Restoration of Complex Biological Components

A Theory of Aging and Working Model for Human Age Reversal

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ABSTRACT

BACKGROUND: Over the years, numerous theories of aging have been proposed. These include the idea that cellular damage accumulates over time, that genetic mutations buildup alongside a decline in DNA repair mechanisms, and that free radicals accumulate. Other theories assert that progressive telomere shortening, a gradual decline in immune function, and the accumulation of senescent cells contribute to aging.

THEORY: In this paper, we discuss a new theory that we believe best incorporates prior concepts, provides a platform for explaining the potential causes of aging, and leads to a more productive strategy for reversing aging for elderly human patients. This theory proposes that aging is best represented by the loss of massive quantities of Complex Biological Components (CBCs) that include mitochondria, RNA, DNA, lysosomes, the epigenome, stem cells, complex folded proteins, and other organelles. We also point out the difference between "delaying" aging (which is most commonly pursued by people under 60 years-old using inexpensive techniques such as diet, exercise, and small-molecule drugs) and "reversing" aging (more important for people 60 or older, the elderly, who need replacement of CBCs).

DISCUSSION: We suggest that the best strategies for reliable, powerful, and reproducible age-reversal in elderly human patients will be based on the restoration of CBCs, which most likely will have to be created externally and transplanted into the body. We discuss existing techniques, such as heterochronic parabiosis and stem cell therapy, which already restore CBCs in limited quantities. We propose that the key to effective age-reversal is to optimize and expand these existing techniques – focusing on restoration and transplantation of massive amounts of high-value cellular materials (CBCs), to return an elderly person to a younger state.

1 INTRODUCTION

The root mechanism behind human aging is one of the mysteries of contemporary biomedicine, with no consensus on a solution. However, there has been no shortage of variant theories of aging seeking to fill this gap. Over the years, it has been suggested that the cause of aging is: accumulated wear and tear on cells[1], accumulated genetic mutations and the decline of DNA repair mechanisms[2,3], accumulation of free radicals[4], progressive shortening of telomeres[5], progressive decline of the immune system[6], progressive accumulation of senescent cells[7], progressive loss of stored information[8], or the presence of an intrinsic "aging clock" that counts down our epigenetic programming, enforcing the transition from young to old[9] and opening the door to the diseases of old age. These theories all have advantages and disadvantages, but none of them explain all of the observations and none has become the mainstream theory of aging.

In this paper, we propose a new approach that we believe combines many prior concepts, provides a better platform for explaining the causes of aging, and provides a more productive strategy for treating and reversing aging. This new theory proposes that aging is best accounted for by the progressive loss of Complex Biological Components (CBCs). Here, CBCs include mitochondria, RNA, DNA, lysosomes, the epigenome, stem cells, complex folded proteins, and a few other organelles. CBCs are lost or degraded in large numbers during the course of aging, including loss of muscle mass, bone density, neurons, and functional organ tissue. These losses account for, on average, 4 kgs (5%) weight loss observed after the age of 65. At the same time, there is the less obvious replacement of complex CBCs with nonfunctional substitutes, e.g., infiltration of the muscle with low quality fat, with about 8kgs (11%) lean body mass lost between age 30 to age 65, plus widespread transition from normal cells to senescent cells and accumulation of random mutations and coding malfunctions into nuclear and mitochondrial DNA[50]. Restoring this lost inventory of CBCs in the body, thus increasing the quality of energy and information, offers an excellent strategy for reversing age and restoring youth to the elderly. (Fig. 1)

1.1 Follow the Energy

In considering the role of CBCs in aging, we emphasize an overall principle: follow the energy. This is because most CBCs require considerable energy to be created and, in many cases, they generate, contain, and/or transport high-quality energy as a part of the process of life. It is the constant, relentless, and unceasing generation of energy and creation and maintenance of CBCs that keeps us alive and youthful.

In this context, we note that mitochondria are perhaps the most important of the CBCs, because they are the distributed "power plant" or energy source that sustains life, and they are the information processors of the cell[32]. When CBCs are donated from an outside source, for example the bone marrow-derived mitochondria transported to tissues in blood platelets, or exogenous mitochondria that we might transplant therapeutically, these CBCs not only contribute useful raw materials but also relieve the recipient tissues of the energy burden of replacement. Thus, they provide a net energy and/or information gain.

1.2 Follow the Information

A second, equally important guiding principle is to *follow the information*, because CBCs, in many cases, generate, contain, encode, transport, and/or process information. An important example of this is the epigenetic code, characterized by a pattern of selectively attached methyl groups that modify the underlying DNA sequence. These modifications regulate the expression of proteins using methylation to turn them "off" or "on", while also providing nuanced instructions for the functioning of living organisms, including aspects of cellular death.

1.3 Delaying vs. Reversing

Note that for purposes of this analysis, we point out the difference between two categories of aging interventions. The first category is *Delaying Aging*, most popular with people < 60 years old, using inexpensive small-molecule drugs and lifestyle changes. These are often called "first-generation interventions". The second category is *Reversing Aging*, most commonly done in the elderly using CBC restoration techniques such as mitochondrial transplantation or stem cell therapy, often called "second-generation interventions[51]".

Although this CBC theory, like all others, perhaps has its own flaws, we emphasize that it is uniquely useful in providing a new framework for viewing aging. Even more important, it provides a useful and productive framework for assessing the relative value of new age-reversal techniques and interventions as they appear.

Fig. 1: Age is Loss

2 WHAT ARE CBCS AND HOW ARE THEY RELATED TO AGING?

2.1 Definition of CBCs

We define Complex Biological Components (CBCs) as *cellular components that require a large amount of energy and/or accurate information to create and are used in large volumes by cells*. As stated above, CBCs may include mitochondria, RNA, DNA, lysosomes, the epigenome, stem cells, complex pre-folded proteins, and other organelles. CBCs are often delivered to cells from stem cells or traded between cells inside exosomes. CBCs are lost or degraded in large numbers during the course of aging, including loss of muscle mass, bone density, neurons, and functional organ tissue. These losses account for a significant fraction of the body weight loss observed in the later years of life[50].

2.2 CBCs are critical to the performance of cells

Because they require time and energy to create (indeed, that is inherent to our definition of them) CBCs are highly important to the survival and peak performance of cells and the organisms. A fully grown adult, at peak health and peak nutrition, is in a state of homeostasis in which the cells are both creating and simultaneously consuming the maximum number of CBCs[10]. As the quantity and quality of CBCs decline, aging results.

2.3 What are *not* CBCs

We define CBCs as complex biological components that require large amounts of energy to build and/or carry large amounts of critical biological information and are used in large quantities as building blocks of the body. Based on this definition, we can identify many things that are not CBCs (Fig. 2). For example, small molecules, simple proteins, peptides, simple signaling or regulatory molecules, heat and cold shock proteins, and enzymes are not CBCs. Foods, nutrients, lipids, amino acids, and vitamins that are ingested through the digestive system are not CBCs, because they are broken down (digested) to low-complexity states by the digestive system. All of these non-CBC substances are simple enough that the human body can make them relatively easily, or they are in the case of nutrients, constantly absorbed as food.

Generally, CBCs are defined as "cellular components," which means they are microscopic. However, there is a special case – transplant organs or tissues, such as transplant of a liver, kidney, skin graft, heart, or lungs. Transplant organs contain massive amounts of CBCs, which upon surgical insertion into the body, can significantly increase the energy and information value of the body. Thus, transplant organs can be considered a type of "macro-CBC".

An important CBCs is the extracellular matrix (ECM). It is the complex network of proteins and other molecules that surrounds and supports cells, essentially acting as a scaffold that provides structure to tissues and plays a crucial role in regulating cell behavior. The degradation of the ECM with age is an important secondary effect of aging.

Transplanted platelets, stem cells, or bone marrow represent another type of macro-CBCs. Note that as we describe below, stem cells and bone marrow can act as manufacturing sites for CBCs in the blood stream. Clearly the exact dividing line for CBCs can be debated and may be changed to suit the preference of the investigator.

2.4 Decline of CBCs and aging caused primarily by accumulated DNA transcription errors

We propose that mammalian aging is caused primarily by the inexorable accumulation of DNA damage/mutation[13]. Both the mitochondrial DNA (mtDNA), the 16.5K base-pair rings of DNA contained by the hundreds within the mitochondria of a cell, and the nuclear DNA, the huge 8 billion base-pair multi-chromosome "library" of DNA contained within the cell's nucleus, progressively accumulate such damage with age[2,3]. The damage to both types of DNA is often caused by replication errors during cell division, among other causes. Every time a cell replicates itself or its mitochondria, the nuclear or mitochondrial DNA may accrue small but inevitable transcription errors in the reproduced DNA structures. The cell's active DNA repair mechanisms, which differ for the two DNA types, can only partially correct such errors. As the decades pass, these "damaged sectors" accumulate.

CBCs	MACRO CBCs (transplant or grown tissues)	Not CBCs
• Mitochondria	• Heart	• Growth factors
• Pre-folded complex proteins	• Liver	• Enzymes
• RNA/DNA fragments	• Skin	• Simple cellular components
• Epigenomes	• Stem cells	• Nutrients/vitamins
• Stem cell exosomes	• Other transplant tissues	• Small-molecule chemicals
• Other complex metabolites		• Simple peptides or proteins
Second-generation aging interventions ⟶		*First-generation aging interventions*

Fig. 2: What are and are not CBCs

2.5 Damaged mtDNA leads to lower energy production and loss of internal operating efficiency

This accumulation of error eventually causes the DNA to fail in its task of directing and managing inter-cellular functions. This leads to reduced inter-cellular efficiency and loss of energy production. For example, a mitochondrion that contains mutated or damaged mtDNA may no longer function properly and thus will produce less energy. This reduced energy flow may be insufficient for the cell to function properly. The cell may no longer be able to replicate, dispose of waste, or perform other needed functions. In the context of aging, cellular processes such as replication, waste disposal, and the execution of essential functions are critical for maintaining organism health. The body also has a broad range of repair mechanisms, but these require significant energy and shut down with age as

their needed energy becomes unavailable and is diverted elsewhere, damage to nuclear DNA may also disrupt these processes, resulting in impaired cellular health. This is analogous to the unavoidable accumulation of bad sectors in computer hard disk drives, an error accumulation rate that may become severe enough that the operating system of the computer can no longer function properly.

2.6 Repair and quality control mechanisms for DNA

The cellular machinery has many repair and quality control mechanisms, for both the nuclear DNA and the mtDNA. However, these quality control mechanisms diminish as we age and may be insufficient over the years to maintain the quality of the DNA. Step by step, over the decades, the inventory of quality DNA in the body gets progressively worse. We note that nuclear DNA and mtDNA have different repair and quality control mechanisms and that the latter, presumably inherited from archaic bacteria, are less efficient, leading to more rapid accumulation of mtDNA damage.

2.7 Errors increase when replication is done under stress

These replication errors may accelerate depending on the level of stress and environmental conditions encountered by the cells. For example, cells that are being stressed by the demands of survival, such as running from predators or encountering famine, may detect stress hormones in the body and consequently choose a replication pathway that is less energetically demanding, faster, but also less error free. Most likely, there is some trade-off at the molecular level between accuracy, quality, and speed of replication, which the body adjusts constantly.

Let's assume there is an animal undergoing severe survival stress in this environment; for example, a grazing animal being forced by changing rain patterns to move quickly from one territory to another. This animal encounters increased predation, gets less sleep, is more guarded and wary, and must be ready to run to escape predators. At the same time, the animal may be forced to change food-seeking habits or adjust musculature, for example by being forced to eat leaves and bark from trees instead of eating grass. In these high-stress survival cases the body may not be emphasizing high quality replication of its cells but is instead forced to replicate cells rapidly and perhaps lacking precision, causing higher amounts of damage and mutation. All of these survival issues have contributed to the evolution of species, leading to their ultimate lifespan.

These high levels of stress in replication, especially in muscles or other means of escape, may be programmed in permanently as part of the animal's normal metabolism; i.e., a mouse may be constantly on the alert because it is so frequently preyed upon, so mouse muscle cells may be permanently upregulated for faster DNA replication instead of higher-quality replication.

2.8 Replication depends on tissue type

Individual tissues within the body may also be more or less likely to replicate DNA via high or low-fidelity replicative pathways. The retina is one of the most metabolically active tissues in the body, particularly due to its high demand for energy to support high visual acuity. Retinas of the eyes are known to have very high levels of energy consumption relative to the rest of the body, which makes sense from an evolutionary standpoint. Since the retina is so small, it can "burn" more energy continuously without significantly reducing the lifespan of the species. At the same time, it must be available instantaneously upon waking. For example, if an animal is awakened by a predator, the eye must be functioning immediately, day or night. An animal must wake up from a nap and instantly run from a predator. It doesn't have time for the retina to ramp up or ramp down; it must be able to perform immediately.

Different tissues in the body have different rates of aging, different rates of energy consumption, and different rates of nuclear DNA and mtDNA degeneration, depending on the function of those tissues. For example, neurons are particularly high-priority survival cells, since the more rapid transmission of signals improves the ability of animals to survive (e.g., allowing prey animals to dodge and weave during escape maneuvers from predators.) At the same time, predators such as dogs must have very fast-moving neurons in order to dodge and weave to catch their prey. The competitive pressures between predator and prey have driven neurons to become more and more specialized over millions of years. For this reason, neurons perform very few of their internal manufacturing and waste-disposal functions. Mitochondria in neurons, for example, are produced and discarded in the astrocytes that support the neurons[11].

2.9 The myth of the self-sufficient cell

While textbooks commonly view cells as completely self-contained systems, we propose that there is a wide spectrum of behavior by different cells depending on their level of importance or criticality to survival. Some cells such as neurons or cardiac myocytes are very single purpose; they do one thing and only one thing very well. For example, they may not manufacture or dispose of their own mitochondria, they may not manufacture their own other CBCs within their cells, instead relying on a network of nearby support cells that manufacture and deliver CBCs to them.

2.10 Remote CBC distribution from bone marrow

We propose that CBCs may be manufactured in central locations in the body and delivered to peripheral tissues via extracellular vesicles (EVs). Tissue such as muscle, brain, liver, kidney, digestive system, etc., what might be called the peripheral or "working areas" of the body, are constantly burning out their mitochondrial DNA due to competitive stress. However, there are remote manufacturing centers of the body, for example the bone marrow, which create replacement materials including mitochondria, complex proteins, or perhaps even RNA fragments. This continuous supply of CBCs, delivered by EVs, contributes to maintaining cellular youthfulness and enhances longevity and survival.

Bone marrow cells are a soft, spongy tissue, found in the medullary cavities of bones, that house the stem cells that produce blood cells and immune system cells. There are two types of bone marrow: red and yellow. Red bone marrow contains hematopoietic stem cells that produce red and white blood cells, platelets, and a variety of exosomes. Red bone

marrow makes up all bone marrow until about age seven, after which yellow bone marrow gradually replaces it. Yellow bone marrow contains mesenchymal stem cells that produce cartilage, fat, and bone cells. Yellow bone marrow also stores fat in adipocytes for energy production.

The CBCs created in bone marrow seem to be mostly in the form of exosomes, which have a variety of contents, and mitochondria, which are contained in platelets[12]. These are released into the bloodstream for delivery to the peripheral tissues. Blood platelets have a lifespan of 8 to 12 days, after which they expel their mitochondria inside extracellular vesicles; these mitochondrial-containing vesicles are then absorbed by nearby cells. The delivery of CBCs from the bone marrow appears to be a critical evolutionarily-conserved determinant of lifespan (Fig. 3).

2.11 Other locations for remote manufacturing

In addition to the bone marrow, there are likely many other locations in the body where such replacement materials are being manufactured. These manufacturing sites are generally in environmentally protected low stress areas like the bone marrow, and they most likely employ slower, more efficient, higher-quality types of replication. So, these manufacturing sites experience lower levels of replication error to the DNA, slower rates of aging. The musculature, digestive system and brain, which are directly involved in the day-to-day stresses of competition, experience much higher levels of aging due to using faster and lower-quality types of replication. The continuous deliveries of supplemental CBCs from the "young" manufacturing sites, to the fast-aging peripheral tissues, may help define the maximum lifespan of the species.

In addition to areas like the bone marrow, there are stem cells scattered throughout tissues of the body, and perhaps traveling freely through the bloodstream, which provide "onsite" replication and manufacturing of CBCs, to help cells that have aged or have become damaged[13].

2.12 Multiple levels of redundancy in manufacturing

Thus, we see that the body has multiple redundant and evolutionarily conserved types of CBC manufacturing. The bone marrow is a major central manufacturing hub for the entire body, delivering it's CBCs via EVs and via platelets. Specific organs, like the brain or the heart, have their own specialized manufacturing cells. For example, many studies show that brain astrocytes trade CBCs with neurons or cardiomyocytes[10]. Finally, we have many types of stem cells embedded in tissues, or roving in the bloodstreams, providing on-site repair.

2.13 Healing of tissues

Note also that cells and peripheral tissues, such as muscle, skin, gut, and bone, are prone to damage from injury or fighting. These injuries can cause significant damage to the functionality of cells in the damage area; for example, tissues that are torn or cut will experience loss of blood flow and loss of oxygen, which will result in damaged or dead mitochondria. In order to heal this damage, the body must have powerful mechanisms for replacing those components of those damaged tissues. Cells must rapidly replicate, in order to repair tears and breaks in the flesh, but they not be able to do so without help.

2.14 The difficulty of rebuilding mitochondria when a cell initially has no mitochondria

One particular example of this is the *ischemia reperfusion* injury and its associated cellular damage. If an animal is cut or torn, the skin or muscle may experience significant blood loss, which causes the death or deactivation of mitochondria within the cells. In many cases, cells can return to glycolysis, which is a much less-efficient form of energy production, simply to stay alive at some low level of function. However, in order to restore functions within the cell, to come back to life so to speak, cells must rebuild their internal mitochondrial inventory

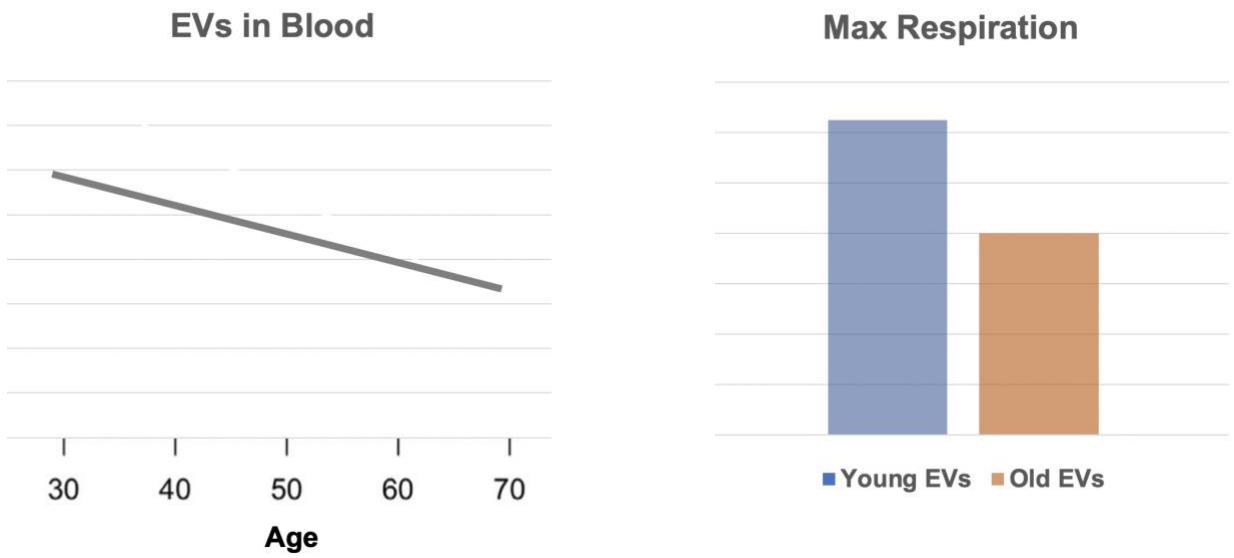

Fig. 3: Mitochondrial DNA in Vesicles Often Decline with Age

so they can again function at full power. Indeed, it is precisely at this time, when the tissue and cells have been damaged, that the cells are being called upon to replicate and to rebuild broken tissue. So, this leads to a dilemma: how does a cell find the energy to recreate its mitochondria, when it has no mitochondria to generate the needed energy?

The problem is that rebuilding the mitochondrial inventory of each cell using mitochondrial biogenesis is highly energy demanding. The cell must rebuild each mitochondrion "from scratch." This requires enormous amounts of protein folding and other actions that are energy demanding. This raises a critical question: how can a cell rebuild its inventory, perhaps 100 mitochondria, when it lacks sufficient energy reserves? The solution, of course, is to get a free mitochondrion from another source; mitochondrial transfer happens because it is more efficient and less energetically costly than *de novo* synthesis for the cell to get new mitochondria "for free" (i. e., from an EV or a stem cell). Free energy is akin to free money: it's always welcome.

Here we can see why the body must provide significant external rebuilding resources to help cells restore damage, heal wounds, and restore normal function. The body supplies these "loaner" mitochondria from EVs, stem cells, and platelets, which coincidentally are programmed by the body to gravitate towards such wounded areas. Another analogy is the way a tow truck might use jumper cables to supply an electrical jump-start to a car with a drained battery.

Mitochondria will also freely transfer between cells, loaned and distributed across cell walls. This is a survival tactic, since the survival of the animal is dependent on the health and healing of a wound. In a similar way, neighboring farmers might loan each other seeds or tools in hard times, so their neighbors can stay in business and loan seed or tools back later. This is a survival tactic for the loaner farms, since they themselves may in some future point need a similar loan. Mitochondrial transfer, through cell walls, via extracellular vesicles or via stem cell distribution, has been called a "Pervasive, evolutionarily-conserved process."

2.15 Intelligent control or pre-programmed distribution

We feel that other parts of the aging and lifespan equation depend on programming of the species and how it distributes, rations, and manages its replacement manufacturing of critical components. The exact proportion of energy in the body that is devoted to remote manufacturing, and the rate at which these materials are "rationed" or delivered to the peripheral tissues, may be programmatically controlled and predetermined for each species, and thus may impact the maximum lifespan of the species.

Humans lose 1% of their skeletal muscle every year after age 30. They lose neurons every year. They see a steady decline in the number of mtDNA in muscles and liver. However, they do not seem to experience the same decline in cardiomyocytes, which are essential for long life. Is it possible that the human species, and large primates in general, are "evolved" for long life, by intentionally reducing leg muscles, so that more CBCs can be routed to key organs like the heart, thus extending lifespan?

2.16 Why does a 150-lb human live longer than other animals of the same weight?

The distribution of CBCs may be a pre-programmed function that determines the lifespan of the species. Why does a 150-lb antelope's life appear to be 30 years old at most, while a human of same size may live to be 90 or so? Perhaps the antelope is metabolically "programmed" to use up its "reserve" of CBCs more quickly, keeping the large leg muscles taut and ready to spring, which is essential for that antelope to survive. Humans and other large primates, on the other hand, might have a clan social structure in which age (and experience) confers significant evolutionary advantages. For humans, weaker leg muscles in the elder years may be a compromise that is acceptable as a way to extend lifespan, so that the clan can benefit from the elder's knowledge.

Thus, we see that all species are an energy equation, tested and perfected by evolution, to fit a given ecological niche. Lifespan, strength, health, brain size, speed, foraging habits, and other variables are balanced against the quantity and quality of CBCs and energy within the closed cycle of the animal's body.

3 THE RISE AND FALL OF CBCs IN YOUTH, ADULTHOOD, AND OLD AGE

Let us now follow the history of CBCs as an individual is conceived, gestated, born, grows to maturity, ages and declines, and finally dies.

We all understand the process of nuclear DNA and chromosome formation during conception: a sperm cell containing the male parent's DNA penetrates the outer wall of the ovum containing the female parent's DNA. The two nuclear DNA components join and coalesce into the nucleus of the pluripotent embryonic cell that has been created. Huge amounts of encoded high-value information is contained in nuclear DNA. The fertilized ovum begins to divide, entering the phase of gestation.

However, there is a second, entirely separate store of encoded high-value information contained within the ovum. This is the mtDNA. There are approximately 500,000 copies of mtDNA in each egg. These mtDNA copies are of the highest possible accuracy, cleaned and perfected by the female reproductive system. Thus, the egg is at a stage where both nuclear DNA and mtDNA are relatively pristine, containing fewer mutations and errors compared to later stages of development.

Interestingly, the Gladyshev group at Harvard[15] has used several epigenetic clock-reading techniques on mouse embryos to learn about how the epigenetic clock is reset. They find that for the first week after the gestating embryo attaches to the uterus its epigenetic age becomes progressively *younger*, being systematically rejuvenated until, around 8 to 10 days after uterus attachment, it reaches the "ground zero" of epigenetic age and then begins to grow older. There is evidence that this down-and-up aging reset scenario is universal, at least among mammals, and that, in particular, it happens to the CBCs involved in human reproduction.

From this point on, the female reproductive system, in concert with the data in the gestating embryo, work together to

create the new high-complexity baby. The embryo is rich in genetic information and cellular building blocks (CBCs), preparing for the further development of the fetus into an adult. During pregnancy the mother makes a huge energy contribution, almost 50,000 dietary calories over nine months, with only about 10% of this energy going directly to the offspring[16]. The womb is a protected and sheltered environment, where the baby can grow isolated from stress, maintaining high-quality, high-fidelity cellular and mitochondrial replication.

Once born, the baby is now generating all energy supplies internally, yet for some period (in humans) the baby is still in a sheltered condition, still protected by adults while it continues to grow to a size where it can obtain its own food and protection. Thus, it may be possible to grow the volume and number of cells, and the CBCs inside them, without as much damaging stress.

Finally, upon reaching physical maturity the young adult stops devoting energy to growth and begins devoting more energy and effort into food and protection. At this point, the cells and CBCs are at maximum levels of power and efficiency, the physical peak of the life cycle. The female reproductive system diverts a significant percentage of this power into the creation and gestation of new young, leading to the renewal of the species, while the male uses all available power to maintain larger body mass, muscles, and strength.

However, these adult forms are now encountering high levels of stress as they fight to survive. At these higher levels of stress, replication of CBCs such as mitochondria are likely to occur with lower quality control and more accumulated damage. DNA errors, which may have slowly begun to accumulate during childhood, now begin to occur more rapidly in adulthood. The accuracy and energy production of the adult cells eventually reaches its maximal point, then tips over and enters the long decline. The body may start to implement "coping mechanisms" such as muscle mass reduction, mtDNA copy number reduction, formation of senescent cells and fatty tissue, and other measures.

Then, as adulthood progresses, the body is fully in decline, losing muscle, neurons, cardiovascular strength, resistance to infection, etc. This decline accelerates over the decades as the person ages, with less energy available for quality-control and waste-disposal processes and greater odds of disease and dysfunction. If they don't die of other causes, they ultimately face mortality when energy levels and cellular functions deteriorate to a point where they can no longer sustain basic metabolic processes. For many of the very elderly, death comes when the high-energy organs of the body, the heart and brain, simply cease to function.

4 EXPERIMENTAL EVIDENCE SHOWING CBC RESTORATION

In recent years, there have been a number of laboratory-based age reversal experiments, usually performed on aged test animals but occasionally on humans, to investigate the effectiveness of various age reversal interventions. We describe them briefly below and discuss how they fit into our concept of CBC-based aging.

4.1 Heterochronic Parabiosis and Plasma Replacement

Are there age-inducing and youth-inducing agents circulating in the bloodstream? Bio-researchers have investigated this question by conducting parabiosis experiments[17] that unite an old circulatory system with a young circulatory system. This is done by joining an old mouse and a young mouse, surgically connecting their blood circulatory systems, and observing the effects on both animals. Researchers observed that the old mouse showed signs of becoming younger while the young mouse becoming younger. The implication of this work is that blood acts as a carrier supplying "replacement" CBCs, for example those sent from bone marrow to peripheral tissues to improve and restore tissue functionality, and also contains degenerated CBCs which induce symptoms of aging.

The hypothesis that old blood contains degenerated CBCs that induce aging has been investigated by the Conboy Group[17] at U. C. Berkeley. In tests, they replaced a significant fraction of the blood plasma from aging animals with a saline solution containing albumin, thus reducing the concentration of damaged CBCs and waste products in the blood of the subject by dilution. They observed a marked improvement in the condition of the subjects, supporting the hypothesis that degenerated CBCs are present in old blood. They have also done plasma dilution tests (or of therapeutic plasma exchange (TPE), on human subjects with similar results, along evidence from their protein expression "noise" technique described in Appendix A.4 below. However, contradictory reports from human participants in some of these trials indicate no significant decrease in epigenetic age as measured with the Horvath technique described in Appendix A.5 below.

Other important parabiosis research is at biotech startup Alkahest Inc.[52] based on work originally from Stanford University, which showed that transfusions of plasma from young donors had preventative effects on neurodegenerative disease in the elderly. Subsequent work identified "roughly a thousand proteins" in blood plasma that changed with age[53].

Fig. 4: Heterochronic Parabiosis and CBCs

Some proteins decreased with age, perhaps indicating a gradual decline of beneficial substances, while other proteins increased with age, perhaps indicating a buildup of detrimental waste products. Alkahest finally identified 4 of these proteins that it would attempt to commercialize as drugs[53].

There are also preliminary investigations of the effects of infusing human blood plasma donated by young volunteers into older human subjects (therapeutic plasma rejuvenation, TPR). There are no final results of this work, but preliminary results indicate improvements in the old subjects, with age-reversal effects that may be more permanent.

The implication of this work is that functional CBCs in the bloodstream can be added and damaged CBCs removed, and that both interventions act to reverse the effects of aging. However, neither of these techniques is likely to be sufficient for large-scale age-reversal, since the mere removal of waste products isn't effective by itself, and the number of young volunteers is insufficient for even a tiny fraction of demand.

4.2 Mitochondrial Transplantation

There is evidence that the number and quality of energy producing mitochondria, an important CBC contained in cells, decreases with age. There is also evidence that an increasing fraction of the mtDNA contained in mitochondria becomes damaged and less functional (See Appendix A.6 below). Mitochondrial Transplantation (MT) is an intervention designed to address this problem. MT has the following steps: (1) obtain a sample of pluripotent stem cells from the subject or from a maternal-chain relative, possibly by liposuction of body fat; (2) amplify the population of stem cells by repeated cell division in a bioreactor; (3) extract and purify the mitochondria from these stem cells and arrange for the extracted mitochondria to be encased in a coating that will deliver the mitochondria to cells without triggering the immune system; and (4) slowly infuse the subject with these mitochondria, into the bloodstream or at particular target sites (eyes, joints, muscles, etc.) where the mitochondria will enrich the mitochondrial count and mtDNA number of the target cells. A form of this MT intervention is now being performed on lab mice, larger animals, and a few rare cases in compassionate-use treatments in human patients, with encouraging preliminary results.

Is the MT intervention safe and effective? Even before the needed safety tests on humans have been implemented, the answer to this questions seems to be YES. Studies applying MT to mice at appropriate dose levels have revealed no negative effects. Further, there is no logical reason to expect that removing CBCs in the form of mitochondria from a subject, externally increasing their number, giving them a hypoallergenic coating, and re-injecting into the same subject should trigger any negative effects. Rather, mice in the test group have showed enhanced energy, more youthful behavior, and extended lifetimes as compared to the control group[18].

The stem cell amplification part of the MT intervention described above might be rather expensive initially because it requires advanced bioreactor and purification technologies, but technique is scalable to societal levels at a reasonable cost. For now, this seems to be the only mitochondrial restoration technique that is available and that has been proven effective. Farther on the technical horizon are perhaps faster and cheaper alternatives, for example, the use of some form of long-range PCR to selectively amplify and replicate only undamaged mtDNA from the subject. A possible problem is that such synthetic mtDNA might lack the epigenetic markers of "natural" mtDNA, perhaps leading to problems. If successful, this development could potentially reduce the cost of mitochondrial restoration even further[19,20].

What is the expected duration of an MT intervention, and how often would it need to be repeated? Research is clearly needed to accurately answer this question, but in the present paper we can discuss expectations. The length of time during which the beneficial effects of an MT intervention would continue will depend on the degree to which the fraction of damaged mtDNA is reduced to a more youthful level. In the testing example mentioned in Appendix A.6 below, a 62-year-old subject had a mtDNA damage level of 11%, while a 93-year-old subject had a level of 25%. A reasonable target for a MT intervention might be to reduce the mtDNA damage level of an aged subject to the 40-year-old level and to repeat the intervention when, with increasing age, it has risen to the 80-year-old level. It is not yet clear how long such an increase would take.

4.3 Stem Cell Enhancement

Stem cells are the body's repair mechanics, ready to repair and replace damaged cells of all types, manufacturing and delivering CBCs of all types. If the supply of stem cells becomes depleted or suffers DNA damage, the health of the body suffers. These new stem cells would the increase their production of CBCs and thus, provide regeneration to the cells they serve.

There are a number of existing stem cell transplant and enhancement procedures. The most common in current use is the bone-marrow transplant procedure, in which blood stem cells are used to treat patients with blood cancers such as leukemia. This procedure is done by clearing harmful blood cancer cells with chemotherapy and then replacing them with healthy stem cells.

There are a significant number of stem cell transplant techniques in testing or near approval in the US, for example for Alzheimer's, retinal or corneal regeneration, wound healing, etc. Outside the US, some nations have more extensive stem cell procedures. Typically, in these procedures, some of a patient's body tissue is removed (stored placental blood, abdominal fat cells, extracted bone marrow, etc.). Embryonic stem cells may have greater regenerative capacity then stem cells from other sources. Then a procedure is used to process the tissue sample to end with pluripotent stem cells, which may then be multiplied in an external bioreactor. Finally, the resulting stem cells are infused back into the patient's body by local injection or slow infusion into the bloodstream.

There is some potential of negative side effects from such stem cell enhancement procedures. Particularly in pathological states like inflammation, stem cells may use various mechanisms, such as active transport or changes in the permeability of the blood-brain barrier, to reach areas that are not targeted for treatment. In some cases, damage can arise if the stem cell dose contains old or genetically damaged cells, if the stem cells are not well separated from other tissue material, or if some fraction of the cells are of an unwanted type. The immune system also may react negatively.

4.4 Exosomes and Extracellular Vesicles

Stem-cell-derived exosomes are membrane-wall vesicles with a diameter of 40–160 nm that are released from stem cells to communicate with and supply CBCs to other cells and may also provide other benefits[48,49]. They contain a variety of components, including proteins, lipids, nucleic acids, and metabolites that can give them therapeutic functions. They are normally non-toxic and do not trigger the immune system. They probably play a role in wound healing and tissue repair. Animal studies with rats[21,22] have shown that significant reductions in epigenetic age can be achieved by administering young-blood derived extracellular vesicles.

There have been a number of human clinical trials on investigating exosome-based therapies for diseases including respiratory exosomes derived from mesenchymal stem cells as an alternative to stem cell therapy. This work suggests that stem-cell-derived exosomes deliver, in part, the same effects as their donor stem cells, without the several drawbacks of stem cell therapy. For example, exosomes cannot self-replicate, preventing tumor formation. They are stable enough for long-term frozen storage or room-temperature storage after lyophilization. Their small size facilitates sterilization by filtration. They can be administered by several routes, including injection and nebulization for inhalation to treat lung disease.

4.5 Senolytics

One key element in the process of aging is the *senescent cell*. As the body ages, cells become damaged and unable to fulfill their intended functions. This damage may be the result of damaged mitochondria, over-short telomeres, transcription errors during cell division, ionizing radiation, oxidation, toxins, diseases, and a long list of other causes. When such damaged cells become senescent, they no longer divide, cease normal functions and behave badly, secreting harmful chemical signals called senescence-associated secretory phenotypes (SASP) that degrade tissue function, generate inflammation, interfere with the performance of nearby cells, and induce neighboring cells to also become senescent, in a type of chain reaction. The inflammation associated with aging is considered to be largely the result of senescent cell accumulation. In principle, the immune system should automatically detect and remove senescent cells, but for unknown reasons it does not. Instead, senescent cells accumulate with age, and their effects show up in many ways: "papery" skin, eye lens cataracts, sarcopenia, poor recovery from exercise, arthritis, organ degradation, a general tendency to form cancers, amyloid buildup in tissues and organs, and so on. Clearance of these cells would make room for the development of new, functional cells, built using CBCs.

Some types of accumulated senescent cells can be cleared by an intervention called *senolytics*, a class of treatments that cause senescent cells to go into apoptosis (programmed cell death) so that the repair mechanisms of the body can replace them with functional cells of the same type. One popular intervention for senescent cell buildup is the "D+Q" senolytic technique, which used doses of small-molecule agents dasatinib (D, an expensive anti-cancer drug) and quercetin (Q, an inexpensive flavonoid found in fruit). The Kirkland group at the Mayo Clinic[23] tested many possible senolytic agents on human senescent cell cultures, identified these two agents as quite effective and synergistic, and tested them on aging mice. The D+Q treatment has been found to extend health spans and lifespans in mice and to produce apoptosis in senescent cells in human cell cultures. Other treatments, such as the flavonoid fisetin (F) has been identified as another powerful senolytic that can be substituted for quercetin. We note that this form of senolytics may be fairly cell-type specific, affecting mainly skin cells, fat cells, intestine-wall cells, blood-vessel lining cells, and perhaps some brain cells. Senolytic alternatives that clear other senescent cell types are currently under development.

Several commercial startups[24] have been seeking and testing a wide range of small-molecule drugs, seeking those that clear specific cell-types of senescent cells as targets, specifically joint, eye, and skin cells. So far, test results on joint restoration were unimpressive, but preliminary trials[25] on diabetic macular edema show promise. Another commercial startup[26] has bio-engineered plasmids that enter cells and trigger apoptosis if and only if the cell is expressing the protein P16 (as do all senescent cells). The overall implication of this work is that non-functional CBCs in the form of senescent cells can be removed and replaced to reverse the effects of aging. We note, however, that senescent cells play a role in the wound healing process. Senolytics and wound repair is nuanced, and there can be improved or delayed healing of chronic wounds[31].

4.6 Epigenetic Reprogramming

An individual who is 20 years old has almost exactly the same DNA as when 80 yet may have a very different body and performance levels. Somehow, the cells of the 80-year-old know that they are old, independent of their DNA. The difference is that the epigenetic programming, the pattern of methyl radicals attached to the CpG sites of his DNA, has been changed, altering which of the DNA-encoded proteins are currently expressed and which are suppressed. This implies that if that epigenetic programming could somehow be reset to the younger methylation profile, his age-related changes would perhaps be reversed.

Are there ways of "resetting" the epigenetic programming of aging cells to a younger state? The answer is: yes. We already know that this is possible in principle. When a man and a woman, perhaps in their 40s, conceive a child, both parents contribute their DNA, and the mother contributes one of her particular cell has had all of her accumulated environmental damage erased and her 40-year-old epigenetic programming reset to zero. The child starts life with new embryonic cells, not 40-year-old ones. How does this happen? There is now a body of research providing the apparent answer to this question: Nature has provided a set of special proteins that accomplish the epigenetic reset task.

In a 2006 paper[27], Takahashi and Yamanaka reported the discovery of what are now called the "Yamanaka Factors", four proteins designated by the acronyms OCT4, SOX2, KLF4, and c-MYC (abbreviated "OSKM") that are actively produced in the cells of embryos. They found that when these proteins were added to older mouse cells, the tested cells were transformed into seemingly brand-new stem cells that were able to develop into many different cell types. In a 2007 paper, Yu, *et al*, reported that aging human cells could be similarly transformed by adding to human somatic cell cultures a slightly different set

of proteins. In a 2019 paper[28], Sarkar, *et al*, a Stanford group, reported that they had combined these two sets of reset proteins into a six-protein cocktail from which a wide variety of aging human cell types are converted into embryonic stem cells. They also found that the same cell cultures could be reprogrammed to be younger but to retain their original functional cell type if the treatment process was halted after four days. They chose four days because that time is just before the observed "point of no turn" was reached, after which the treated cells reverted to completely undifferentiated embryonic cells. In other words, they found that human cells can be rejuvenated. The implication of this work is quantitative evidence that functional CBCs in the form of DNA epigenetic programming can be reset to reverse the effects of aging.

So how is this rejuvenation done? How does one put selected proteins into cells for such treatments? To answer this question, let's discuss a bit of the mechanics of cell biology and look into how a cell actually produces a protein like those discussed above. If a cell wants to express (i. e., produce) a protein, it generates a transcription factor that has been coded to find within the library of the cell's DNA sequences the particular "promoter region" (a unique identifying DNA base sequence) indicating the location of the instructions for producing the targeted protein. The transcription factor finds and lands on the DNA's promoter region, then zips down the connected sequence of bases, producing a chain of messenger RNA (or mRNA) containing the information. The mRNA that has been generated finds its way to a ribosome (a protein-assembly organelle located within the cell) and threads through the ribosome like a punched paper tape, delivering the instructions and causing the ribosome to assemble the requested protein out of amino acids. That protein is then released and does its job within or outside the cell.

What the Sarkar group did in demonstrating that human cells can be "reset" was to use the new laboratory bio-machinery of RNA sequencing to mass-produce mRNA sequences coded for each of the six reset proteins of the OSKMLN cocktail, using a process similar to that recently developed for the mRNA COVID vaccines. They then introduced this six-element cocktail of mRNA sequences into the cell interiors of their cell cultures, using several specialized commercial transfection compounds that were optimized for the different cell types investigated. The ribosomes of the cell interiors used the mRNA to generate the desired rejuvenation proteins, and the proteins then went to work reprogramming the cells to a more youthful state. The cell cultures that had been "reprogrammed" by this procedure were then tested to determine their biological state. The researchers found that in the aging cells tested, even those obtained from elderly humans, seven of the eight hallmarks of aging had been reset to the state expected for young cells, with only telomere length (see below) remaining unchanged.

This shows that it is possible, in the laboratory petri dish and perhaps even in living organisms, to restore the epigenome to a youthful state. The implication of this work is that functional CBCs in the form of DNA epigenomes can be reset to reverse the effects of aging. A large number of teams are hard at work converting this technology into practice.

4.7 Lengthening Telomeres

In 1965, the late Leonard Hayflick (1928-2024) was a young cell biologist working in Philadelphia, discovered that fibroblast cell cultures from human skin, which he was growing in his laboratory, showed a definite limit to the number of times they could divide. Further, he found that tissue from embryos could divide more times than tissue from young human adults, which in turn could divide more times than tissue from older humans. This limiting condition became known as the Hayflick Limit. It has a value of about 50 cell divisions in human embryonic tissue. Later it was discovered that the limit has some exceptions. Cancer cells and cells in the male reproductive system associated with sperm production are exempt from the Hayflick Limit and can continue to divide indefinitely.

Until 1990 the biological mechanism behind the Hayflick Limit was a mystery. It was clear that some biological clock was ticking along within each cell, and that when that clock ran down the cell ceased to divide and went senescent or died. It also was clear that cancer cells were somehow resetting or ignoring this clock. But it was not clear what the clock was or how it worked.

A clue was finally provided by the Hutchinson-Gilford (HG) syndrome, a childhood pathology when a few children (about 1 in 8 million) show signs of greatly accelerated aging. The HG child begins to show early aging symptoms in the first year of life and typically dies of what looks like advanced old age by the age of 13. The HG syndrome appeared to be a case where the Hayflick limit was significantly reduced, but its origins were still mysterious.

Then in 1990 with the work of Cal Harley (McMaster University) and Bruce Futchter and Carol Greider (Cold Springs Harbor Laboratory), the telomere was discovered and the mechanism identified. A telomere is a special nuclear DNA structure with a repeating nucleic acid pattern, found on the ends of chromosomes within the cell nucleus, rather like the plastic tips on the ends of shoestrings. It provides a "docking zone" or starting sequence for the enzymes that perform chromosome duplication when a cell divides, and it also provides an inert region preventing one chromosome end from sticking to another.

Young cells, it was found, have long telomeres containing perhaps 1,500 repetitions of the TTAGGG sequence. But each time a cell divides, the telomere region is only partially reproduced and becomes shorter. Finally, the telomere is used up, the duplication enzyme cannot dock, the cell cannot divide, and it becomes senescent, mutates destructively, or dies. There is a very good correlation between the incidence of cancer and short telomere length[38,39]. When this happens to enough cells, many symptoms of old age are produced and finally the whole organism dies. The HG children had a genetic disease that gave them short telomeres.

In the 1990s, it was widely believed that diminishing telomere length was the fundamental cause of aging[5]. The enzyme telomerase, which has the function of rebuilding and lengthening telomeres, became a target of interest in the antiaging community.

Experiments testing the telomere-exhaustion idea and its cure with telomerase found that trans-genetic mice engineered to have elevated levels of telomerase showed higher cancer

incidence but did not live longer. However, later human tests[37,40] with the supplements *cycloastragenol* (CAG) and *astragaloside* IV (AG-IV), both derived from *astragalus membranaceus* root, showed that with daily doses for about a year, telomeres could be lengthened by ~30% without increasing cancer risk[38,39]. An even greater therapeutic increase in telomere length and other benefits could, in principle, be achieved by infusion into the subject of the protein TERT or its mRNA template[33,34,35].

We note that because of its ring structure, mitochondrial DNA *has no telomeres*. For this reason, it is "immortal" and not subject to Hayflick-type disruption during replication.

The implication of this work is that insufficient telomere length in its nuclear DNA is correlated with incipient cell senescence and damage, and that the reduction in telomere length that accompanies cell division could be a roadblock for extending the human lifespan. However, the condition is likely treatable and is not the fundamental "countdown timer" mechanism causing aging.

5 "NOT-CBC" INTERVENTIONS: SMALL MOLECULES, GROWTH FACTORS, NUTRIENTS, LIFESTYLE

In addition to the CBC-restoring interventions described above, there are many small-molecule drugs and supplements that have been adopted by health-conscious individuals. These anti-aging interventions are not themselves CBCs, but they might have significant value if they can slow the loss of CBCs, for example by slowing or changing the metabolism. They may *delay* aging, however they are unlikely to have the impact needed to *reverse* aging for the elderly.

Thus we propose that small molecule, diet, lifespan, and other inexpensive aging interventions are primarily useful for adults 40+ who wish to delay the aging process, while large-scale CBC replacement, which we discuss later in this paper, is needed to reverse the age of people 60+, who want to "go back to being 40 again."

5.1 Rapamycin

Perhaps the most common non-CBC intervention is rapamycin, an immuno-suppressant drug often prescribed to prevent organ transplant rejection. It was initially developed as an anti-fungal agent, but this use was abandoned when it was found to have strong immunosuppressive and antiproliferative properties due to its ability to inhibit mTOR.

The mechanistic target of rapamycin (mTOR), specifically mTOR C1, was first shown to be important to aging in 2003. Since then, it has been shown to inhibit and slow aging in worms, yeast, and flies, and to improve the condition in mouse models of various diseases of aging. Rapamycin was first shown to extend lifespan in wild-type mice in a study published by NIH investigators in 2009, with results further supported by the finding that genetically modified mice with impaired mTOR C1 signaling live longer.

Rapamycin was first shown to extend lifespan in wild-type mice in a study published by NIH investigators in 2009[41,42]. Their results show that it can extend mouse lifespan by 30% and is effective even when given late in life for a limited period. These results were further supported by the observation that genetically modified mice with impaired mTOR C1 signaling live longer.

There is limited data on rapamycin in humans[45,46]. Because of this it is used generally by "early adopters." In some cases, it is prescribed off-label as an anti-aging intervention.

Although the high dose of rapamycin used for organ transplant patients acts as an immunosuppressant, the low weekly rapamycin dose used in anti-aging interventions has been shown to inhibit mTOR C1 but not mTOR C2, and does not seem have significant immunosuppressive effects[43,44]. More research on humans would seem needed before it could be widely prescribed for anti-aging by the medical profession[47].

5.2 Metformin

Another potential small-molecule anti-aging intervention is metformin, a low-cost prescription drug commonly used to treat type 2 diabetes. Metformin has anti-inflammatory effects that are claimed to help protect against age-related diseases. It may also stimulate bone formation and reduce resorption, which could help maintain facial bone density and improve facial contours, and may also stimulate autophagy, which is the removal of damaged proteins and cells, potentially slowing aging.

Human subjects taking metformin have shown anti-aging potential. However, most research on metformin has only included people with diabetes or pre-diabetes, so it is not established that it can also benefit non-diabetics. Metformin can have mild side effects, including nausea, stomach upset, or diarrhea, but more serious side effects are rare.

5.3 NAD+, NMN, and Apigenin

Sirtuins are a very important family of seven proteins that regulate many cellular processes including DNA expression and aging. However, they function only in conjunction with NAD+ (nicotinamide adenine dinucleotide), a coenzyme that is present in all living cells. By middle age, our NAD+ levels have dropped to half that of our youth. Some studies have shown that boosting NAD+ levels increases insulin sensitivity, reverses mitochondrial dysfunction, and extends lifespan. NAD+ levels can be increased, at least in principle, by activating enzymes that stimulate synthesis of NAD+, by using supplements like *apigenin* that inhibit enzyme CD38 (which degrades NAD+), or by supplementing with NAD precursors, including nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN).

However, work by the Kirkland Group and others have shown that the action of enzyme CD38 frustrates efforts to boost NAD+ by taking precursor supplements like NR and NMN orally. Again, more research is indicated.

5.4 Klotho and GABA

Klotho is an enzyme that in humans is encoded by the KL gene. Subfamilies of klotho are highly expressed in the brain, liver, kidney, and skin. Although the majority of research has explored the effects of klotho's absence, it was demonstrated that klotho over-expression in mice extended their average life span between 19% and 31% compared to normal mice.

Klotho is an antagonist of the Wnt signaling pathway, and chronic Wnt stimulation can lead to stem cell depletion and aging. Klotho inhibition of Wnt signaling can inhibit cancer. The anti-aging effects of klotho are also a consequence of increased resistance to inflammation and oxidative stress. Extracellular vesicles (EV) in young mice carried more copies of klotho-producing mRNA than those from old mice.

Transfusing such young EVs into older mice helped rebuild their muscles. The presence of senescent cells decreases α-klotho levels. Senolytics clears these cells, allowing α-klotho levels to increase. A research group in Toronto[30] has found that the production of klotho is stimulated by the supplement GABA (gamma aminobutyric acid), which plays a role as a brain neurotransmitter and helps to regulate nerve activity and to maintain a balance between excitement and relaxation.

5.5 Resveratrol

The French Paradox is the 1980s conundrum that, despite a typical diet high in saturated fat and other rich foods, the French people appear to have lower rates of coronary heart disease. One 1980s theory asserted that the paradox could be explained by the high consumption of red wine, which contains resveratrol, a compound that is reputed to have anti-hypertensive effects and help relax blood vessels. Studies indeed show that resveratrol has anti-inflammatory properties, suggesting that it may be an effective anti-aging supplement, relieving oxidative stress and inflammation, improving cell function, and regulating cell aging and death.

However, much of the initial enthusiasm about resveratrol as an anti-aging agent dissipated in the 2000s, following test results indicating that heath improvements from regular resveratrol dosage were moderate at best. Nevertheless, research continues into resveratrol's effects.

5.6 Vitamins, Minerals, and Supplements

The role of vitamins, minerals, and supplements as agents of anti-aging and CBC-restoration has long been promoted and confusing, in part because there is a large and well-funded industry that, since the 1950s, has been aggressively promoting the need for them. The problem is that our foods contain most of the vitamins and nutrients needed for good health, so that accurately identifying areas that are in need of supplementation is difficult and controversial.

5.7 Heat and Cold Shock Proteins

Historically, human cultures around the world have developed traditions of immersing the body in very hot or cold water. The enthusiasm for hot baths goes back to the Roman Empire and today includes Japanese hot tubs, Scandinavian saunas, Turkish baths, and European hot springs. At the other end of the temperature scale, there are many traditions of "polar bear clubs" with participants that plunge into icy waters.

As it turns out, there is a good biological reason behind these practices: stimulating the production in the body of heat shock and cold shock proteins, which seem to be associated with longer lifespan. In particular, it has been shown that Bowhead Whales, which have two or three times the human lifespan, have DNA coding that expresses the cold shock protein CIRP (known to promote DNA repair) at several times the human level.

5.8 Exogenous GDF11 Supplementation

Although they may be considered CBCs by some, we consider growth factors to be non-CBCs. For example, Growth Differentiation Factor 11 (GDF11) supplementation has been tested on mice and shown to have the capacity to restore aging muscles, hearts, and brains.

Recently, human experimenters have been self-testing with very small (~10^{-6} mg/kg) periodic injections of GDF11. The reported results have been impressive, with anecdotal improvements in reaction time, HRV, BP, pulse, naive and NK T cell counts, spirometry, eGFR, H1AC, total cholesterol, and many other key biomarkers. The implication of this work is that GDF11 triggers or up-regulates the production of CBCs, and thus can slow or partially reverse some of the effects of aging.

5.9 Other Options

Lifestyle changes, e.g., giving up smoking, adopting healthy eating, and/or regular exercise can also slow the loss of CBCs. In fact, such simple health solutions are much less expensive, and thus should always be emphasized by medicine. However, at age 60 or older, or in the case of someone with premature aging (for example, following chemotherapy), the loss of CBCs seems to be unstoppable. Then we must resort to the much more expensive solutions like transplantation of bioreactor-grown CBCs.

6 HOW CBC THEORY INSTRUCTS AGE-REVERSAL THERAPY

We've shown that aging is caused by the loss of CBCs and that the body is in a constant state of flux, with cells constantly rebuilding themselves and trying to achieve more vigor and health. Since there are a number of techniques that have demonstrated successful age-reversal by restoring CBCs on a limited scale, then logically we should be able to gain much greater degrees of age-reversal by restoring CBCs on a larger scale.

Researchers have uncovered techniques that give glimpses of age-reversal: parabiosis, mitochondrial transplantation, epigenetic reprogramming, etc. We must combine all those techniques together and optimize them in order to restore CBCs on a large enough scale to achieve lasting age-reversal.

The body loses CBCs because it slowly loses the accuracy of its information, loses the ability to generate sufficient energy for cellular activity, stops using quality control, and fails to maintain its inventory of CBCs (either by failing to create them or converting them to lower-quality waste materials.) Thus, our age restoration process must focus on:

- Restoring energy via mitochondrial restoration;
- Restoring inventory of complex biological materials via exosome or stem-cell therapies;

- Removing waste products or converting them back into higher value complex materials, using senolytics, plasma replacement, and other techniques;
- Restoring information quality, using epigenetic reprogramming, telomere elongation, and genome repair.

6.1 Restoration of mass is the key issue

Most other longevity treatments attempted in the past have been too small to have the needed effect. Treating with growth factors, simple small-molecule drugs, or a few hundred million stem cells, adds only a few grams of materials at best.

Our theory of "Loss of CBCs" forces us to focus on the mass-loss issues of aging. An aging person loses kilograms of biologically important components by old age, with many more kilograms of materials still in the body converted to useless waste products such as fat or senescent cells. The loss is massive. If the elderly body had the energy and ability to restore those lost materials, *it would already have done so*.

Thus, when evaluating the potential for true age-reversal for the elderly person, we must always ask these questions:

- "How will this treatment restore kilograms of high-quality tissues?"
- "Where will the energy come from, to rebuild and reintegrate these materials?"
- "How will the elderly cells, which are already chronically energy-depleted due to declining mitochondrial efficiency, find the energy needed to replicate to fill the gaps left by removal of waste products?"

Some researchers might answer as follows: "By improving the performance of the cells with Compound XYZ (whatever it is called), we are improving the performance the cells sufficient to they to grow back these materials in-situ."

However, this answer is unconvincing. It seems unlikely that any simple small-molecule, peptide, or growth factor could repair massive systemic genetic damage to the epigenome and the mitochondrial DNA, to enable such growth. If so, evolutionary pressures would have incorporated this magical substance into our body chemistry millions of year ago.

We propose that the elderly body has lost massive amounts of CBCs because it has no choice. The elderly body no longer has the high-quality energy or resources it had when it was young; the bone marrow is exhausted and stem cells depleted. Therefore, to reverse age, we must find techniques to artificially grow and restore CBCs with exogenous medical treatments. We must literally "create" young components or tissues and transplant them into the aging body, just as we might transfuse a unit of whole blood to replenish blood count or transplant a new kidney to replace an aging kidney.

Above all, this treatment must be capable of growing and restoring kilograms of materials – by the standards of modern regenerative medicine – massive amounts.

6.2 Use of external sources (i.e., externally grown mitochondria or stem cells)

Since the bone marrow and other manufacturing sites in the aged body are no longer capable of creating CBCs in sufficient quantities to restore youth, we most likely must do this work outside the body, using external nutrients, energy sources, containment, quality control, and human ingenuity to create additional biological components. We are in essence creating an external manufacturing site, a form of external bone marrow, to grow new CBCs for transplantation. This can be done using bioreactor-grown cells, chemical manufacturing, or other biological equipment. These CBCs are generally microscopic in size and transplanted into the tissues for take-up by cells. Transplantation of such large volumes can create immune system reactions, so we must use coatings, extracellular vesicles, or stem cells as vectors to carry and protect these materials. Fortunately, the human body already uses these vesicles in the amounts needed; we need only provide supplements to the existing cycle of vesicle-mediated material transfer by external production and transplantation.

6.3 Large tissues (i.e., entire replacement organs)

Alternatively, we can use external equipment to grow larger structures, such as full-size organs or replacement tissues. This type of work is already being performed by many labs, who are using a variety of complex 3-D scaffolding, embedded with stem cells, to create such tissues. When ready, they are transplanted into the body. These larger organs require a significant amount of energy to create, and thus create a significant net energy gain for the body.

We can envision times where such replacement organs might be superior compared to the regeneration of existing organs with CBCs. For example, if the liver has degraded into cirrhosis (and has thus been converted into scar tissue) then adding CBCs may not regenerate the liver. In this case, the growth of an entirely new liver, externally, which is then transplanted into the body, may be the better option. Other examples of this are new teeth (tooth buds) or transplantable hair follicles.

6.4 Use of accelerated waste-removal, in concert with the above, may speed up process

During aging, CBCs can convert into low-value materials. For example, muscle tissue or bone marrow may become infiltrated with fat. Active cells may convert to senescent cells. One may view this as a coping mechanism by the body: as it loses energy, it cannot maintain the inventory of CBCs and complex tissues, so it substitutes low-quality fat or inactive cells to fill the space. Also, a large variety of waste products may be flushed into the bloodstream or lymph glands, and these waste products may not be promptly removed by a liver and kidney that have become energy depleted. As a result, these waste products may build up as fat deposits, plaque, or mineralization.

As we gradually restore the energy and CBCs of the body, it may be that these waste products and low-value materials will be automatically broken up, cleared out, or restored to full function. However, it is also quite possible that such deposits, once created, are not addressed by the body; in other words, there is no mechanism for going "backwards" with some waste products, once they are created. In this case, we may need to supply chemical or biological treatments, such as the senolytic treatments described above, to assist in this break-up and clean-out process.

6.5 Use of growth hormone etc. may be beneficial to trigger states of regrowth

We don't completely preclude the use of small-molecule drugs, growth hormone, etc. It is not clear if all tissues in the body will be regenerated back to a "young" state once the additional CBCs, energy, and information are restored to them. Perhaps some parts of the body, for example bones, will stay old and weak, even while the other tissues surrounding them are restored to youthful strength. It would not be desirable to have newly restored muscles and tendons supported by bones that are weak and prone to damage. In that case, it might be necessary in this example to use growth hormone or other substances in combination with CBC restoration, or perhaps senolytic treatments as described above, to stimulate regeneration of bone tissue.

6.6 Physical therapy

Once CBCs and other materials are transplanted back into the body, thus restoring lost materials, it is still very likely that the patient will be required to exercise aggressively, stretch, or undergo other physical actions to "grow" the new muscles, tendons, heart strength, vasculature, and neural materials, to achieve a state of youth. Growing young again won't happen overnight; it may take months and require effort to achieve

7 CONCLUSION AND DISCUSSION

There have been many theories of aging, based on mitochondria, damage, evolution, and information. However, we have found that these theories do not lend themselves well to the design or testing of age-reversal treatments. To better support such treatments, we have proposed a new theory that incorporates elements of past theories together with what we term the "Loss of Complex Biological Components". This theory defines aging primarily as a process of massive loss of complex biological materials from cells and from the body over time, leading to loss of energy, loss of accurate cellular performance, tissue decline, and eventually death.

This concept of complex biological components fits nicely within a range of age-reversal techniques, such as parabiosis and stem cell therapy, which have historically shown glimpses of successful age-reversal.

This theory has limitations. It is challenging to create any single theory that completely represents aging in all of its extraordinary complexity. There may be other and better approaches yet to be developed. Nevertheless, we believe that by using CBC theory as a central mental model and by optimizing relevant age-reversal techniques on large scales, we have the best chance of developing a truly powerful, practical, and scalable age-reversal process.

APPENDIX - TESTS FOR INTERVENTION EFFECTIVENESS

One critical element in investigating CBC enhancement techniques is to have available quantitative ways of measuring the effects. In evaluating interventions that can boost or restore CBCs, we want to consider the question of how we know that a given intervention is actually working.

A.1 Test Animal Activity, Fitness, Healthspan, and Lifespan

Many of the CBC-restoring interventions discussed above have been tested with animal models, usually laboratory mice. The standard laboratory mouse is widely used in age-related experiments. They have an average lifetime of about 26-30 months and a maximum lifetime of about 3 years. The age-related effect that sets the hard limit on the maximum lifetime in mice is not known. However, that maximum lifetime limit for short-lived mice (~3 years) probably has a different root cause from that for long-lived humans (~120 years).

Nevertheless, the researchers investigating CBC-restoring interventions that may combat the effects of human aging are very interested in the impact of their interventions on the lifetime, health-span, and maximum lifetime of their laboratory mice. It is also fairly standard to test and record the state and changes in mental and physical acuity of the animals as interventions are being tested.

A.2 Blood Work and Phenotypic Age

Another measure of CBC-restoring intervention effectiveness is to observe before-and-after values of quantities that are readily measurable in a blood sample, e. g., the concentrations of albumin, creatinine, glucose, alkaline phosphatase, and C-reactive protein, as well as lymphocyte count, white blood cell count, mean cell volume, and red cell distribution width. A paper[29] by M. E. Levine, *et al* presents a technique for combining these blood-work values to obtain "phenotypic age", i.e., apparent biological age as implied by blood variables. They find that this quantity correlates well with other measures of biological age.

A.3 Telomere Length Assay

As was discussed in Section 2.7 above, telomeres are special regions at the ends of chromosomes that provide a "docking zone" for the enzymes that perform DNA duplication during cell division. They grow shorter with each cell division until they are too short to function, cell division is blocked, and the cell becomes senescent or dies.

There are now standard tests that measure telomere length in white blood cells from a blood sample as an indication of the biological condition of the subject. Telomere length can be taken as a rough indication of biological age, but it does not correlate well with some of the other measurements.

A.4 Protein Expression "Noise"

In their work on plasma dilution as an anti-aging intervention, as described in Section 4.1 above, the Conboy

Group at U. C. Berkeley identified 10 marker proteins, the "noise" in the expression of which can be used as a measure of biological age[17]. In particular, they find that in the distribution functions of the degree of expression of these 10 proteins from cell to cell, the standard deviations characterizing the widths of the distribution curves correlate very well with other measures of biological age.

A.5 Epigenetic Clocks

Epigenetic age has lately become a standard for biological age. Let's begin with a rough description of the mechanism behind epigenetic programming in cells. The human genome is the net DNA content of cells, encoded using the GCAT sequences of nucleotides (guanine, cytosine, adenine, and thymine). These sequences encode instructions for assembling proteins, folded chains of amino acids that are the fundamental structures of life. These instructions are stored in the 23 chromosomes inherited from the father and the 23 from the mother, containing over three billion nucleotide base pairs forming about 49,000 genes.

Each protein-coding gene within the DNA is preceded by a "promoter region", essentially an ID code used by the cell's transcription enzymes to find and identify the gene and its DNA code that is to be transcribed. One can think of this as if each gene is a book stored in the DNA library, with the title and call number on the book's spine represented by the gene's promoter region. Some of these books are locked away on inaccessible shelves while others are wide open on the library table.

Typically, each promoter region of a gene contains an "island" containing a repeating cluster of sequences of the nucleotide cytosine (C) followed by guanine (G). These are called CpG islands, with "p" indicating pyrimidine, the 4 carbon + 2 nitrogen ring structure that is part of cytosine. The significant feature of the CpG sequences is that one specific carbon atom in the pyrimidine ring can be methylated, i. e., a carbon-hydrogen CH_3 methyl radical can be attached to it in place of a hydrogen atom.

When such methylation is present, it encourages that region of the DNA chain to "close" and to be wrapped around spool-like histone structures for inactive storage, and it also discourages any wandering transcription factors from landing on the promoter region and transcribing the DNA coding into messenger RNA that will lead to ribosome-implemented protein production. When a sufficient number of methylations are present in the CpG islands of a promoter, it has the effect of completely silencing the gene and preventing production of its encoded protein. In other words, while all body cells have the same genes, ready to express their proteins, only those genes with the promoter region relatively clear of methylation will actually express their proteins. This selective methylation is the principle epigenetic mechanism by which cells become specialized to perform specific functions and by which cells are "set" to be either young or old.

DNA methylation now provides a very important analytic tool, because equipment suppliers have developed CpG methylation microarrays that allow simultaneous determination of the methylation condition of many thousands of CpG sites within a DNA sample. In 2013, Prof. Steve Horvath (UCLA) used data from two sizes of Illumina microarrays that identify the methylation states of 27,000 CpG sites. Using advanced statistical techniques and machine learning, he isolated 353 CpG sites that were highly correlated with human calendar age. This analysis was based on 7,844 tissue samples that spanned 51 different tissue types. He found that methylation decreased with age in 55% of these sites and increased with age in the other 45%. As the subjects aged, genes were being systematically switched, with some genes being brought to expression and others silenced. This work became the Horvath DNAm Clock, and it has become the standard for aging research. In 2018 Horvath improved the technique with a new clock algorithm with 513 CpG sites based on larger Illumina microarrays.

One central question raised by the discovery of this methylation clock is whether the epigenetic programming revealed is the *result* of aging or the *cause* of aging. In other words, if one could provide a subject with treatment that would reset the Horvath Clock to a younger DNA methylation profile, would that rejuvenate the subject? (Or would it be the equivalent of attempting time-travel by setting back your kitchen clock?) The answer seems to be that a reset produces some rejuvenation. Since the methylation profile tends to remain in place once it is established, any intervention that produces a DNAm change can be taken as relatively permanent.

A.6 mtDNA Damage Assay

Mitrix Bio has developed a "MitoClock" test procedure for testing the condition of mtDNA in mitochondria (Fig. 5). Using the newly available long sequence PCR technology, they employ a technique for quantitatively characterizing the degree

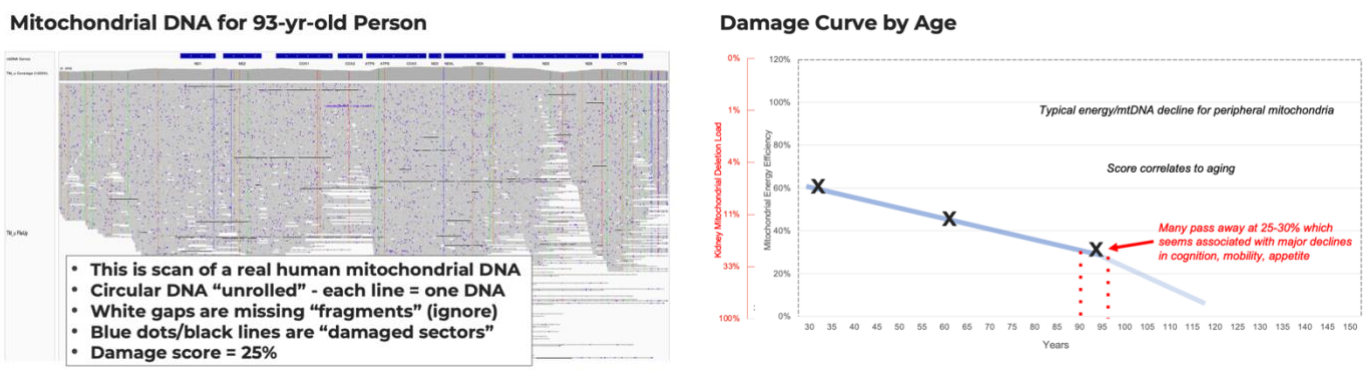

Fig. 5: MitoClock Test

of DNA damage present in mitochondria from a subject's urine, spit, or blood samples. In one example, they show damage plots for the same mtDNA pattern taken from three maternal-line subjects that are 32, 62 and 93 years old. These plots show mtDNA damage scores of 7%, 11%, and 25%, respectively. There are indications that a subject is not likely to survive if the general mitochondrial damage level reaches 25% or more, because energy production by the mitochondria becomes too inefficient to sustain life. In other words, there is now evidence that mitochondrial damage, which will severely limit the energy available to otherwise healthy cells, may be a principal contributor to the maladies of old age.

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