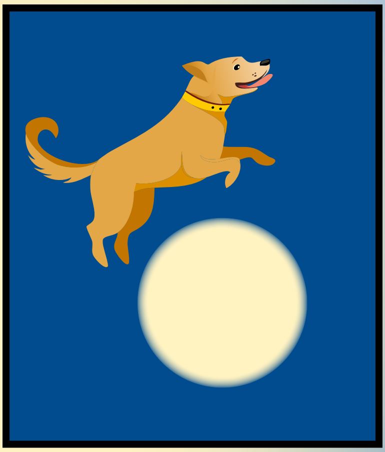
Researchers have rushed to meet the challenges. Since the hallmarks were first proposed in 2013, aging research has exploded. Recent studies extend beyond simple organisms to mice and even primates, showing that these hallmarks apply to mammals—including humans. And we now have the age reversal successes of the ICE mice and NAION monkeys, with more on the way!

The upshot: aging isn't inescapable. The hallmark changes aren't set in stone. Scientists now believe they can be slowed, stopped, or even reversed.

As of 2024, the team has identified 12 hallmarks:

1. **Genomic instability** is about our DNA getting damaged more as we age, affecting cell function.
2. **Telomere Attrition** involves the shortening of telomeres, the ends of DNA strands housed in chromosomes, which leads to aging cells.
3. **Epigenetic Alterations** are changes that affect gene activity without altering the DNA sequence, influencing aging.
4. **Loss of Proteostasis** refers to our body's declining ability to manage protein creation, folding, and disposal, leading to diseases.
5. **Disabled Macroautophagy** is about cells losing their ability to clear out damaged parts, causing aging and illness.
6. **Deregulated Nutrient-Sensing** shows altered cellular responses to nutrients, impacting growth and metabolism.
7. **Mitochondrial Dysfunction** describes our cellular powerhouses becoming less efficient, decreasing energy output.
8. **Cellular Senescence** means cells stop dividing and don't function right, contributing to aging and diseases.
9. **Stem Cell Exhaustion** indicates our reduced capacity for self-repair due to stem cell loss.
10. **Altered Intercellular Communication** involves changes in how cells talk to each other, affecting inflammation and tissue repair.
11. **Chronic Inflammation** is ongoing, low-level inflammation that harms cells and tissues.
12. **Dysbiosis** is an imbalance in our microbial communities, impacting health and disease resistance.

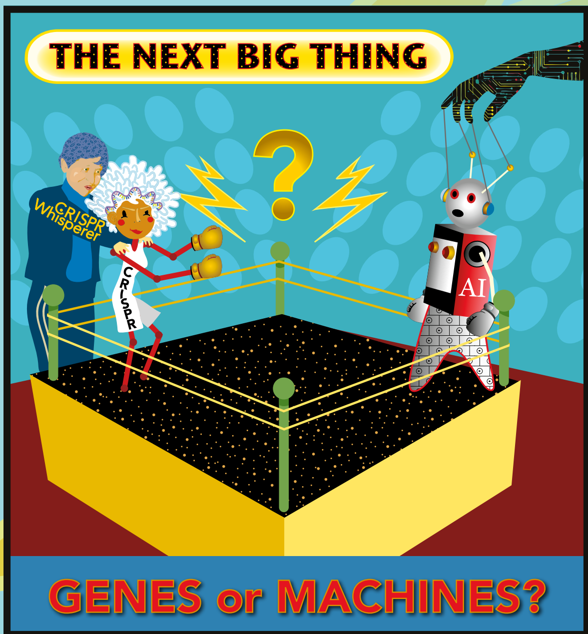
Fun Game: Create short, catchy, and humorous titles for each hallmark of aging! For a competitive twist, have fellow players score the titles based on laughs and applause.



The "Young-Again" Dog Jumps Over The Moon!

No Tinkering Allowed UNTIL...
You've read the fine print and deposited your lifeblood!

We close this episode with CRISPR pitching her uniting message—it's your invitation to visit the upcoming episodes of The CRISPR Whisperer Picture Series. We look forward to seeing you there!



Refreshed and recharged, she's set to go—
Next Stop: "From 'Bugs' To Labs!"

CRISPR Whispers Picture Series
EPISODES LIST

01 CRISPR Smarts Via the Arts
02 Mentoring, Creativity, and Women Entrepreneurs
03 Age Reversal
04 CRISPR Origins & History
05 "Dear CRISPR" Plea Letters
06. Critics Cavern
07. Science Lab
08. DNA Repairs & Upgrades Mall
09. Pest Control Corps
10. Extinction Controls & Returns Center
11. Super Seedy Greenhouse & Bioreactors
12. Indispensable CRISPR Support Centers
13. CRISPR Roots
14. CRISPR Foundation: Science Power
15. CRISPR Fun Activities

CRISPRwhisperer.org
and
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Glossary
Episode 03: Age Reversal

Aging
Time-related deterioration of the physiological functions necessary for survival and reproduction

Base Editor
A type of genome editing that uses CRISPR components to directly change a limited set of single DNA letters to other letters without making breaks in the DNA. Base editors are made by fusing (attaching) DNA-modifying enzymes to DNA-targeting Cas proteins like Cas9.

Epigenetic
Refers to changes to a cell's gene expression that do not involve altering its DNA code. Instead, the DNA and proteins that hold onto DNA are "tagged" with removable chemical signals. Epigenetic marks tell other proteins how to read the DNA, which parts to ignore, and which parts to transcribe into RNA.

Gene
A segment of DNA that encodes the information used to make a protein. Each gene is a set of instructions for making a particular molecular machine that helps a cell, organism, or virus

Genetic Engineering (Genetic Modification)
The use of laboratory-based technologies to alter the DNA makeup of an organism. This may involve changing a single base pair (A-T or C-G), deleting a region of DNA or adding a new segment of DNA.

Genome
A full set of chromosomes; all the inheritable traits of an organism.

Genome Editor
Tool to alter the genetic code of a living organism—ZFN, TALEN, or CRISPR. These systems are used to create a double-strand break at a specific DNA site. When the cell repairs the break, the sequence is changed. Can be used to remove, change, or add DNA.

Induced Pluripotent Stem Cells (iPSCs)
Cells that have been reprogrammed from mature, adult cells (such as skin or blood cells) into an embryonic-like state. This means that they have the potential to differentiate into any type of cell in the body.

Mutation
A change from one genetic letter (nucleotide) to another, which gives rise to the incredible diversity of species or differences between organisms of the same species. Some mutations have no consequence, while others directly cause

disease. Mutations may be caused by DNA-damaging agents such as UV light or may arise from errors that occur when DNA is copied by cellular enzymes. They can also be made deliberately via genome engineering methods.

Nuclear
Refers to the structure in a cell that contains the chromosomes, where DNA resides.

Primates
A group of mammals that includes humans, apes, monkeys, lemurs, and more. They are known for their large brains, opposable thumbs, and forward-facing eyes.

Protein
A string of amino acids folded into a three-dimensional structure. Proteins are each specialized to perform a specific role to help cells grow, divide, and function. One of the four macromolecules that make up all living things (protein, lipids, carbohydrates, and nucleic acids).

Regeneration
Process of regenerating or being regenerated, in particular the formation of new animal or plant tissue—e.g., animal muscle.

Senescent Cells
Cells that stop dividing but don't die, and can build up in the body as people age. They are also known as "zombie cells".

Telomeres
Protective caps at the ends of chromosomes that are made of DNA and protein. They shorten with age and can indicate how quickly a cell ages

Virus
An infectious entity that can only persist by hijacking a host organism to replicate itself. It has its own genome, but is technically not considered a living organism. Viruses infect all organisms, from humans to plants to microbes. Multicellular organisms have sophisticated immune systems that combat viruses, while CRISPR systems evolved to stop viral infection in bacteria and archaea.

Yamanaka Factors
A set of 4 genes for 4 proteins (Oct4, Sox2, Klf4, and c-Myc) that can reprogram differentiated cells into induced pluripotent stem cells (iPSCs).