Engineering *Longevity* and the Reversibility of *Aging*

INTERVIEW WITH DR. IRINA CONBOY

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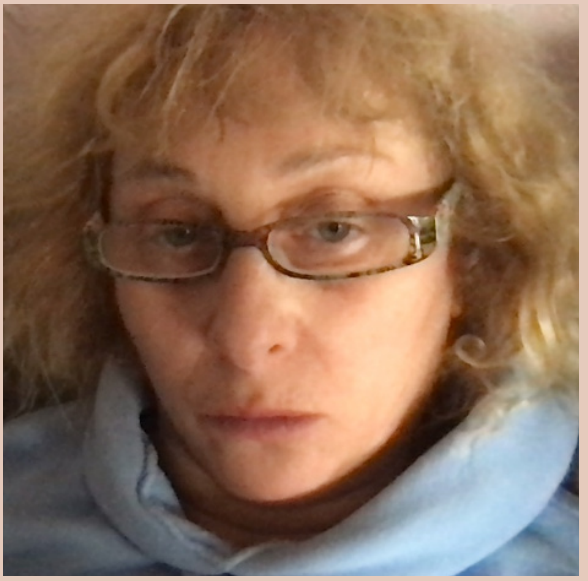

Dr. Irina Conboy, PhD, *is a professor in the Department of Bioengineering at the University of California, Berkeley. Her research focuses on the physiological basis of aging and potential therapeutic solutions to aging with the goal of applying these treatments to various degenerative diseases. Dr. Conboy's lab aims to "engineer longevity" by way of rejuvenation of tissues through plasma exchange and through gene editing with CRISPR technology.*

BSJ: What is the physiological basis behind aging?

IC: Some factors of aging are still under dispute, and many of them are unknown. Some say that aging is due to shortening of the telomeres. Others say that it is due to damage to the DNA throughout the genome, or accumulation of reactive oxygen species which damage proteins, or accumulation of senescent cells (clusters of cells throughout the body that make the rest of the tissue unhealthy). Very interestingly, there is no definitive proof that any of those factors, or a combination of them, is what drives aging.

BSJ: How does your research contribute to our understanding of aging?

IC: If you start with a very old animal, such as a two year old mouse, which is analogous to a 75-80 year old person, and you apply our "engineering longevity" approach, that animal becomes rapidly young with respect to tissue repair. There is improvement in the muscle, liver, brain, cognitive capacity, agility and strength, which means that whatever cumulative damage happened—telomere attrition, accumulation of DNA damage, mitochondrial damage, or something else—is not the driver of aging, because we were able to reverse it. Once again, it points to the conclusion that aging is determined by the rate of tissue repair, so if we increase the rate of tissue repair, not only can we stop aging or slow it down, we can also gradually reverse it.

BSJ: What does it mean to "engineer longevity"?

IC: Some people perceive the aging process of humans as something similar to how a new car ages—as you use it, it becomes less functional. What we started to realize is that aging depends on the efficiency of damage repair and not how much damage can accumulate. Since it is a process of repair, we can try to extend the process and then tune that to our advantage, and that is synonymous with "engineering longevity." Our hope is that we will find ways to not only treat age-related diseases like Alzheimer's, but to prevent them.

BSJ: What is therapeutic plasma exchange, and what is its significance in medical treatments?

IC: Therapeutic plasma exchange (TPE) has been used in the medical field for around 35 years. The main goal of TPE is to purify blood plasma of toxins or autoreactive antibodies that can attack the person's body. In general, it can purify the circulatory system. For example, if someone ingested a toxin or if they overdosed on drugs and no other treatment works, TPE could save their lives by replacing part of the blood plasma with saline and albumin. It also works in the case when the body generates an antibody which attacks its own proteins. For example, in multiple sclerosis, in which the antibodies attack the myelin sheaths that wrap the nerves, TPE works by removing those overactive antibod-

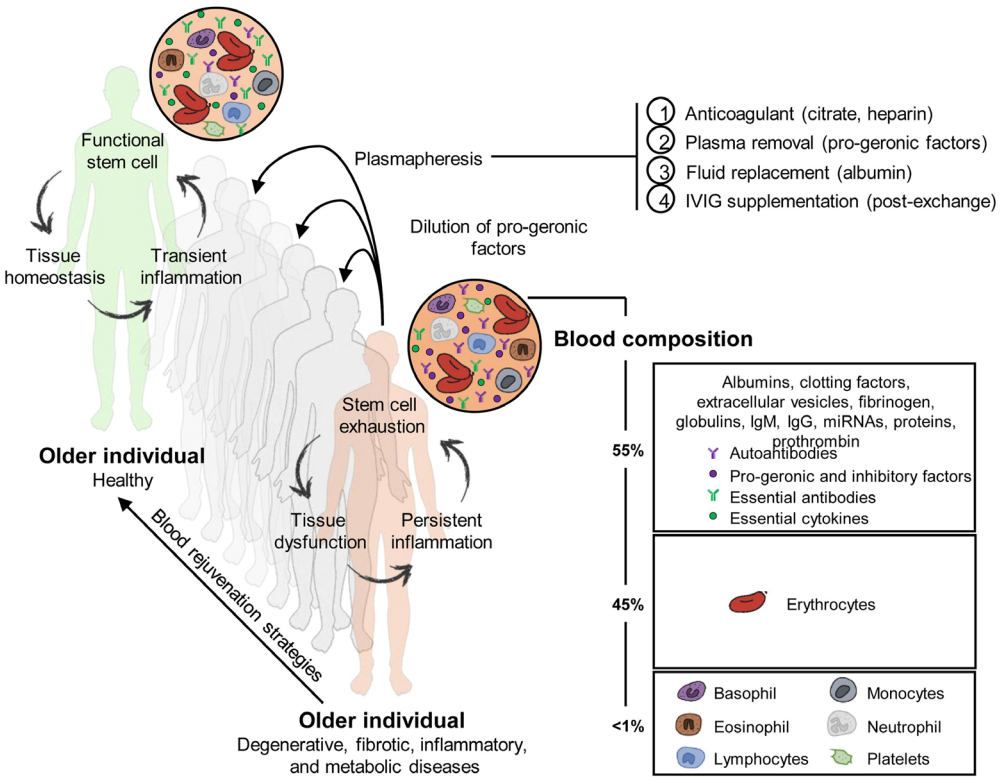

Figure 1: Blood Rejuvenation by Plasmapheresis. *Pro-geronic factors that accumulate with age can be removed by plasmapheresis treatment.*

ies from the blood. This scheme also applies to most autoimmune diseases. TPE returns blood cells back to the person, but the cells are resuspended in physiologic solution, like saline and albumin. Albumin is an abundant protein in our blood which is needed for protein transport and blood rheology, which allows our blood vessels to keep their shape. When blood plasma is removed, some of the albumin is lost, so we have to replenish it. That is the entire procedure of TPE.

BSJ: You found that TPE decreased the levels of proteins that accumulate with age, and surprisingly, also elevated the levels of certain proteins. What is the significance of this observation?

IC: When we decided to apply TPE to "engineer longevity," we thought that we were simply removing accumulated senescent cells or inflammatory proteins. When people grow older, they have multi-tissue inflammation and fibrosis, which comes from damage of organs and tissues over time and the inability to repair them. The immune system becomes chronically activated instead of having a productive immune response that eliminates bacteria and viruses. What we thought is that TPE will be useful for people who are older because it will simply remove excessive inflammatory proteins, but what we discovered about one month after procedure and control is that all the typical regulators of health—proteins that maintain tissues, repair tissues, and make blood vessels better—which typically declined with aging, now came back. Not only was there the first wave when we diminished inflammatory proteins, there was also a second wave when the age-diminished proteins again became restored. What we realized is that when many of the proteins are elevated with disease/age, they suppress the productive homeostatic gene expression and, consequentially, healthy blood proteome. All the genes in our body are interconnected for us to function as organized systems, and when some of them are expressed in excess, they suppress many of the other genes/proteins that we need for healthy tissues. Therefore, an acute large plasma dilution allowed those other proteins to come back.

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BSJ: What are the physiological processes that accelerate aging? And how does TPE alleviate the effects of these processes?

IC: The main mechanisms are cellular senescence, immunosenescence, and systemic chronic inflammation. Cellular senescence is an interesting concept that was pioneered by Judith Campisi, a professor at Buck Institute for Research on Aging. She

published works describing senescent cells. Senescence evolved as an anti-cancer phenomenon where if a cell's normal functions are impaired, such a cell does not divide. At the same time, senescent cells produce senescence-associated secretory phenotype (SASP), such as harmful inflammatory proteins. These senescent cells affect tissue around them in many ways, including making the tissue become more prone to cancer spreading. Senescent cells themselves do not turn into cancer, but they allow cancer cells that spontaneously appear in the tissue to metastasize and grow better.

As for the role of TPE, we found that TPE reduces tissue senescence. For example, for the brain, we show that there are fewer cells with a particular marker of senescence, senescence-associated (SA) Beta-Gal. That is quite interesting because we did not use the drugs called senolytics; instead, we studied senescence in parallel with the plasma dilution procedure. It is interesting that lowering the levels of excessive systemic proteins could attenuate senescence. We do not know if plasma dilution removed them or perhaps made them healthier and less senescent.

BSJ: A potential treatment for aging is the attenuation of senescence-associated secretory phenotype (SASP). Why did you choose to attenuate SASP, instead of directly removing senescent cells?

IC: Direct removal of senescent cells can lead to bad things happening, such as wound healing becoming worse. That was also published by Judith Campisi's lab. When they looked at skin and wound healing with and without senescent cells, healing without senescent cells did not take place as well as with senescent cells. Additionally, some markers of senescent cells are similarly expressed on normal cells in young animals when cells differentiate. Cells that regenerate tissue start as stem cells and differentiate into precursor or progenitor cells before differentiating further into the final tissue. During that differentiation process, cells express p16, another marker of senescent cells. What we published is that p16 is expressed even in young animals in tissues that are very healthy. So it will not work if you start ablating cells based on p16. In contrast, if you make cells less senescent or healthier through plasma dilution, this might be a milder and safer approach.

BSJ: What further research needs to be conducted to establish attenuation of SASP as an actual treatment for aging?

IC: In the lab, we work with neutral blood exchange (NBE) instead of TPE, which is done by our collaborator, Dr. Dobri Kiprov. He thought that TPE could be used for rejuvenation back in 2014. What needs to be done is reputable clinical trials with placebo controls. There will be some subjects between 60 and 80 years old undergoing TPE but also a placebo group, and we would then measure numerous parameters of tissue health, inflammation, regeneration, fibrosis, degeneration, and other hallmarks of what are called the comorbidities of aging. Through this clinical trial, one will see whether TPE could be repositioned to treat diseases that increase with aging. There are numerous diseases without cures right now, and there is a suggestion that TPE, which is already approved by the FDA, could be prescribed to those patients. Right now we are trying to fund-raise for that clinical trial.

BSJ: In addition to this study, you also developed a graphene-based biosensor for detection of bio-orthogonally labeled proteins, which aims to identify circulating biomarkers of aging during heterochronic parabiosis. What is the purpose of heterochronic parabiosis? Are there any challenges or ethical concerns associated with this procedure?

IC: Heterochronic parabiosis is a very ancient approach that was introduced 200 years ago. In this approach, different animals are surgically sutured together. The animals are of different

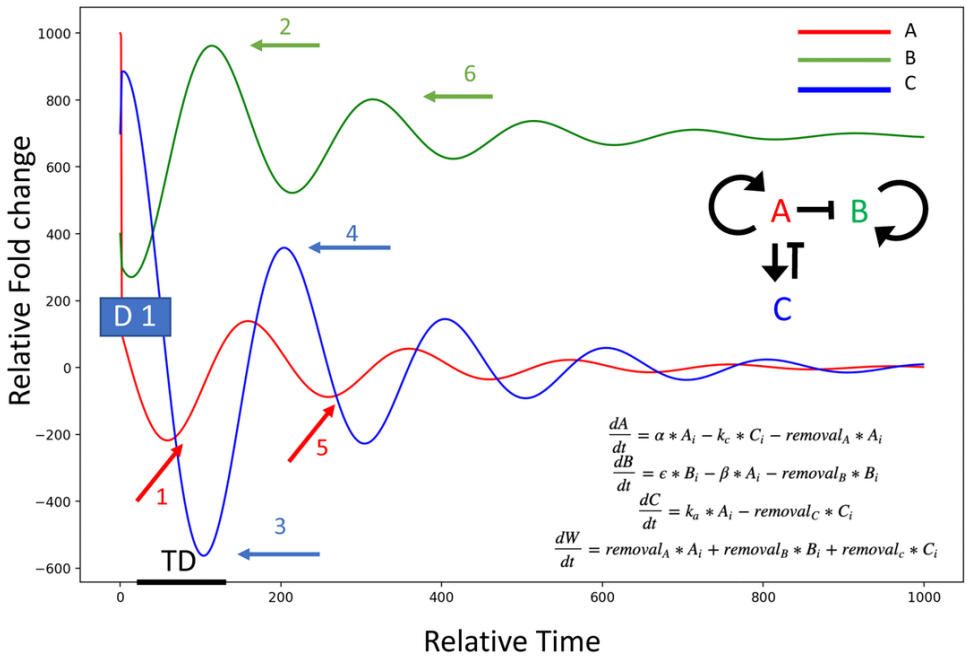

Figure 2: Model of the dilution effect in resetting of circulatory proteome. *A induces itself and C; A represses B; C represses A. Dilution of an age-elevated protein, A, breaks the autoinduction and diminishes the levels of A. The secondary target of A, B, becomes de-repressed and elevated. The attenuator of A, C, has a time-delay of being diminished, as it is intracellular and was not immediately diluted, and some protein levels persist even after the lower induction of C by A. C is no longer induced by A and decreases, and a reboot of A results in the re-induction of C by A, leading to the secondary decrease of A signaling intensity/autoinduction and a secondary upward wave of B.*

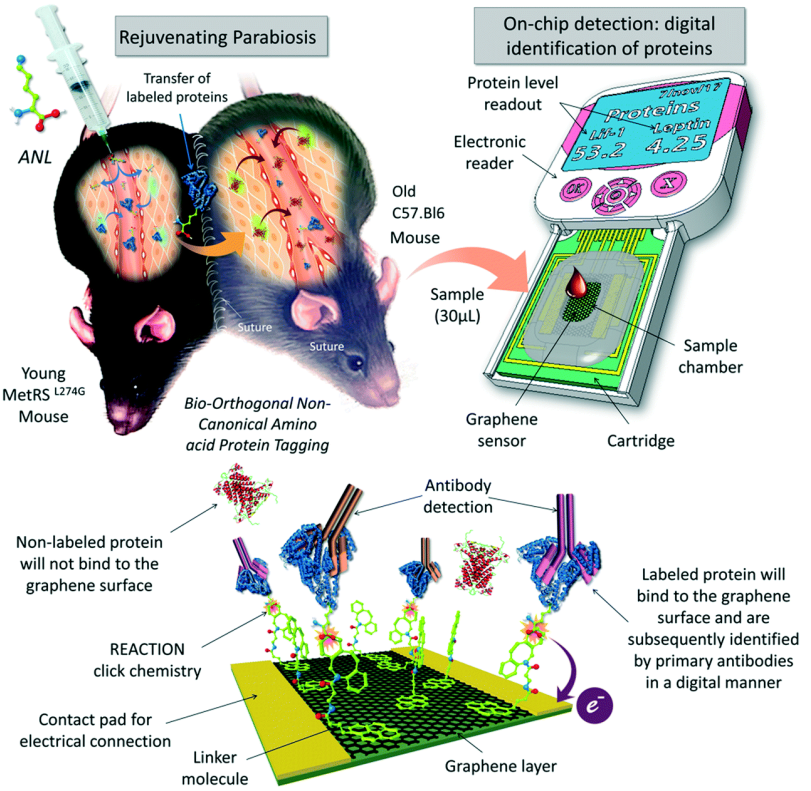

Figure 3: Function of Click-A+Chip. *Binding of the bio-orthogonally labeled proteins with a linker molecule on the graphene surface can be detected by the sensor.*

ages, or they could have different diseases or health status. It was applied to the idea of rejuvenation by trying to see if connecting one old rat with six young rats can "dilute" the aging.

However, these approaches are not well controlled. Although animals are monitored every day and are given analgesics, it is a very poorly controlled procedure because you do not know when the positive and negative effects take place. There is organ sharing and environmental adaptations, not just blood exchange.

But, overall, this procedure helps us to understand the general concept of aging and rejuvenation. An old mouse which has already accumulated intrinsic damage is rapidly rejuvenated through parabiosis to a young partner while the young mouse ages when it has no previous intrinsic accumulation of tissue or cell damage.

BSJ: What is bio-orthogonal non-canonical amino acid tagging (BONCAT), and how does it allow us to label proteins of interest?

IC: BONCAT was developed by Professor David Tirrell from Caltech, and I did my sabbatical in his lab where I learned about this technology. He developed it in bacteria, and we then applied it to the field of mammalian aging in vivo. In this technology, there is a mutant enzyme of methionine tRNA synthetase. Methionine tRNA synthetase is the enzyme that attaches the amino acid methionine to its specific tRNA for translation. If this enzyme is mutant, it can incorporate a non-canonical amino acid instead of methionine during the synthesis of polypeptides. One single mutation, L274G, in the sequence of the enzyme allows one to incorporate azido-nor-leucine (ANL) instead of methionine, hence, metabolically label cells or tissues with ANL. If you have two mice that are parabiotically connected and exchanging blood with each other, but only one of them is expressing the transgene, only the proteins of that one animal will be tagged with ANL. One can then see how the proteins go through the shared heterochronic blood circulation and where they end up, whether in the muscle, brain, or liver. Moreover, one can specifically identify the proteins which came from the young animal into the old animal, or from the old animal into the young animal. That is what we did and published in 2017 in Nature Communications, and with the help of Professor Kiana Aran, we then developed the more sophisticated, digital graphene-based biosensor called Click-A+Chip.

BSJ: How does Click-A+Chip work, and what are some of its key features?

IC: The chip is a graphene model device where graphene is used as the electro-conductive material to detect increase in resistance through binding of analytes to its surface. It is transistor-based so the current and resistance can be tuned. If anything is bound to the surface of the graphene, resistance to the circuit is introduced, which one can detect as a drop in electric current. The device was developed by Professor Kiana Aran. With these collaborative efforts, it is possible to accurately identify every single "young" and "old" circulating protein that is important for tissue

aging and rejuvenation.

BSJ: What were some critical observations when you applied Click-A+Chip to heterochronic parabiosis?

IC: We found that rejuvenation is not based on a silver bullet- one blood protein. Why would it be? Instead, there are numerous young proteins that traverse from the blood of young mice to the tissues of the old mice that work together and interact with each other. Some of them diminish inflammation, some of them participate in remodeling of the extracellular matrix, and others activate muscle stem cells to divide and differentiate. Some of these proteins, such as Leptin and Lif1, were previously implicated in aging and rejuvenation but not in the effects of heterochronic parabiosis.

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BSJ: Aside from identifying factors of aging, are there other potential applications of Click-A+Chip?

IC: In our study, young mice expressed the mutant methionine tRNA synthetase, so we looked at young proteins in old tissues. Since aging is driven by an excess of old proteins, we are also aging the methionine tRNA synthetase transgenic animals for the reciprocal study: for example, to identify the systemic proteins of old animals that make young tissues pro-geronic.

BSJ: You have expertise in various scientific fields of immunology, bioengineering, therapeutics, and more. How do these fields intersect in your research?

IC: I think immunology is something that everybody should learn because it is one of the most well-developed and oldest areas of science that is quite complex and easy to misunderstand. It also has technological applications in the area of immune engineering. Knowledge of immunology contributes to our current studies on how TPE rejuvenates the old immune system, making individuals more resilient to viral illnesses such as COVID-19. Old people succumb to COVID-19 more than young people, so TPE could be used to improve recovery from COVID-19.

There are also many ways to combine BONCAT with understanding immune system responses, such as looking at what cancer cells make in vivo; for instance, how they change immune responses in young versus old animals. Another example is a recent published paper on a blood brain barrier organ chip, which allows screening of all secretory molecules either using BONCAT or Click-A+Chip. Instead of doing very difficult studies in vivo to see what happens in the brain, they use chips which could be humanized.

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