Curing Aging: Mainly a "Garbage" Disposal Issue?

Introduction:
Recently, a novel anti-aging idea that could substantially reverse and prevent aging[1] and a novel cancer therapy idea[2,3,4] that could possibly treat many cancers without side effects were proposed.

To explain these therapeutic approaches, I must first give a bit of an introduction or refresher in biology. You are made up of cells and the molecular scaffold that they sit in.

Cells have DNA inside them, which is the set of instructions that they use to perform necessary tasks. Cells also have sub-compartments within them called "organelles" that perform different functions.

The nucleus, where the DNA resides, is a considered an organelle. Mitochondria are also organelles - they produce ATP, which is the energy currency for the cell. Cells also have lysosomes; these are like recycling centers, where old molecules go to be digested into their component pieces. The pieces are then used as building blocks for new molecules.

Cancer therapy:
In the very near future, thanks to new research, virtually all blood cancers may be curable using chimeric antigen receptor (CAR) T-cells[5]. CAR T-cells are genetically modified immune cells that attack cells with certain cell surface proteins. Because blood cells can be replenished by stem cells in the bone marrow, entire types of white blood cells, like T and B cells, can be eliminated without an issue. Solid tumors are much more difficult to cure because that same strategy clearly does not apply to the brain or heart, for example. While around 30% of childhood cancers are solid tumors, around 90% of cancers in adults are solid tumors. Additionally, adults are far more prone to developing cancer than children.

The proposed cancer therapy, called "OVERCOME", is mainly applicable to solid tumors. With certain modifications, it could potentially also work for blood cancers - which is important in case a given patient cannot be treated by existing immunotherapies or the new CAR T-cell strategy referenced earlier, i.e., if escape variants arise.

At the very least, it could be used for "purging" autologous hematopoietic stem cells, as allogeneic transplants often lead to graft vs. host disease.

OVERCOME involves first performing DNA sequencing on a patient's tumor or tumors. Circulating tumors cells and circulating cell-free tumor DNA can also potentially be analyzed in this context. The DNA sequencing will identify if a patient has one or more mutations that are shared between all the biopsied regions, which almost always seems to be the case. However, a number of patients may have no such mutations in their cancers. In this scenario, a small set of mutations that together are present in all of their cancer cells could be targeted.

After sequencing, clinicians or researchers would then bioengineer a virus or intracellular bacterium to be unable to replicate until it senses the target mutation(s) in a host cell that they infect. Bacterial vector replication upon mutation detection should be transient in order to preclude systemic infection.

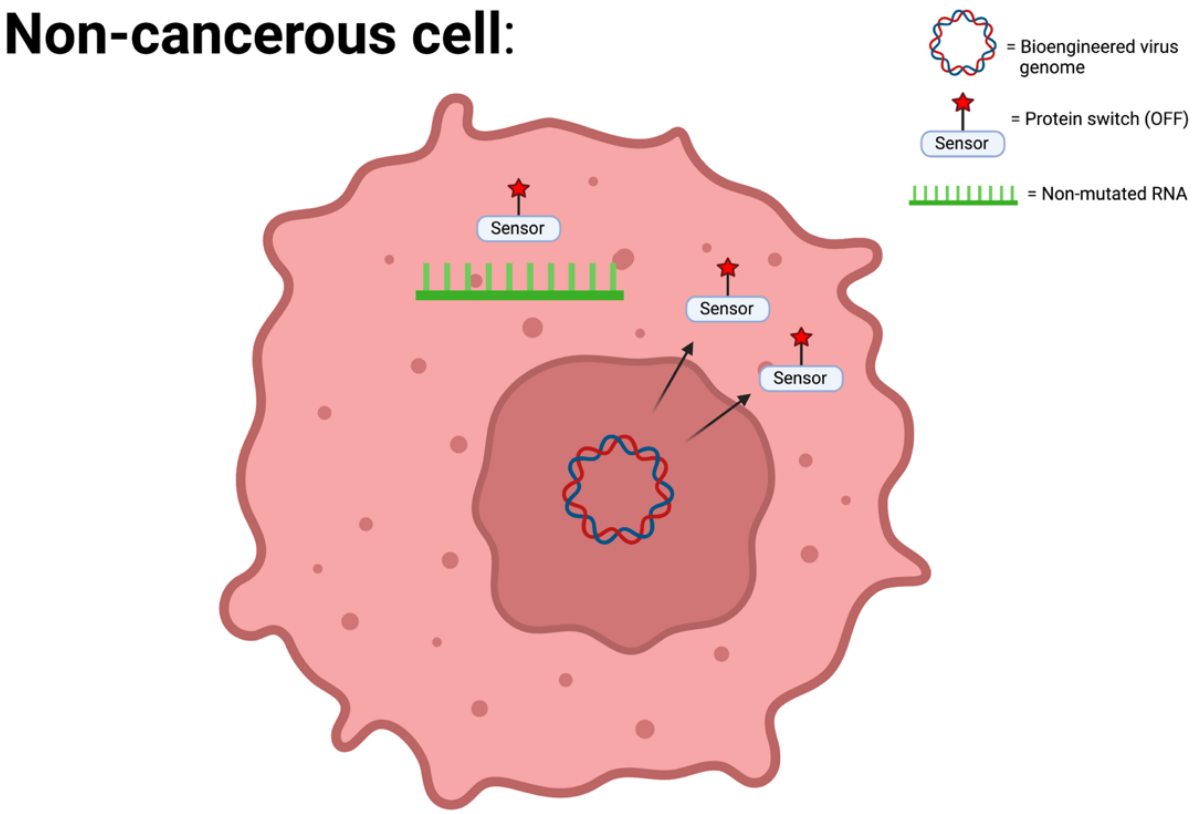
A bacterium, as opposed to viruses, can contain a large amount of foreign DNA to be used for therapeutic purposes. Thus, intracellular bacteria would make better vectors. A "facultative" intracellular bacterium might be best; such bacteria can survive outside of human cells, but can also enter them and detect the target mutation(s) from the inside. Ones that detect the mutation(s) can transmit the "signal" to nearby intracellular or extracellular bacteria in a given tumor.

The virus or intracellular bacterium could be injected intravenously into a patient. They would infect many non-cancerous cells as well as cancerous cells. But they would not create more copies of themselves until they detect the targeted mutation(s), so normal cells would be safe from their negative effects.

Upon detection, however, the virus or intracellular bacterium would become hyper-virulent and replicate aggressively, lysing their host cell and spreading throughout the patient's tumor or tumors.

This process would be repeated in each new cell that is infected - keeping normal cells bordering their tumor(s) safe from harm.

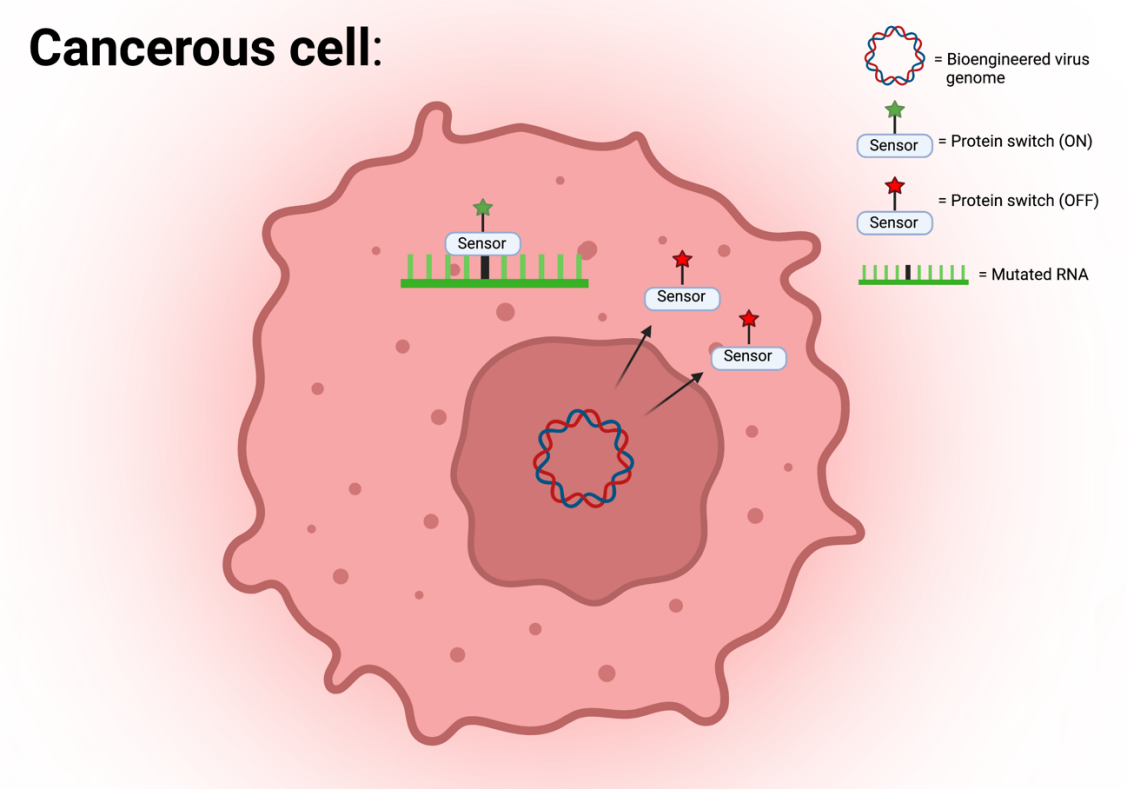
Figure 1:



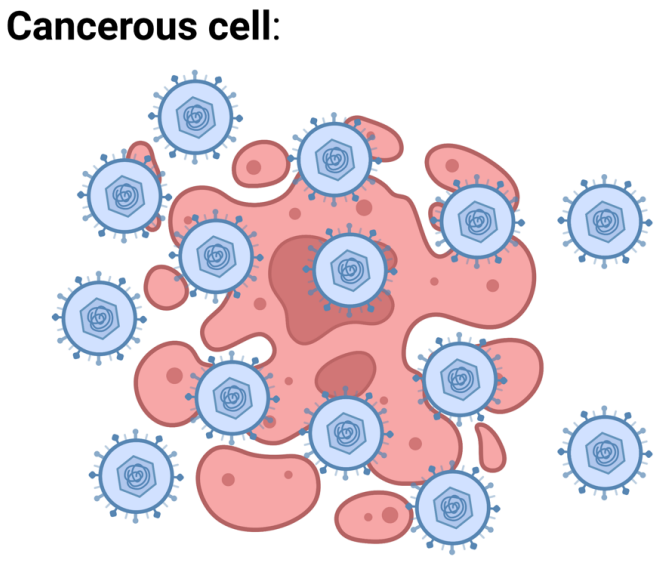
Caption: With regard to a non-cancerous cell, the bioengineered virus will still be able to enter and get its genome to the nucleus. However, once there, it will not replicate - it will simply send out sensors to look for the target mutation(s). Since the target mutation(s) will not be present in the non-cancerous cell, the virus will remain dormant. The bioengineered

viral genome can be programmed to self-destruct via after a certain period of time or in response to a small molecule after the therapy is complete.

Figure 2:
A)



B)



Caption: A) With regard to a cancerous cell, the bioengineered virus will enter and get its genome to the nucleus as well. Once there, it will also not replicate yet - it will simply send out sensors to look for the target mutation(s). The target mutation(s) will be present in the cancerous cell, and the sensors will detect it or them. B) Upon detection of the mutation or

mutations, the virus will activate and replicate uncontrollably - eventually lysing the cell and spreading to neighboring cells.

Lipofuscin as the main cause of age-related diseases currently afflicting us:
With regard to aging, we will focus on lysosomes. Although there are multiple theories about why we age, one stands out to me at least as being the most plausible based on the evolutionary and mechanistic evidence. This theory is called the "garbage catastrophe theory of aging."

Drs. Brunk and Terman posited years ago that aging can essentially be summed up as a "garbage disposal issue." The main idea is basically that old molecules are sometimes damaged in ways that prevent the enzymes inside of lysosomes from breaking them down properly. Over time, these warped, old molecules accumulate inside the lysosomes. As one ages, many lysosomes eventually become full of this indigestible garbage, i.e., "lipofuscin", and cannot perform their normal function. At this point, there is a garbage back-up - and the cell starts to decline health-wise.

In our bodies, we have cells that divide and cells that don't divide. For a dividing cell, consider a scenario where it has twenty lysosomes full of lipofuscin, but then proliferates into two "daughter" cells. Each of the resulting cells would only have ten lysosomes full of lipofuscin. Thus, cell division "dilutes out" the garbage problem.

This is why *Hydra vulgaris* is essentially biologically immortal. It has stem cells in its "body column" that continuously divide to replenish the cells at its extremities, and the ones at the extremities continuously slough off. Thus, indigestible garbage is continually expelled from its body.

However, for non-dividing cells in human brains, hearts, skeletal muscle, etc., the garbage has nowhere to go. There is a surplus of data showing that aged, non-dividing cells in the human body become densely packed with lipofuscin-laden lysosomes.

One clear issue with non-dividing cells becoming full of lipofuscin is that they cannot recycle damaged mitochondria effectively. They also will not be able to degrade various proteins as effectively, leading to hyper-phosphorylated tau accumulation intracellularly and beta-amyloid deposits extracellularly. (You may have heard that those two molecules, tau and beta-amyloid, are implicated in causing Alzheimer's disease, etc.)

Additionally, cancer is more likely to initiate or progress if many areas of the body are burdened by high levels of lipofuscin, preventing them from working efficiently to repress malignant growths. Similarly, the cells that support stem cells in their "niche" can accumulate lipofuscin - potentially preventing the stem cells from replicating efficiently to replenish tissues.

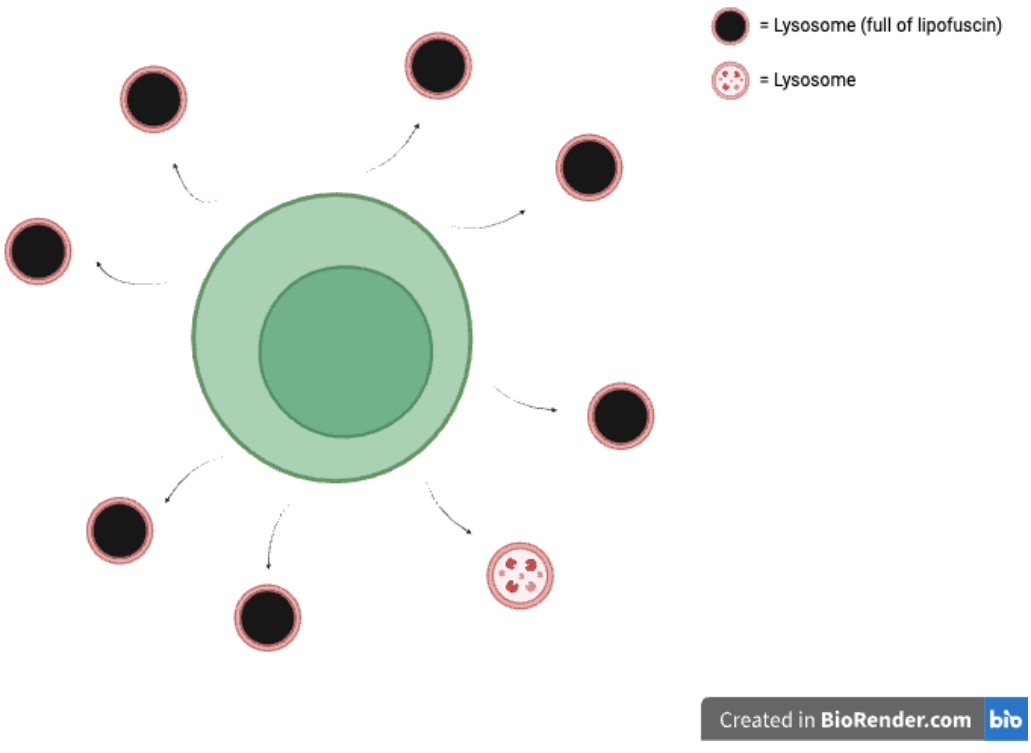
You may have heard of "senescent" cells. They are permanently arrested in their division potential as a safety mechanism to prevent cancer after accumulating mutations. However, they sit in tissues and secrete inflammatory factors that contribute to aging. In youthful organisms, there is evidence to suggest that the immune system destroys them. They may start to accumulate when bone marrow, spleen, and thymus aging occurs due to lipofuscin accumulation.

Lipofuscin clearance:
The proposed anti-aging therapy details a way of clearing lipofuscin-laden lysosomes out of our cells and out of our bodies. It involves eliminating our tissue-resident macrophages (TRMs) and replacing them with edited variants. When a small molecule is administered, these edited TRM variants would become hyper-motile and secrete RNA or DNA to surrounding post-mitotic cells to "instruct" them to make more lysosomes and export some of their mixed-aged lysosomes.

The edited TRMs would also collect extracellular lysosomes, counting each time one is picked up. After they have phagocytosed an experimentally determined number of lysosomes, they would asymmetrically divide, wherein one progeny cell retains all of the collected lysosomes. This progeny cell would then migrate to an extraction point in the body, where it can autonomously leave the body or be easily extracted from the body.

For reasons that are a bit too complex to go into here, reversing aging with this therapeutic approach may not be quite as effective as it is in preventing aging, but it will likely be substantially rejuvenative for those who are very elderly at the time this therapy has been developed - and could prevent further aging in those individuals.

Figure 3:



Caption: A cell exporting some of its lysosomes.

Other anti-aging therapies:
Other anti-aging therapies could help elderly individuals live long enough to benefit from the cancer and lipofuscin removal treatments. For example, being able to destroy senescent

cells, i.e., those that have lost replication potential due to the activation of an oncogene - would be very helpful. CAR T-cells may be able to achieve this[6]. Small molecule activators of TFEB and perhaps telomerase could also be helpful[7]. Adeno-associated viral or cytomegaloviral gene vectors could also be used to overexpress telomerase in some of our cells[8,9].

The other theories of aging:
There are a few other, well-established theories of aging. One is the mitochondrial free radical theory of aging. Mitochondria, as I mentioned, are the organelle in the cell that produce ATP, or the cell's energy currency. Free radicals are defined as an atom, molecule, or ion with at least one unpaired valence electron. Mitochondria generate free radicals as a byproduct of normal functionality - and they can cause chain reactions that lead to electron-mediated damage of biomolecules.

The idea here is that mitochondrial free radicals produced as a result of normal metabolism damage our cells and cause aging. But the thing is - our cells can recognize damaged molecules and sequester them in the lysosomes, even if they end up as part of a lipofuscin deposit. The point here is that there are active repair processes at play that scout for electron-damaged biomolecules and deliver them to lysosomes. Of course, if damaged mitochondria accumulate later in life as a result of lipofuscin accumulation, it is more likely that they will perform in a substandard manner and produce more free radicals, which could eventually be toxic to our cells. However, if we periodically remove lipofuscin, our cells will be able to recycle old, damaged mitochondria and generate new ones will be that are better at their jobs - and which only produce acceptable levels of free radicals.

You may have heard of "telomeres" in the context of aging. They are non-sense DNA sequences at the ends of our chromosomes - that are necessary to protect our genomes from damage. Each time the cell divides, they shorten a little. There is an enzyme called telomerase that extends them in youthful organisms to account for this issue. The cells that divide to replenish our tissues are called "stem" cells.

It has been shown that the level of telomerase activity in stem cells decreases with age. Unsurprisingly, it has also been shown that the telomere lengths of stem cells decrease with age. If stem cell "niches" - i.e., the microenvironments surrounding stem cells that are involved in their maintenance and care - begin to build up lipofuscin, those partially or entirely corrupted niches could cause dysfunctional proteostasis in their resident stem cells leading to lower levels of telomerase activity.

(If telomere shortening does end up being an issue, the delivery system that I am proposing we develop for lipofuscin removal could help us deliver telomerase-encoding gene vectors to our stem cells - to be periodically induced.)

Epigenetics has to do with which parts of our DNA instructions are "read" in which cells and at what times. There is a concept that is talked about nowadays called the "epigenetic clock". It has been shown that with age, our cell's epigenetic patterns shift to a more pathological state. However, this is much more likely to be an effect of aging rather than a cause. Clearly if the autophagy efficiency of a cell declines steadily with age due to lipofuscin accumulation, the epigenetic signature of the cell is going to change as well. In other words, some proteins will be over- or under-expressed to compensate for this deficit.

If you remove lipofuscin from a cell, the epigenetic signature will probably revert to a youthful state.

Indeed, "epigenetic reprogramming", which changes a non-dividing cell into a stem cell via the continued generation of Yamanaka factors, a set of four proteins, promotes autophagy (i.e., cellular recycling) and cell division. When a cell is reprogrammed, the epigenetic clock reverts, and it has been shown that the cell's telomeres become substantially elongated.

Future issues:
Eventually, we will need to be able to address nuclear DNA mutations/damage and mitochondrial DNA mutations. The mitochondrion is the only organelle aside from the nucleus that contains DNA - and its DNA is different from nuclear DNA. There is also much less DNA in a mitochondrion than the nucleus.

To be able to fix nuclear DNA mutations and mitochondrial DNA mutations on a fundamental level in the future, we must have reference sequences that are as pristine, i.e., close to the individual's original sequences, as possible. This means that ideally we would obtain these reference sequences when we are as young as possible. However, having a few mutations scattered throughout our sequenced genomes probably won't matter too much. With the price of whole-genome sequencing dropping rapidly, cost shouldn't be an insurmountable obstacle. Freezing cells with at least relatively pristine DNA would be even better than simply having a digital copy of one's genomic codes, and may actually be necessary.

Whole-body lipofuscin removal performed once every decade or so will likely substantially slow down the rates of nuclear and mitochondrial DNA mutation accumulation. Additionally, the DNA mutation issues can probably be staved off even longer via the systemic inhibition of the DREAM complex[10] and overexpression of mitochondrial DNA repair enzymes.

It is possible that mitochondrial DNA mutations will be an issue before nuclear DNA mutations/damage, as mitochondrial DNA has a mutation rate 10 to 20 times higher than that of nuclear DNA. However, in elderly humans, it appears as though not many cells have mutated mtDNA molecules. That being said, the ones that do can have a large percentage of their mitochondria being mutated. Of relevance, flies and mice with substantially more mutations in their mitochondrial DNA than their normal counterparts do not show signs of premature aging.

To replace mitochondrial DNA systemically, edited TRMs could secrete new mitochondria with pristine DNA in microvesicles, as well mRNA contained in enveloped protein nanocages. The mRNA would encode DNA-cleaving enzymes that target sites missing in the new mitochondrial DNA.

TRM replacement could also be used in combination with whole-body induced cell turnover to replace cells with mutated/damaged nuclear DNA[11]. If done slowly, this could even work for the brain. This will likely be the most difficult part of aging to cure. (Of course, mutated mitochondrial DNA can also be replaced in this manner - provided we can wait until this therapy is developed to do it.)

Concluding thoughts:
Please be/stay healthy and happy until we can figure this all out and live in peace and bliss for eons together.

If you would like to join in on the anti-aging effort, you may wish to watch the TED talk given by Dr. Aubrey de Grey in 2006, "A roadmap to end aging" - as well as the book written by Dr. Aubrey de Grey and Michael Rae, "ENDING AGING: The Rejuvenation Breakthroughs That Could Reverse Human Aging in Our Lifetime." John Furber also designed a poster with a lot of age-related information: https://www.legendarypharma.com/furberchart.pdf. Finally, a list of institutions that are doing great work in this field can be found here: https://www.lifespan.io/institutions-working-on-aging/.

Acknowledgments:
The figures in this piece were created with BioRender.com.

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