Therapeutic Plasma Exchange as a Tool for Reversing Age-Related System Dysfunction With A Special Emphasis On Reversing Brain Dysfunction

By Patrick Cox, Research Analyst, Lifespan Edge

Brief Foreword

By Dr. Michael Roizen & John Mauldin, Co-Founders, Lifespan Edge

This brief paper by Patrick Cox does the important job of summarizing thousands of pages of significant research introducing the benefits of Therapeutic Plasma Exchange (TPE). It does so by outlining the history of the procedure and the important individual discoveries that have been made over the last century and especially the last 20 years. We believe this demonstrates that TPE is not only beneficial in treating specific human medical conditions but also has significant impact on cognitive dysfunction and ***the entire aging process*.**

We think this paper is a well-written introduction for professionals researching the state-of-the-art, including links to the journal articles that many will want to analyze. It also summarizes key data for the layman who is considering using TPE for their own family members, friends, or personal well-being; and it serves as a resource they can share with their personal physicians to help educate the physical benefits and the hypothesized mechanisms of action for the benefits shown in the gold standard of studies, randomized double blind studies.

Thanks for taking the time to read this paper; feel free to reach out to any of our trained staff who can help you with your own personal or family needs, and desires

to know more. Please note that there are numerous other research papers on TPE at the Lifespan Edge website.

The History and Benefits of Therapeutic Plasma Exchange

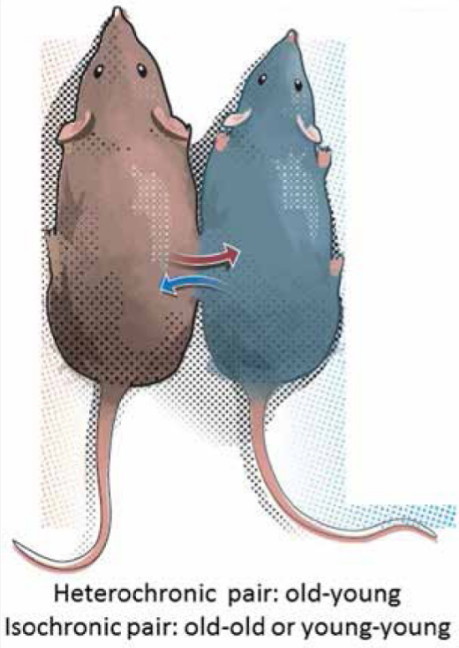
Therapeutic Plasma Exchange (TPE) is a 75-year-old FDA-approved medical procedure in which each treatment removes and replaces about 50% of a subject's plasma, the carrier medium that makes up about 55% of the bloodstream, with saline and a few other substances, especially proteins. Other blood components (such as platelets, and red and white blood cells) are removed but returned washed with new saline and albumin to the subject. Though TPE is used to treat a large and growing number of conditions, more recent research is motivated by evidence that TPE decreases genetic age and improves biological and cognitive functions.

The best way to understand the growing scientific interest in TPE's anti-aging potential is to briefly review the scientific backstory, which started in the 1860s when French physiologist Paul Bert surgically joined two rats, stitching veins and arteries from both animals together so that blood flowed freely between them.

The Evolution of Parabiosis to Plasma Replacement

Bert named this process "parabiosis," using the Greek prefix para (παρά), "beside" and biosis (βίωσις), "living." The term was literally descriptive because the parabiotic animals were fated to live like permanently conjoined twins. The process allowed experiments that contributed significantly to biological knowledge, and Bert won the French Academy of Science's prize for Experimental Physiology in 1866.

In 1956, almost a hundred years after Paul Bert first described parabiosis, Cornell University scientist Clive McCay used a similar procedure to further his investigations into the aging process. McCay linked young and old rats' circulatory systems together, which is now known as heterochronic (different age) parabiosis. After 9 to 18 months with bloodstreams linked, measures of the old animals' health, including bone strength and density, improved significantly.



From *The Fountain of Youth: A Tale of Parabiosis, Stem Cells, and Rejuvenation*, Open Medicine, 2017.

At that time, McCay was a member of a small minority of scientists who were interested in learning how and why we age. His experiments were not designed to investigate the impact of heterochronic parabiosis on lifespans, but two University of California scientists, Frederic Ludwig and Robert Elashoff, picked up that ball. In 1972, they published evidence that heterochronic parabiosis extended the lives of the older parabiotic mice by four to five months, which approximately equates to 8 to 10 human years.

Highlighting how much attitudes about aging have changed, McCay's experiment was generally ignored and largely forgotten. Parabiosis continued to be used but primarily for stem cell research.

Thirty years later, in 2002, two married researchers at Stanford University, Irina and Michael Conboy, were exposed to heterochronic parabiosis in a faculty presentation. They instantly realized that it could help them answer a key question.

Specifically, the Conboys were trying to understand why all cells seem to deteriorate simultaneously with age. One theory was that cells degrade collectively due to something happening within the cells themselves. The other theory was that

something outside cells, an external milieu, triggered systemic cellular dysfunction.

To answer that question, the Conboys and a few other Stanford researchers (including Thomas Rando, Amy Wagers, and Irving Weissman) decided to expose old mice to the youthful milieu of younger mice's bloodstreams. If the old mice exposed to young blood continued to age normally, they reasoned, it would indicate that collective cellular aging is due to some internal cellular clock. If the cells of old mice exhibited younger characteristics, however, it would mean that cellular aging is effected by factors outside of the cell.

In fact, there experiments yielded the same results found by Ludwig and Elashoff in the 1970s. The older parabiotic mice lived significantly longer than expected.

As reported later by a science historian in the journal Nature, the authors of the study concluded that the primary mechanism of rejuvenation working in the older animals was the repair of their stem cells' ability to replicate and repair other tissues. This theme, stem cell rejuvenation by TPE, would return decades later, notably in the 2005 Nature article, *Rejuvenation of aged progenitor cells by exposure to a young systemic environment*, authored by the Conboys and associates.

Crucially, they observed significant improvements in the activity of aged progenitor cells; these cells are descendants of embryonic stem cells. The reversal of normal age-related "replicative senescence," the researchers believed, explained why exposure to young blood had significantly rejuvenated muscles, the liver, and the brain.

Repairing Stem Cell Function and the Big "Young Blood" Mistake

While their conclusion was technically accurate, they would later realize it was flawed by serious omission. Nevertheless, the 2005 publication of their paper in the prestigious journal Nature provoked an explosion of interest in what many were calling "young blood therapy."

Other scientists replicated the study, producing evidence that heterochronic parabiosis repaired spinal cord injuries in older mice and reversed age-related thickening of the heart walls.

Media coverage of young blood therapy escalated, and several startups began offering transfusions from young donors. Critics also responded, calling heterochronic parabiosis "vampire therapy" and warning it would exploit young blood donors.

The FDA, cautious by design and insisting on human safety and efficacy data, opposed the practice and highlighted the lack of any long-term data. An official warning would eventually be issued, effectively ending the young blood therapy business in the US, though not elsewhere.

Another consequence of the Conboy et al. 2005 study was a scientific gold rush for discovery of specific proteins in young blood that could be responsible for the rejuvenation of the old mice. The Conboys themselves joined this search and identified oxytocin as a possible stem cell activator. Their colleague Amy Wagers' work centered on Growth Differentiation Factor 11 (GDF11), though that avenue of research was confused by wildly differing trial results.

It's Not the Whole Blood, It's the Plasma

Coincidentally, the office of Tony Wyss-Coray, a Stanford neurologist with a background in Alzheimer's research, happened to be located next to Rando's lab. As a result, he closely followed the progress of the study and Rando shared unpublished data with him showing that the older parabiotic mice (those receiving the young blood and getting rid of their old blood) had experienced increased neuron growth.

Convinced that heterochronic parabiosis had produced real rejuvenation, Wyss-Coray founded a company to explore therapeutic options. He repeated the parabiosis experiment to verify neuron growth in the old mice and identify possible therapeutic proteins. His results were extremely successful, and he submitted them in a paper to the top journal Nature.

The scientists assigned by Nature to review the article rejected it, but not because they found errors or problems with the study design. Rather, they believed the results were simply too good to be true.

In response, Wyss-Coray had the experiment replicated by different researchers at another university using different instruments. After a delay of a year, the reviewers accepted and published the paper in the August 2011 issue of Nature.

Given the seriousness of the Alzheimer's threat to individual lives and the economy combined with the absence of any effective treatments for the disease, results initially believed to be "too good to be true" were certainly stimulatory of more research and longer trials. In retrospect, however, the most consequential aspect of Wyss-Coray's research may have been the inclusion in the paper of a minor point that might have been easily missed. Specifically, he discovered that old plasma was harmful to young mice.

This was a critically important breakthrough in the ongoing process of understanding how young blood therapy actually worked. However, it would soon become apparent that even this discovery didn't go far enough.

Grifols Joins the Quest

At the same time that Wyss-Coray and his colleagues were planning the animal trial that rebutted the young blood hypothesis, a group of scientists in Barcelona, Spain were formulating a series of human trials that would challenge both the young blood and proteins in young plasma concept.

The research was funded by Grifols, a global supplier of albumin and other blood products. The company committed the significant funding needed for a large randomized blinded controlled clinical trial based on the current theory that Alzheimer's disease is caused by amyloid proteins produced in the liver and carried by albumin to the brain, where they form harmful plaque structures.

Albumin has several functions other than transporting substances through the bloodstream, including control of blood pH and oncotic pressure, which prevents blood vessel leakage. For that reason, albumin is often included with saline in the replacement fluid used in TPE procedures.

If amyloid-carrying albumin plays a major role in Alzheimer's disease, they reasoned, replacement of patients' existing albumin with purified albumin could remove amyloids and suppress AD progression. As a leading supplier of albumin, Grifols hoped to prove the theory correct and gain regulatory approval for a procedure that would benefit millions of people threatened by Alzheimer's disease as well as the company's bottom line.

To that end, scientists planned a series of trials, starting with a small pilot study to work out details needed for a full-scale trial. The first trial, an open-label pilot study, was performed with ten subjects diagnosed with mild to moderate AD. In a three-week trial, seven of the ten received up to six TPE procedures with 5% Albumin and an anticoagulant in saline replacement fluid. The three control subjects were given a sham procedure using modified TPE machines that only appeared to function.

As measured by brain scans, cognitive testing, and blood flow within the brain, the treated patients' AD stabilized while the placebo group's condition worsened. The treated patients and family members were so happy that they asked for a continuation of treatment, which led to an extension study one year later. This second phase of the pilot study utilized the same TPE procedures as the first in the six treated subjects who were able to participate.

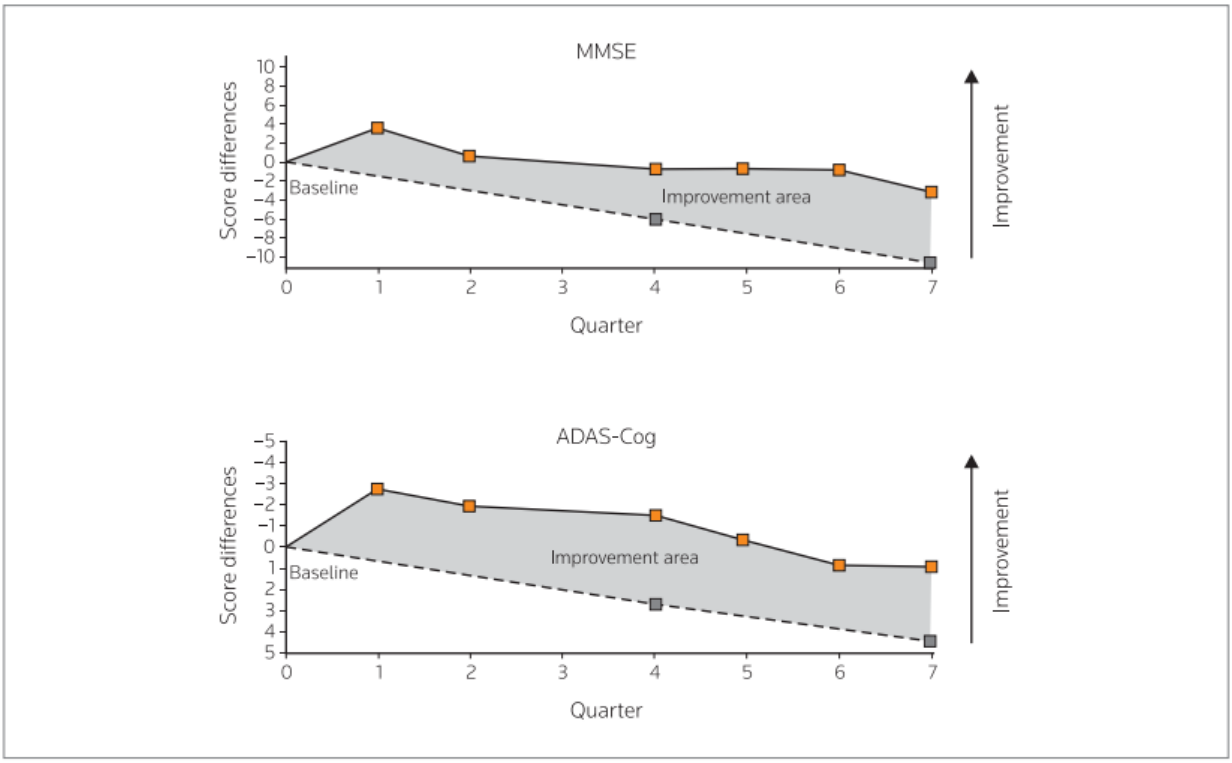


Figure 7. Changes from baseline scores (average from the patients included in both the pilot and the extension study) of the Mini-Mental Status Examination (MMSE) and the Alzheimer's Disease Assessment Scale, cognitive subscale (ADAS-Cog) measured during 2 years of follow-up at weeks 15, 27, 52, 60, 75 and 87. For clarity, the negative values of ADAS-Cog have been represented upwards. The dotted line represents the expected progression for this type of Alzheimer's disease patients at 2 years of follow-up. The surface lying between the two lines can be considered as an indicator of improvement.

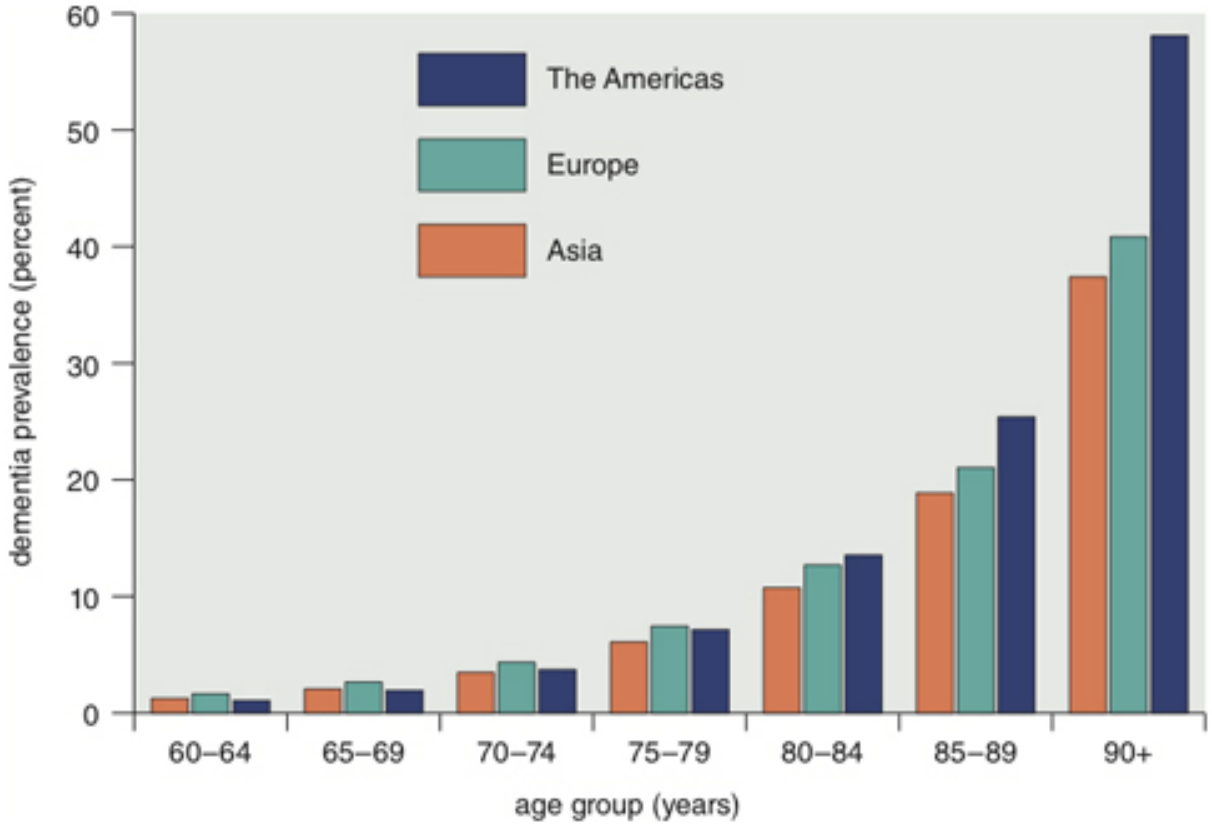
Though the pilot studies were successful, more test subjects were needed to achieve statistical confidence, so a Phase 2 randomized, controlled, parallel, single-blind clinical trial was carried out in 36 patients with early to mid-stage Alzheimer's. Each arm involved three periods of six TPE procedures.

Unlike the pilot studies, which took place solely in Spain, Phase 2 was performed in two Spanish centers and two US centers. The endpoints included comparison of amyloid levels in spinal fluid, cognitive status, and neuroimaging.

Outcomes matched those produced in the pilot studies, with treated patients exhibiting significantly better cognition and brain scans than the placebo group. When pilot and Phase 2 results were published in the July/August 2009 edition of

Drug News Perspective, they changed the way researchers viewed parabiosis and TPE.

Though the Grifols TPE studies were designed to test efficacy on Alzheimer's disease alone, there was reason to believe that the results could be extended to the aging process in general. AD rates are strongly correlated to age and the odds of getting the disease rise sharply over time. The following chart demonstrates that correlation as well as the existential risk posed by increasing dementia in societies with increasingly older populations.



From *Alzheimer's Disease: The Great Morbidity of the 21st Century*, American Scientist, 2012.

The Grifols studies were a serious blow to the young plasma theory, raising the possibility that the source of TPE's benefits was purified albumin, which is commonly included in TPE procedures. This explanation supported Grifols' premise for conducting the clinical trials, as is reflected by the title trial for the nest

study (Alzheimer's Management by Albumin Replacement or its acronym AMBAR).

A few scientists, however, began to think about another possibility. Specifically, they focused on the removal of old plasma (plasmapheresis) to make room for the replacement fluid. Plasmapheresis is needed to prevent a dangerous excess of fluid, proteins, or inflammatory mediators.

The Major AMBAR Trial

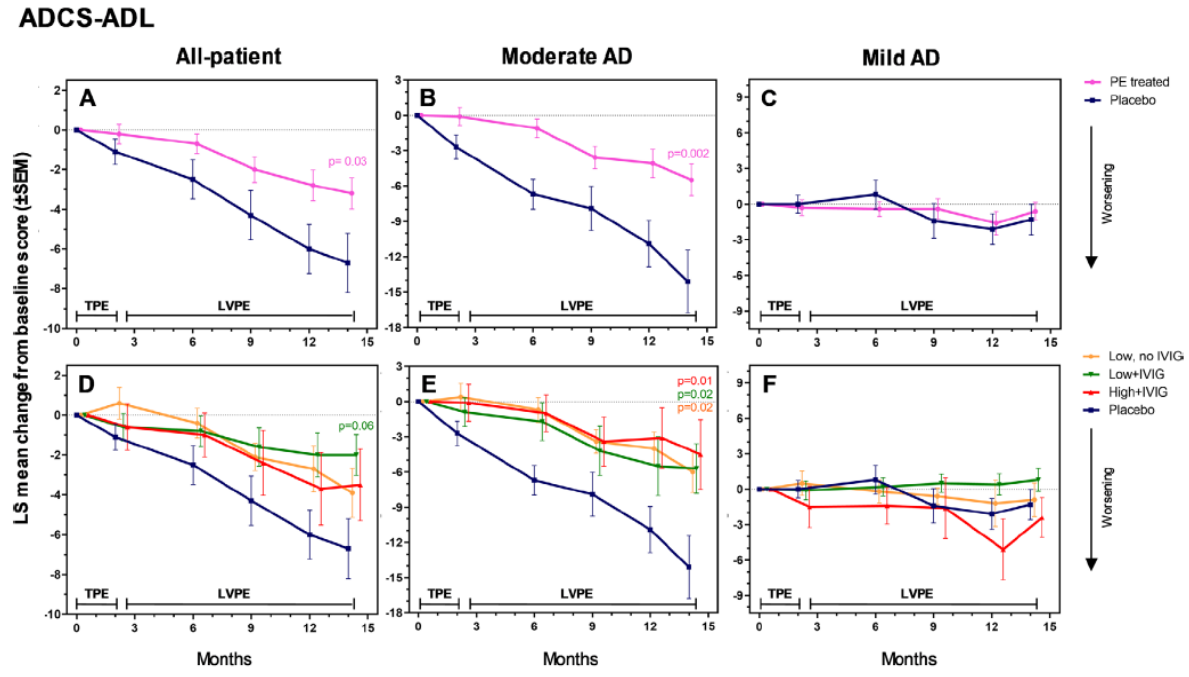
Following the successful pilot and Phase 2 trials, the team moved ahead with the Phase 2b/3 AMBAR study. Scientists who worked for Grifols participated in the trial design and the company provided funding.

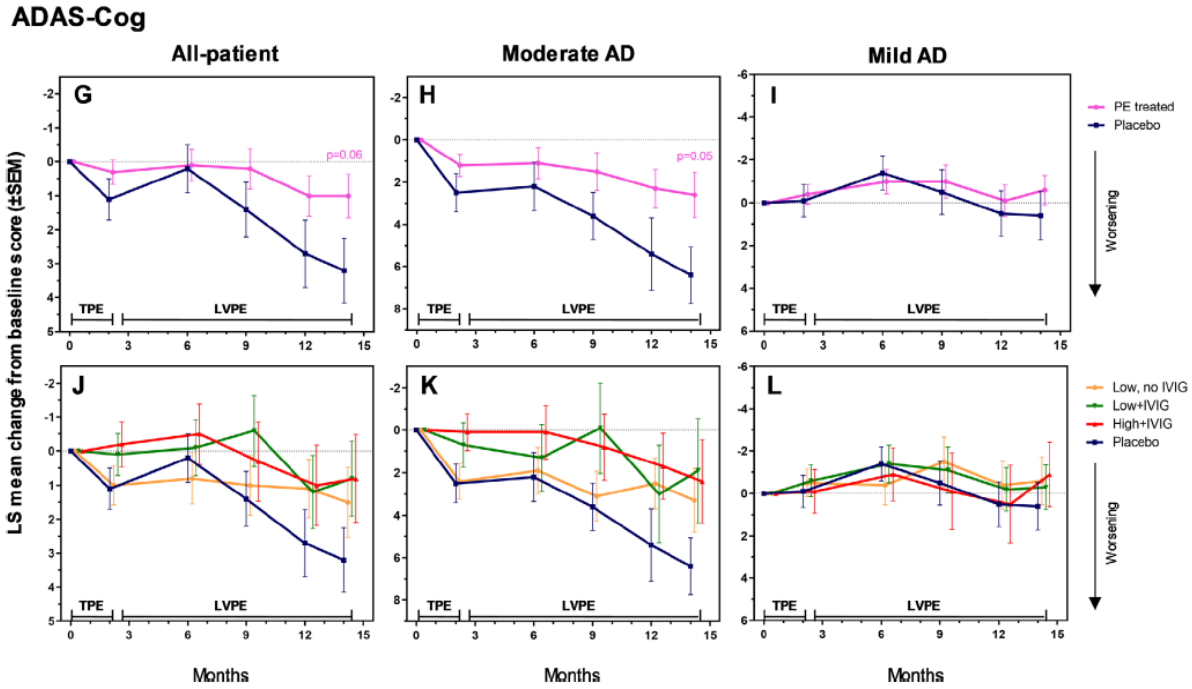
Three hundred forty-seven patients with mild to moderate Alzheimer's disease were enrolled in 19 sites in Spain and 22 in the US, including the Cleveland Clinic. Subjects were randomly allocated into a control group and three plasma exchange treatment arms with different doses of albumin and intravenous immunoglobulin replacement. The protocol included a 6-week period of weekly TPE followed by a 12-month period of monthly low-volume plasma exchange and a sham placebo procedure.

The results, once again, matched or exceeded those produced in early trials. Unsurprisingly, multiple analyses of the trial data have been performed, with the resultant papers published. A primary journal article is *A randomized, controlled clinical trial of plasma exchange with albumin replacement for Alzheimer's disease: Primary results of the AMBAR Study*, Alzheimer's & Dementia, 2020.

The researchers show that patients with moderate Alzheimer's experienced a 60 to 70% reduction in progression of the disease as measured by detailed assessments of quality of life and cognitive tests. Neuroimaging also suggested less neural damage. At the end of the study, patients' cognitive scores continued to improve, indicating that TPE had not only stopped degradation but had effected permanent changes that were somehow improving biological functions.

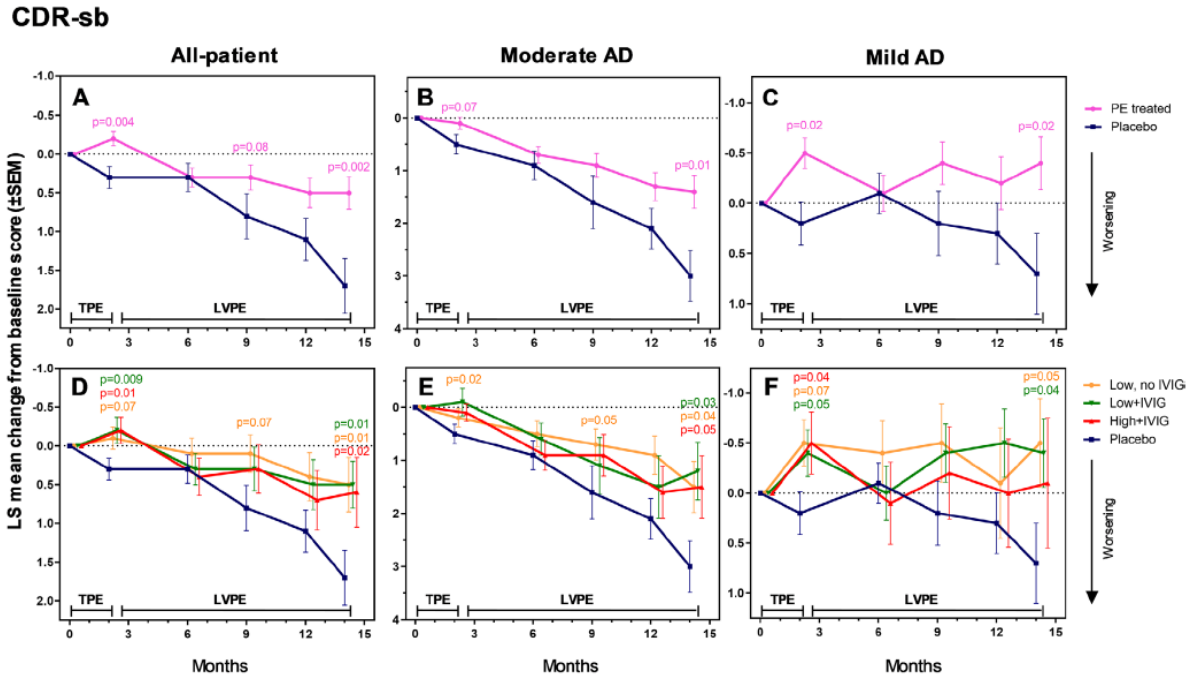
Data from the study indicate that treatment groups averaged 50% to 75% less worsening of scores of cognitive functions such as ADAS-Cog scores and 42% to 70% less worsening of ADCS-ADL scores than control subjects.

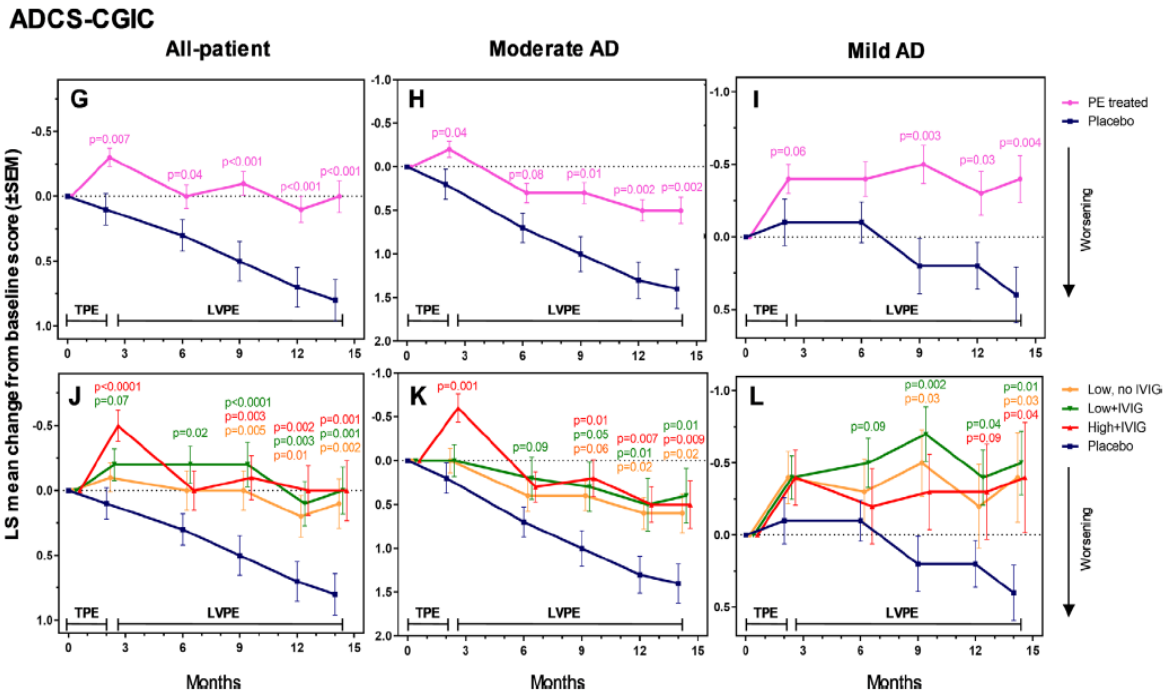




Pooled data showed that treated patients declined, on average, 66% less than control subjects on other cognitive function styles such as those based on ADAS-Cog scores ($p = 0.06$) and 52% less based on ADCS-ADL scores ($p = 0.03$). Analyses of changes from baseline to endpoint in patients with moderate AD found 61% less disease progression, based on both ADAS-Cog and ADCS-ADL scores, than sham-treated moderate AD patients ($p = 0.05$ for ADAS-Cog, 0.002 for ADCS-ADL).

Though reversal was not an endpoint of this study, data indicate that mild Alzheimer's was reversed at 16 months. See charts I and L above. Early treatment almost always produces superior results in most diseases, but these data suggest strongly that TPE for AD be initiated before onset or as soon as possible once detected.





Not surprisingly, given the results and the urgent need for effective Alzheimer's treatments, interest in the AMBAR study has continued. One group of medical statisticians reexamined the data and concluded that the use of better mathematical methodologies **produces evidence of even greater magnitudes of improvements than those reported in the initial paper.** Their work focused on evaluating endpoints in the most relevant stage of AD. See *Application of a Novel Endpoint Staging Framework: Proof of Concept in the AMBAR Study*, Journal of Alzheimer's Disease, 2024.

Comparison with the Currently Approved Monoclonal Antibody Treatment for Alzheimer's

When compared with the currently approved treatments for early or any stage of Alzheimer's, TPE is much superior in improving or reducing cognitive function and activity of daily living cognitive decline. It has virtually none of the serious adverse effect listed in the table as ARIA (adverse results requiring intervention), otherwise known as bleeding into the brain associated with the approved monoclonal drug treatment. TPE is also less expensive we believe even if just considering co-pays of cost:

Table 1: AD Treatment Pipeline: Efficacy results of mAbs anti-Ab and AMBAR

Effect size comparison across outcomes - % effect size is largest for AMBAR

	Alzheimer's treatment candidates	N	ADCS-ADL Effect Size	ADAS-Cog Effect Size	CDR-Sb Effect Size	ARIAs
mAb anti-Aβ	Aducamumab ENGAGE	1647	18%	11%	-2%	40%
	Aducamumab EMERGE (10mg/kg)	1638	40%	27%	22%	
	Lecanemab (BAN2401)	1795	37%	26%	27%	12%
MULTI-MoA	AMBAR	347	52%	66%	71%	0%

Efficacy comparative Table elaborated by the authors

25-49% Effec. 50-80% Effec.

ADCS-ADL: Alzheimer's Disease Corporative Study-Activities of Daily Living; ADAS-Cog: Alzheimer's Disease Assessment ScaleCognitive subscale; CDR-Sb: Clinical Dementia Rating-Sum of bones

To summarize, AMBAR provided more benefit and less harm from potential side effects.

The End of the AMBAR Trials

Despite strong safety data and evidence that TPE is the more effective treatment for Alzheimer's, reversing and presumably preventing progression and maybe even the occurrence of early stages of the disease, Grifols ended the AMBAR effort to gain regulatory approval for treatment of AD. There are several possible motives behind Grifols decision.

One is simply cost. The price tag for a large Phase-3 trial, a prerequisite for gaining regulatory approval, would be extremely high. On average, the price of Phase-3 trials is more than $20 million. Multiple apheresis procedures per patient, a relatively expensive and complex medical procedure, could have pushed costs up to $100 million or more.

Because of their high cost, Phase-3 trials are usually funded by big pharmaceutical companies with very deep pockets. These companies, however, typically want exclusive patent rights to the biotechnologies they are testing. Because TPE is already an approved and commonly prescribed medical procedure for other indications in most major markets, regulatory approval of TPE for AD would yield uncertain and probably little value.

Another factor may have been that the basic hypothesis that AD is caused by amyloids was under serious attack and continues to be challenged today. Trials targeting amyloids had not proved anyplace near as successful as hoped.

Additionally, Grifols may have been concerned that albumin added to the replacement saline solution in the AMBAR program did not yield benefits in a dose-dependent manner. This called into question the role of albumin, a Grifols' product, in TPE's benefits.

Purified human albumin added to the plasma replacement fluid may assist in the clearance of unhealthy proteins, including amyloid structures, just as does the body's own newly synthesized albumin. Humans have, on average, between 96 to 137 grams of albumin in the bloodstream. The liver replaces albumin at a rate of about 9–12 grams per day in healthy adults so total replacement normally takes about ten days. If half of the plasma volume is removed, albumin replacement takes about five days.

Repeated TPE procedures within a short time period could therefore result in low albumin levels, hypoalbuminemia, which is often well tolerated in patients, including rare cases of analbuminemia, a genetic disorder that results in zero albumin production, where it seems over time their bodies may adjust at least short term to a very low albumen level. This does not mean that albumin plays no beneficial role in TPE.

Clinicians have observed that some patients may experience low blood pressure following multiple TPE procedures in a short period unless they receive albumin. For that reason, albumin is routinely delivered in plasma replacement fluid during TPE treatments.

It's Not What's Added, It's What Is Removed

What is certain, however, is that scientists who had studied the young blood and young plasma phenomena were increasingly focused on the removal of old plasma as the primary source of TPE's health benefits. This changed the way that heterochronic parabiosis was viewed, even in retrospect, and the Conboys' research team began to refer to the procedure as "plasma dilution" rather than "exchange."

Though this phase of the Conboys' research was performed on animals and human cells, the AMBAR trial provided strong indications that TPE produces similar results in human subjects. This conclusion has been reinforced by ongoing human trials specifically investigating the effects of TPE on the aging process.

Before discussing the results of recent human trials, we'll summarize some of the important science regarding the anti-aging impacts of TPE using animal trials.

The Berkeley Animal and Human Cell Trials

Informed by the AMBAR trial, the Conboys and colleagues, including TPE pioneer and Lifespan edge Chief Medical Officer Dobri Kiprov, examined the effects of a single TPE with albumin on mice. The title of the controlled 2020 study, *Rejuvenation of three germ layers tissues by exchanging old blood plasma with saline-albumin*, summarizes its most important findings.

The three germ layers referred to in the title are the first three types of cells that emerge from the embryonic stem cells (ESCs) that form upon fertilization in humans and other mammals. Those ESCs are "pluripotent," meaning they can differentiate to become any of the body's many hundreds of final cell types.

The embryo's first differentiation is the formation of the three foundational cell types, called germ layers. These are "progenitor" cells, the stage of development between embryonic stem cells and the final forms that the cells of the body take.

Once committed to one of these three germ layers, cells are "multipotent," meaning they can form multiple, but not all, cell types. The breakthrough heralded in the title of this paper by the Berkeley researchers is that all three cell types are rejuvenated by TPE. Because all the body's tissues come from these three original progenitor cell types, this set of experiments demonstrated that all the body's cell can be rejuvenated by TPE.

While this discovery is sufficient to impart landmark status to the study, it's not the only ground-breaking finding reported. Another important outcome was proving that TPE in mice provides all or more of the benefits imparted by past young blood experiments.

The researchers wrote that a single TPE procedure, which they call neutral age blood exchange (NBE), meets or exceeds "the rejuvenative effects of enhancing muscle repair, reducing liver adiposity and fibrosis, and increasing hippocampal neurogenesis in old mice, all the key outcomes seen after blood heterochronicity [young blood exchange with old blood]." (Note: hippocampal neurogenesis refers to the growth of new neurons in the brain's memory center, which helps explain the cognitive improvements documented in the AMBAR trial.)

How Does TPE Work?

The paper is also noteworthy for characterizing the mechanism responsible for TPE's cellular rejuvenation. Broadly, the researchers analyzed proteins in the blood of mice and humans, before and after TPE, and observed "a molecular re-setting of the systemic signaling milieu."

This research conclusion deserves special emphasis, starting with the definition of the "systemic signaling milieu." This phrase refers to the fact that the bloodstream carries information in the form of hormones, growth factors, cytokines, neurotransmitters, and other signaling molecules between the many different cells and systems of the body.

The purpose of this signaling milieu, as described in the Nature article, is to "broadly coordinate tissue maintenance and repair and promote immune

responses." The Berkeley team expanded this concept significantly, however, by presenting evidence that excess signaling molecules accumulate in the bloodstream with age.

This accumulation confuses molecular sensing mechanisms, degrading the body's ability to respond to the information carried in the bloodstream. The Berkeley team employs an electronics metaphor, "crosstalk," to describe the injurious impact of excess signaling molecules.

Crosstalk happens when a signal from one channel or circuit gets unintentionally coupled into another. In context of our signaling milieu, we can think of this phenomenon as biological static or interference that confuses our bodies' complex maintenance and repair systems. Those systems include the cardiovascular, respiratory, digestive, nervous, endocrine, immune, musculoskeletal, integumentary, urinary, and others.

Crosstalk immediately degrades many tissues' abilities to respond correctly to molecular signals, but it has broader and more profound impacts, including age-related inflammaging. Muddled signaling degrades the systems that keep various tissues and organs operating optimally. This increases susceptibility to disease.

The hypothesis is that TPE helps restore signaling by removing crosstalk; that restoration of more normal signally of the many regulatory systems lets them work in an integrated manner to restore and maintain the health of the body.

Rejuvenating Stem Cell Function with TPE

The most important problem caused by signaling crosstalk, according to the Berkeley team's analysis, is "inhibition of progenitor cell proliferation." Unfortunately, this concept may be confusing for those who are not well-versed in this area, so we will discuss some important concepts that you may already know—we apologize for being verbose if that is the case.

Let's begin at the beginning, when fertilization sparks formation of embryonic stem cells (ESCs) that form the embryo. ESCs are pluripotent, meaning they have

the potential to become any cell in the body. Moreover, they have an unlimited ability to make copies of themselves because they can regrow telomeres whenever they divide. Adult cells, on the other hand, have a limited telomeres. When they're used up by repeated cell divisions, stem cells cannot replace themselves or further repair tissues.

The first major change that the embryo goes through is the formation of the three germ layers. At that point, ESCs transform into the three primary cell types that make up our bodies. (The ectoderm cells become skin, nervous system, hair, nails. The mesoderm becomes muscle, bone, blood, heart, kidneys. The endoderm becomes the lining of digestive and respiratory tracts, liver, pancreas.)

What Are Progenitor/Stem Cells?

Initially, all the cells of the three germ layers' are progenitor cells, sometimes called progenitor/stem cells. The word "progenitor" comes from the Latin term for ancestor, which is a good way to look at these cells. Progenitor cells are the intermediate step between embryonic stem cells and their fully differentiated final forms which make up most of your body.

Unlike ESCs, progenitor cells do not constantly regenerate telomeres so their ability to create new cells is not infinite. To preserve their ability to survive and replicate, progenitors utilize a kind of trick to avoid using up their telomeres.

What Is Propagation?

Instead of dividing to create all the new cells needed to repair damage, they create other progenitors called daughter cells that divide into many copies. This spares the original progenitors' telomeres for future proliferation and tissue regeneration. This process of creating daughter cells to make additional cells is called propagation.

Adults maintain supplies of these important progenitor cells in many different parts of the body. These progenitor reserves play a critically important role in your health because they can be called into action when your tissues are damaged.

When cellular injuries are otherwise unrepairable, progenitors are activated to supply replacement cells. Later in life, they can replace cells that have reached the limits of lifespan due to aging.

Not surprisingly, tissue regeneration by progenitor cell propagation is significantly more efficient in younger people. In older people, progenitor cell dysfunction increases significantly, which results in diminished cellular repair and replacement. Eventually, it leads to cell senescence and death.

This phenomenon has frequently been attributed to inflammation, also known as inflammaging because it accelerates aging. The cause of inflammaging, however, has never been definitively determined. The Berkeley group's contribution to this research was finding evidence to support the hypothesis that the accelerated aging associated with inflammaging is caused, in large part, by progenitor cell dysfunction due to signaling crosstalk.

The consequences of this insight are far-reaching. The inhibition of progenitor cell promulgation prevents adequate healing of tissues in need of repair. Worse, unrepaired tissues continue to generate signals that provoke additional progenitor proliferation, which expends those cells' telomeres and reduces their ability to propagate in the future.

As a result of this vicious circle, regeneration is degraded by signaling crosstalk. Critically, however, the Berkeley team also presented evidence from animals and now humans that removing excess signaling molecules by TPE rescues progenitor cell proliferation.

Examining the Role of Albumin

Having established that TPE likely rejuvenates progenitor cell proliferation, the researchers turned to the task of explaining how it happens. Having eliminated young blood and young plasma as the sources of TPE's benefit, they logically considered the role of albumin, which has been included in transfusions, including TPE, since the 1940s. See Cohn et al., 1944.

The Berkeley researchers first tested different doses of albumin on myoblasts, the progenitor cells that become different muscle cell types, including heart and skeletal. Muscle cells that had gone through TPE as well as those that had not gone through TPE were tested. Only those that had previously undergone TPE demonstrated superior myoblast proliferation.

They then exposed both TPE-treated and TPE-untreated cells to human albumin (HSA), producing no effects on progenitor proliferation in either cell type. This led the authors to conclude that TPE's positive effects on adult myogenesis [muscle cell growth] are not caused by albumin. **This reinforced the view that TPE's impact on health is attributable to what it removes from blood.**

Because of evidence in animals and humans that TPE rejuvenates the hippocampus by proliferation of neural cells, they also examined the effects of albumin on neural progenitor cells (NPCs). The hippocampus is essential for maintaining cognitive functions such as memory formation, spatial navigation, learning, emotional regulation, and stress response.

In younger animals, including humans, older hippocampal neural cells are regularly replaced by progenitor cells. During the 40s and 50s, however, hippocampal cell replacement wanes, leading to an average annual loss of about 0.5% to 1% of the organ's volume. This shrinkage is associated with age-related memory loss and, in the worst case, dementia.

They then tested the effects of albumin on neural progenitors and, paradoxically, found that albumin did, in fact, have a positive effect on NPC proliferation in cell cultures. Critical, however, the journal article notes that albumin does not cross the Blood Brain Barrier (BBB) in healthy individuals and is not, therefore, a direct contributor to hippocampal regeneration.

In fact, albumin in the brain is an indicator of a leaky BBB which is associated with serious health problems, including dementia. Direct infusion of albumin into the brain causes neuro-inflammation and neuronal dysfunctions. The authors

emphasize that they were careful not to increase total albumin levels but only to replenish those diminished by NBE/TPE.

Though the study minimizes albumin's contribution to TPE's health benefits, the authors state that it might be beneficial to immune and other functions when multiple plasma dilutions are performed.

Genetic Engineering Via TPE

To summarize, excess signaling molecules create crosstalk that blocks critical stem cell functions needed to fight senescence and aging. Worse, unrepaired damage may provoke stem cells to compensate by dividing to create additional progenitor cells, depleting their limited ability to replicate.

The solution, they posit, is TPE to reboot the signaling milieu, allowing progenitor cells to multiply and heal damaged and aged tissues and regulatory systems. The paper presents evidence that this occurs by comparing the serum proteomics (proteins in the bloodstream) in animals and humans before and after TPE.

TPE does not simply reduce the levels of signaling proteins, which would be expected. Rather, it lowers some protein levels but increases those that coordinate tissue repair and promote immune responses. In other words, the reduction in bad signals allows an increase in good signals. According to the authors, this results in "changes in gene expression" that "have long-lasting molecular and functional effects that are consistent with our observations."

This is, incidentally, consistent with anecdotal evidence from participants in the AMBAR TPE trials who demonstrated continuing improvements in various physical conditions well after treatments. Ongoing human trials have also produced evidence of enduring epigenetic changes.

The authors characterized their own results in the following way. **"This work shifts the paradigm of blood heterochronicity away from dominance of young blood factors and establishes that replacement of a large volume of old blood**

with a neutral age physiological fluid (saline supplemented with 5% purified albumin), is sufficient for most if not all observed positive effects on muscle, brain, and liver. Importantly, it shows that a currently approved FDA procedure promotes molecular and functional rejuvenation of the blood in older people, with improved proteomic profile and support for myogenic responses."

The authors do not exclude the possibility that factors in young blood or plasma might also contribute to the rejuvenative effects of heterochronic parabiosis. They conclude, however, that larger benefits can be explained by what is removed from older people's bloodstreams.

Reducing Biological Age with TPE

The team led by Irina and Michael Conboy of UC Berkeley have continued to do breakthrough research into the mechanisms and benefits of TPE. In 2021, they published results of animals trial in GeroScience, *Plasma dilution improves cognition and attenuates neuroinflammation in old mice*.

The opening sentences of the abstract are, "Our recent study has established that young blood factors are not causal, nor necessary, for the systemic rejuvenation of mammalian tissues. Instead, a procedure referred to as neutral blood exchange (NBE) that resets signaling milieu to a pro-regenerative state through dilution of old plasma, enhanced the health and repair of the muscle and liver, and promoted better hippocampal neurogenesis in 2-year-old mice (Mehdipour et al., Aging 12:8790–8819, 2020). Here we expand the rejuvenative phenotypes of NBE, focusing on the brain. Namely, our results demonstrate that old mice perform much better in novel object and novel texture (whisker discrimination) tests after a single NBE, which is accompanied by reduced neuroinflammation (less-activated CD68+ microglia)."

The team also presents evidence against dilution of the senescence-associated secretory phenotype (SASP), the target of senolytic drugs, may not be a major mechanism behind TPE's benefits. The evidence supporting this conclusion is that

the senolytic drug ABT 263, unlike TPE, had limited effects on neuroinflammation and did not enhance hippocampal neurogenesis in the old mice."

Expanding the Berkeley TPE Research into Human Age Reversal

In 2022, the Conboys and colleagues expanded their analysis to demonstrate that TPE provides similar benefits to humans as well as mice. Published in GeroScience, *Old plasma dilution reduces human biological age: a clinical study* compares markers of biological age in humans and mice before and after TPE.

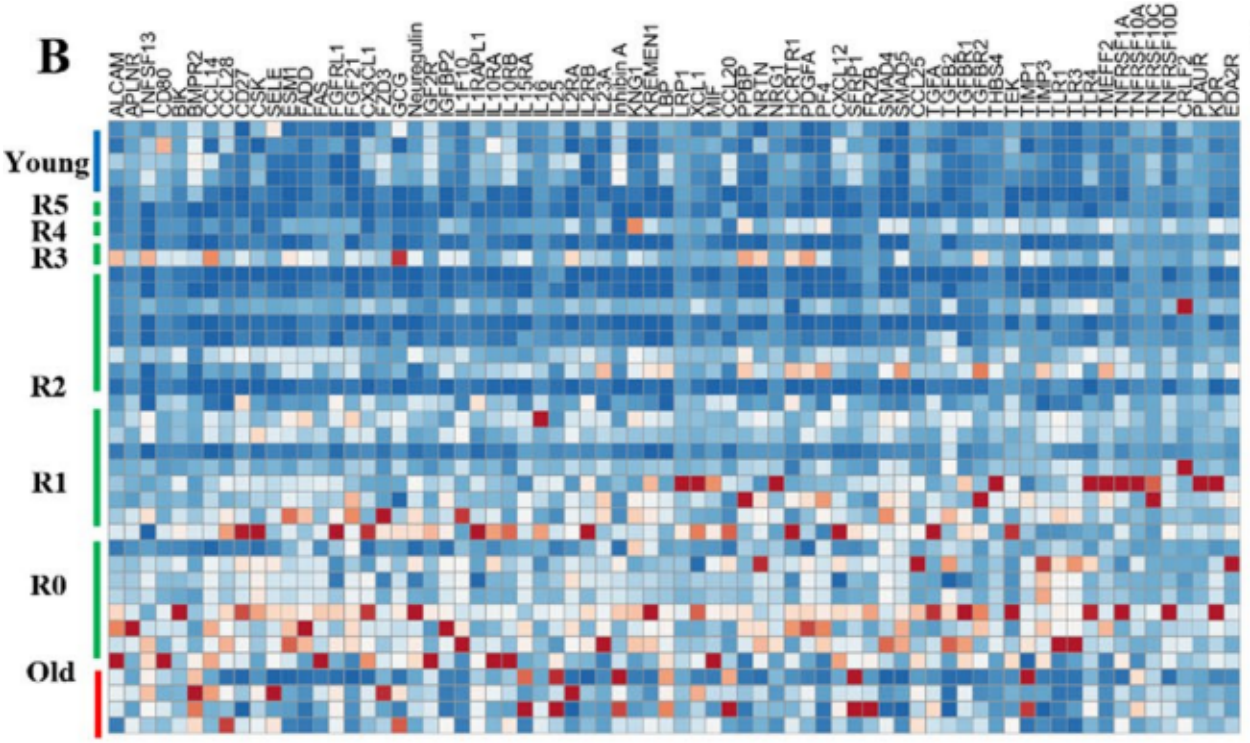
The abstract succinctly summarizes its conclusions. "This work extrapolates to humans the previous animal studies on blood heterochronicity and establishes a novel direct measurement of biological age. Our results support the hypothesis that, similar to mice, human aging is driven by age-imposed systemic molecular excess, the attenuation of which reverses biological age, defined in our work as a deregulation (noise) of 10 novel protein biomarkers. The results on biological age are strongly supported by the data, which demonstrates that rounds of therapeutic plasma exchange (TPE) promote a global shift to a younger systemic proteome, including youthfully restored pro-regenerative, anticancer, and apoptotic regulators and a youthful profile of myeloid/lymphoid markers in circulating cells, which have reduced cellular senescence and lower DNA damage."

Genetic analysis was performed in mice and human participants in pilot studies, before and after multiple TPE procedures. They wrote, "The results demonstrate significant and lasting rejuvenation of both humoral and cellular blood compartments in people who underwent repeated plasmapheresis. **The rejuvenative changes are not limited to a reduction of inflammaging but encompass diminished circulatory protein markers of neurodegeneration and cancer, as well as reduced senescence, lower DNA damage, and improved myeloid/lymphoid homeostasis. Mechanistically, these and previously reported positive effects of TPE become better understood through longitudinal comparative proteomics of the blood plasma, demonstrating a youthful recalibration of the canonical signaling pathways, broadly regulating tissue health … ."**

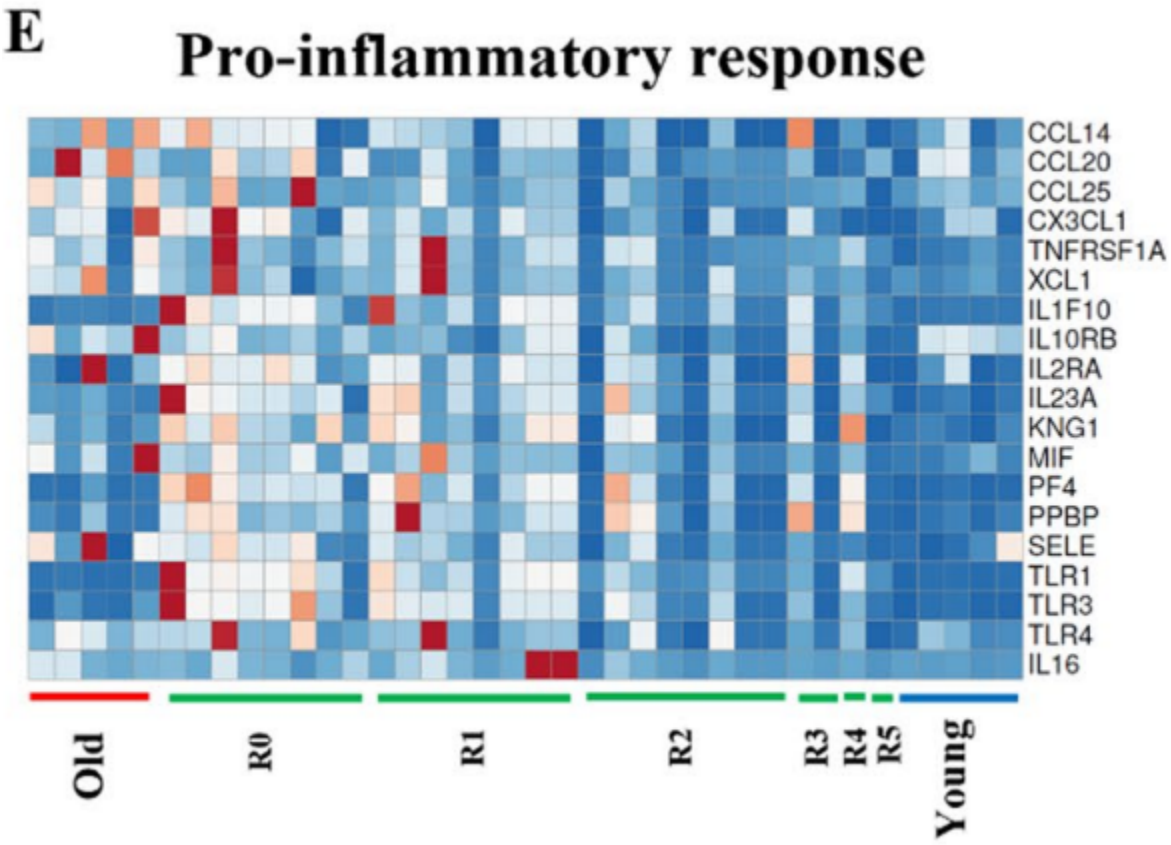
We can view the impacts of TPE using a gene expression heatmap, which shows changes in the level of gene expression for multiple genes associated with age and aging. Specific gene expression may increase or decrease in old subjects.

The following heatmap presents expression levels of 72 proteins that are significantly different in young and old humans and animals. The bottom row shows gene expression in old individuals, with red and orange representing high levels of expression and white and blue being lower. The top row shows gene expression in young individuals.

The R0 row represents subjects' gene expression before TPE. R1 represents gene expression following a single TPE procedure. R2 represents gene expression after two procedures, etc. This heatmap clearly shows the gene expression of old individuals increasingly resembling young gene expression.



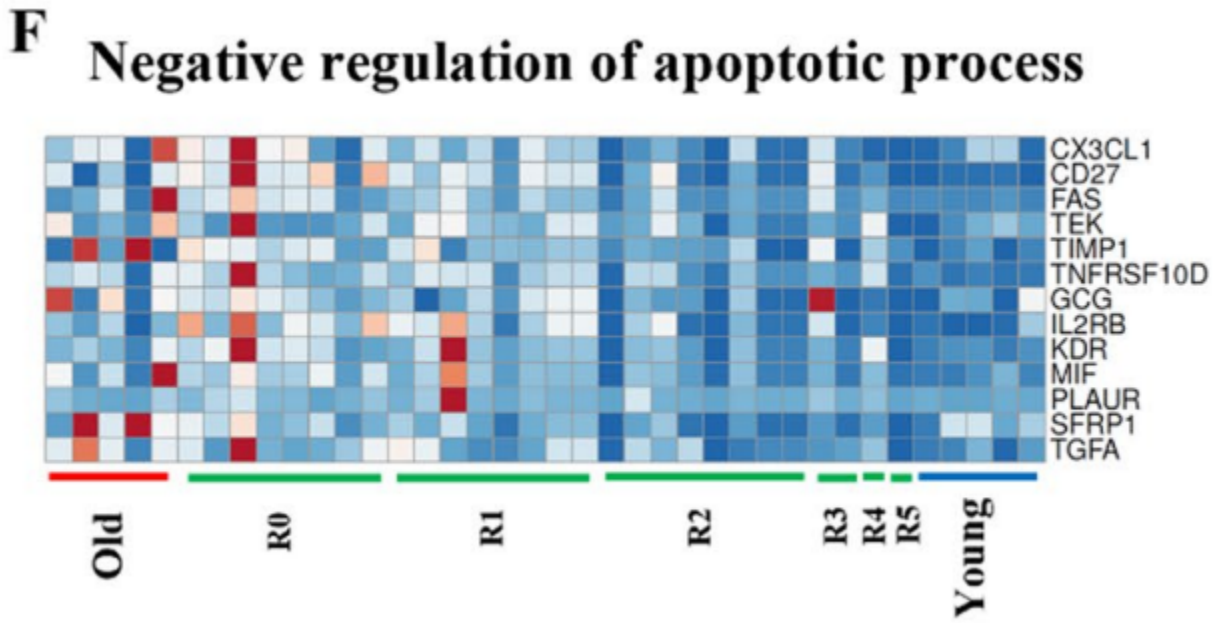
The following heatmap focuses on genes that control or reflect the pro-inflammatory response, which correlates to immune function. Once again, we see that old gene expression becomes increasingly young following multiple TPE procedures.



Another heatmap that reflects the state of immune system health is apoptosis protein levels. Apoptosis is natural programmed cell death, which becomes increasingly dysregulated with age.

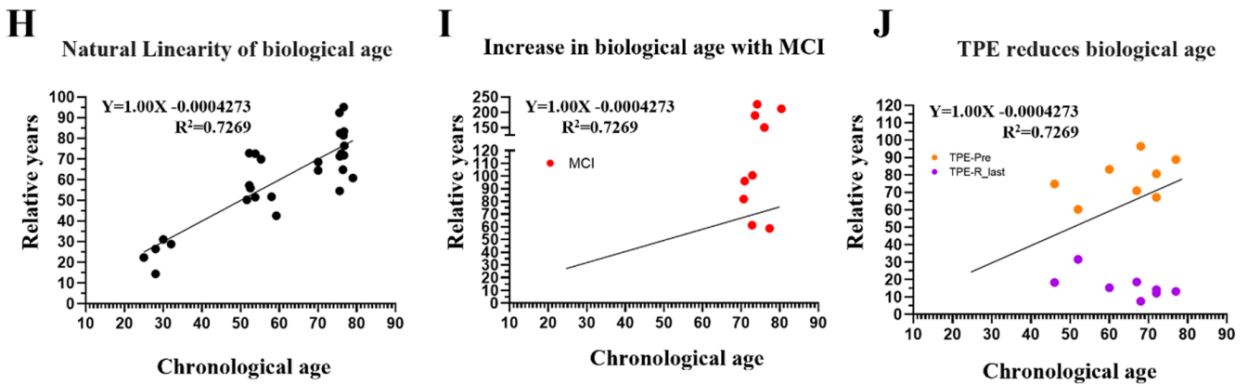
As the authors explain, “Apoptosis plays an important role in immune responses and tissue homeostasis. However, with advancing age, resistance to apoptosis is increased through an enhanced negative loop of anti-apoptotic signaling, leading to senescence, inflammation, fibrosis, and a propensity for cancer.”

The following heatmap shows the de-aging of the apoptotic process as gene expression following multiple TPE procedures increasingly resembles youthful gene expression.

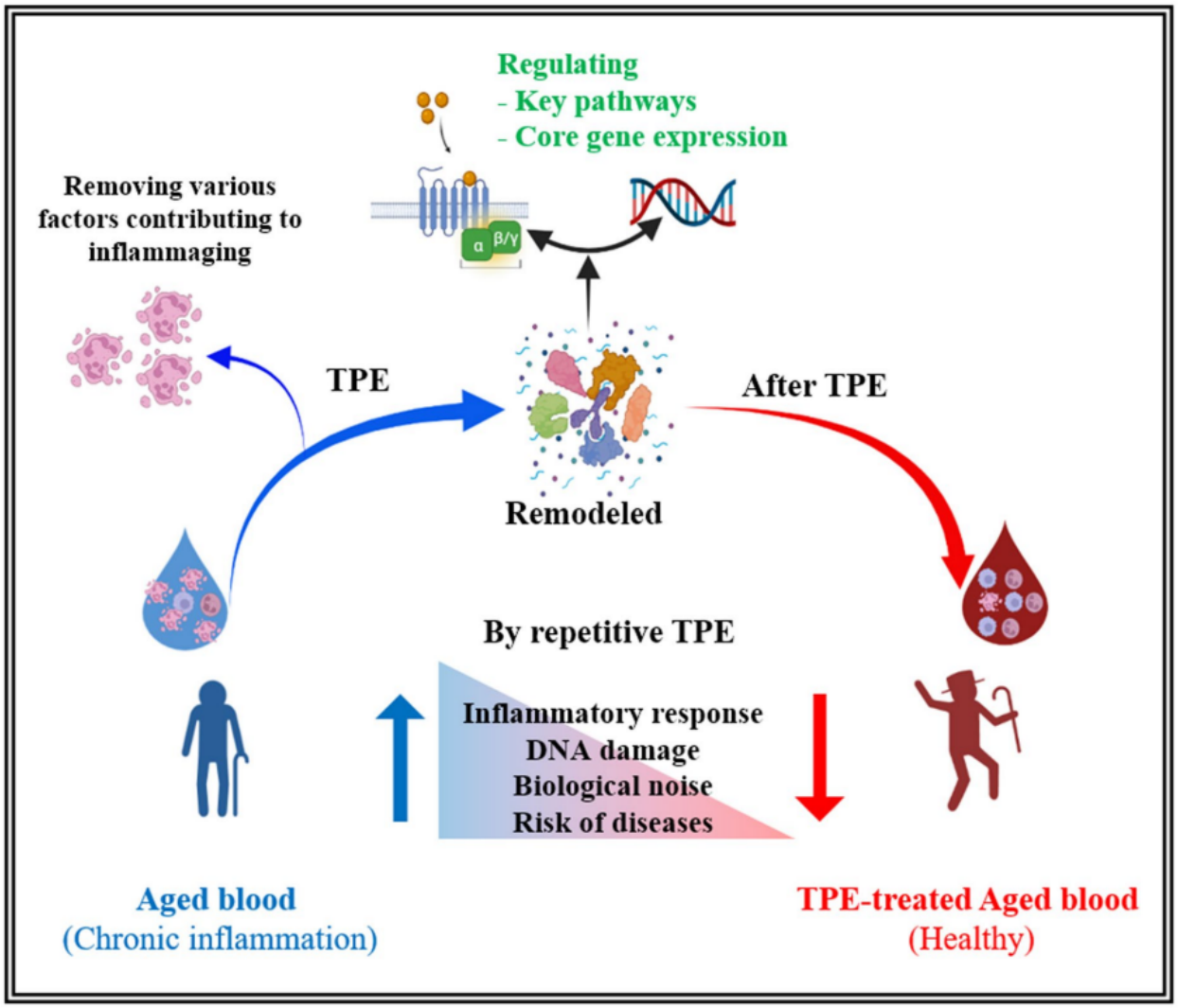


Though this paper contains far more information, we'll include here just one more group of charts comparing actual chronological age to biological age based on analysis of proteins.

The first graph H demonstrates the correlation between chronological age and biological age in people who have not had TPE. The second graph I shows the biological age, based on protein analysis, of people with mild cognitive impairment (MCI). Note that proteins in MCI patients are so dysregulated that biological ages of some patients had to extrapolated into impossible ages. The final chart J shows the extent of age reduction based on protein profiles of patients before TPE (in orange) and after the last TPE procedure (in lavender). Note that the biological age of many of those who have undergone multiple TPE procedures matches those of teenagers.



The following graphic from the same study summarizes the impact of TPE.



Ongoing Human TPE Trials

A key participant in the AMBAR trial as well as the Berkeley research, Dobri Kiprov, (who serves as the Chief Medical Officer of Lifespan Edge) has launched an additional human TPE trial in conjunction with the prominent Buck Institute for Aging Research.

In 2023, preliminary results were published in Annals of Clinical and Medical Case Reports, titled *A Paradigm Shift in the Utilization of Therapeutic Plasmapheresis in Clinical Practice*. The complete study is currently available as a preprint titled *Multi-omics Analysis Reveals Biomarkers that Contribute to Biological Age Rejuvenation in Response to Therapeutic Plasma Exchange*.

This trial collected epigenetic data from treated subjects and calculated DNA methylation age using 35 different epigenetic clocks. According to these measures, all participants experienced reductions in biological age, with the largest reduction in those who had TPE with immunoglobulin, which improves immune response.

Those who had six monthly TPE with IVIG treatment displayed the largest reduction in biological age of 2.61 years, but this may not reflect the long-term benefits of restored rejuvenation documented by the Conboy's Berkeley team. In fact, biological age reduction based on epigenetic analysis was significantly less dramatic than that shown by proteomic analysis, which also supports the thesis that long-term healing by repair of progenitor propagation and immune function is understated by immediate changes in DNA methylation, measured by epigenetic clocks. Moreover, no long-term trials of epigenetic clocks support their predictive accuracy.

TPE Summarized

Therapeutic plasma exchange (TPE), the replacement of part of a patient's blood plasma with saline and albumin, is used to treat a large and growing number of immunological, neurological, connective tissue, hematological, nephrological,

endocrinological and metabolic disorders. Though clinical trials of plasma exchange for rare disorders are extremely difficult and costly, a growing body of evidence supports the treatment of individual indications.

In the medical community, it is well established that patients with autoimmune and chronic inflammatory conditions require removal of malfunctioning immune system factors in blood plasma. Decades of work by immunologists with specific chronic inflammatory disorders such as chronic inflammatory demyelinating polyneuropathy (CIDP), a chronic inflammatory disease of the peripheral nervous system (PNS), and acute inflammatory demyelinating polyneuritis, the most common presentation of Guillain–Barré syndrome (GBS), demonstrate a dramatic relief of chronic inflammatory conditions as a result of removal or reduction of disease-causing plasma. Significant evidence also supports the successful treatment long Covid. Recently, however, the focus of biogerontologists has shifted to the use of TPE to prevent chronic diseases of aging and extend health spans.

Therapeutic Plasma Exchange has been used for almost 75 years to treat diseases by removing excess proteins from the bloodstream. Finally, in the 21st century, scientists began to take seriously widespread reports of remarkable improvements in the general health of patients prescribed TPE for critical life-threatening diseases. Now, a growing body of scientific evidence has emerged supporting TPE's power not just to treat the diseases of aging, including Alzheimer's, but to slow and reverse aging itself.

Lifespan Edge was founded to expand the availability of this breakthrough biotherapy by reducing costs, training physicians and clinicians, and gathering data through clinical trials and studies to understand how to optimize TPE's benefits. We are motivated not only by our commitment to improving the quality of life for all aging people but also to help solve the crippling financial costs imposed by a growing older population.

Family and government budgets alike are straining under the growing costs of aging, but we are convinced that the price of TPE will yield enormous returns by delaying or preventing the extraordinarily high price of age-related illness. For example, the total cost of care for an Alzheimer's patient, based on Alzheimer's

Association estimates, is about $400,000. Most of that cost falls on families through out-of-pocket expenses like nursing homes and unpaid caregiving.

No price tag can measure the value of improving the quality of life for those who are aging, along with their families and friends. You can contribute to this mission by participating in our TPE program, either for yourself or loved ones.

If you would like to talk to a Lifespan Edge representative, click here and leave your name and contact information and a brief description of what we can help you with. We look forward to serving you.