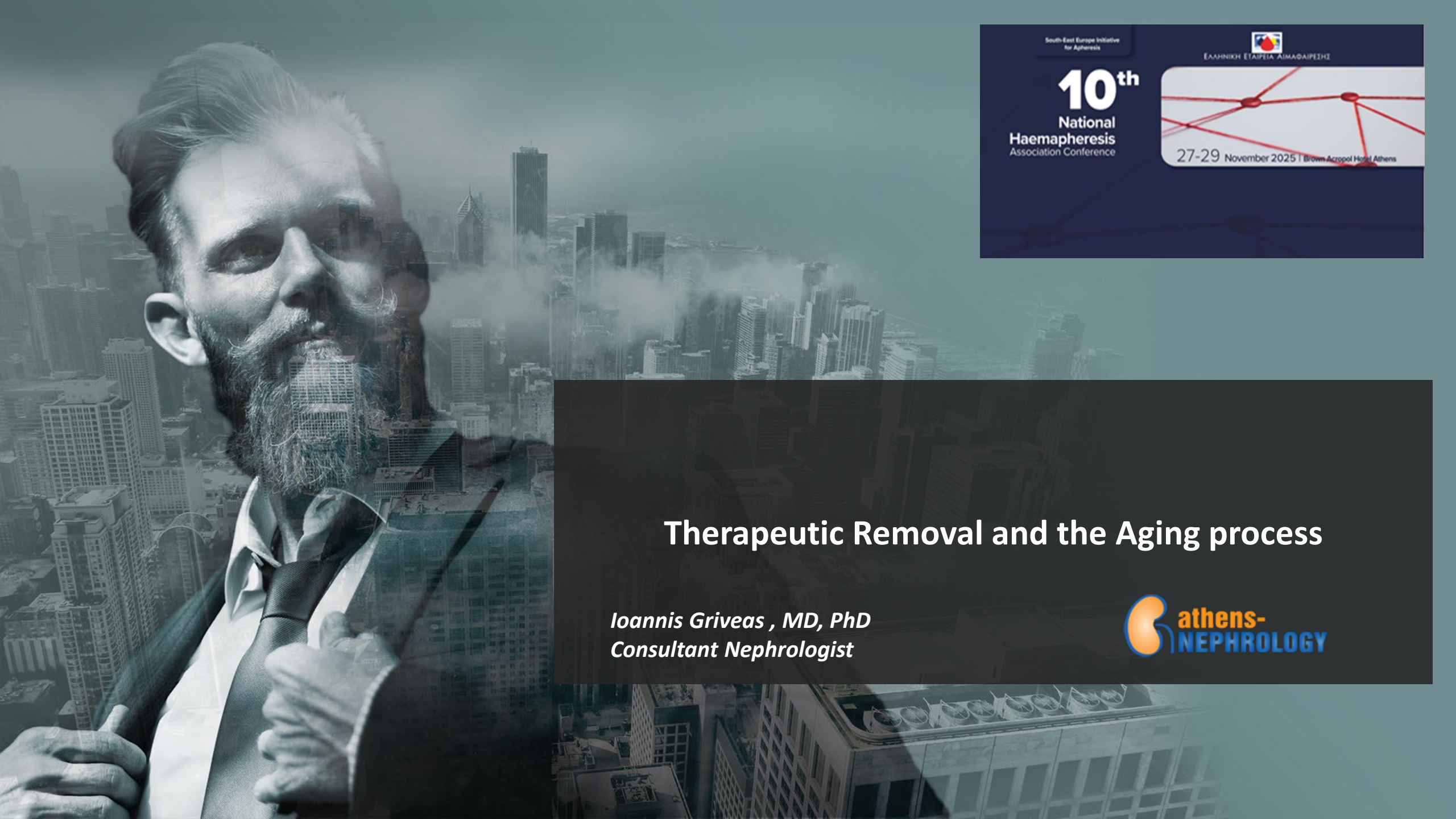




Therapeutic Removal and the Aging process

*Ioannis Griveas , MD, PhD
Consultant Nephrologist*



A man in a dark suit and light shirt is smiling and looking to his right. His face is partially obscured by a dark, stylized overlay of a city skyline, with skyscrapers appearing to grow out of or replace the right side of his head and shoulders. The background is a hazy, high-angle view of a city with many skyscrapers, including the Empire State Building prominently in the center. The overall color palette is muted, with greys, blues, and the dark tones of the man's suit and the city overlay.

In studies on aging, the primary objective is to improve the quality of life, not necessarily with the goal of extending life for a much longer period, but by combating disease.

Therapeutic removal and aging process are linked through strategies that first remove "bad" components like senescent cells and inflammation, and then replenish damaged tissue with new cells, such as through stem cell therapy or young blood components.

This "remove and replace" approach aims to combat age-related decline by clearing out cellular damage and regenerating tissues to improve healthspan and, potentially, lifespan.

Cold Spring Harb Perspect Med. 2016 Mar;6(3):a025908.
doi: 10.1101/cshperspect.a025908
Translating the Science of Aging into Therapeutic Interventions
James L Kirkland



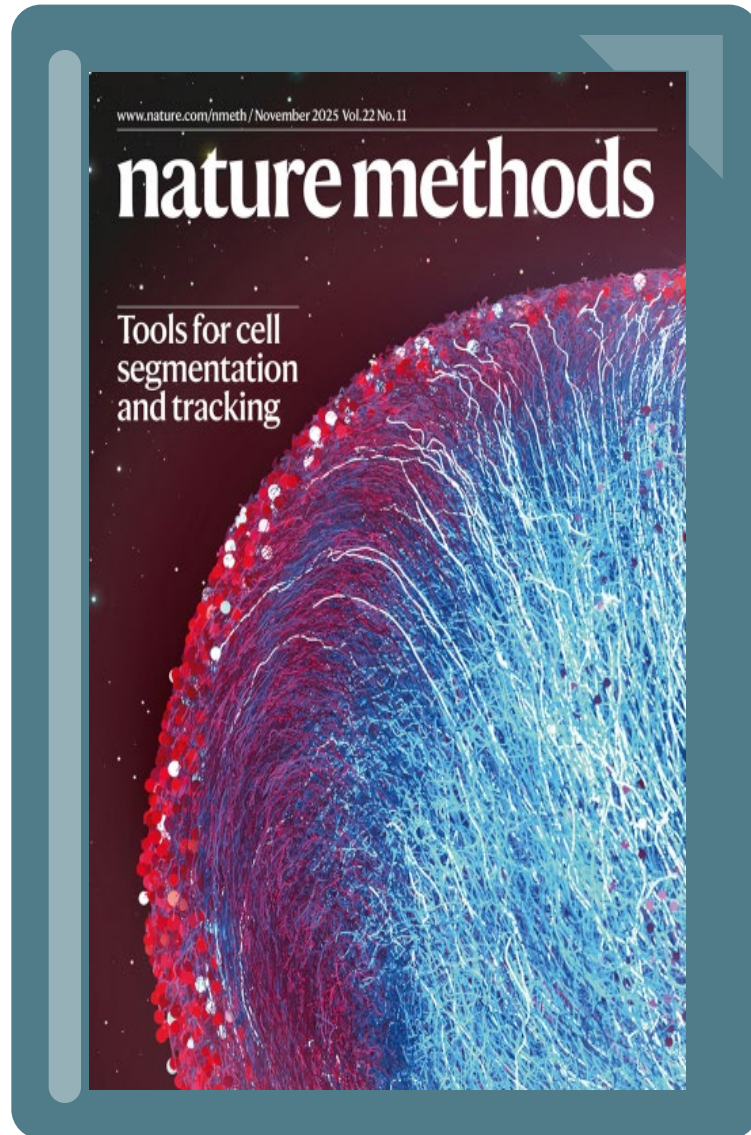


Step 1: Therapeutic removal of aged or damaged components

Senolytics: Therapies that specifically target and remove senescent (aged and non-dividing) cells, which accumulate with age and cause inflammation.

Plasma exchange: Aims to remove harmful molecules from the blood by replacing it with saline or albumin, which can bind to and help remove toxins. Studies in mice have shown this can improve markers of aging, particularly in the brain, liver, and muscle.

Targeting inflammation: Addressing chronic inflammation, known as inflammaging, which is a key driver of age-related diseases.



Step 2: Replenishing with new, healthy cells and factors

Stem cell therapy: Using stem cells to repair or replace damaged tissues. This can involve transplanting stem cells or using therapies that restore the function of endogenous progenitor cells.

Young blood factors: Identifying and administering specific molecules found in young blood that have anti-aging effects, such as oxytocin, TIMP2, and osteocalcin.

Extracellular vesicles (EVs): Using extracellular vesicles from young blood, which contain beneficial factors like growth factors and proteins, to enhance tissue function and promote regeneration.



01

Reversing genome damage caused by aging

02

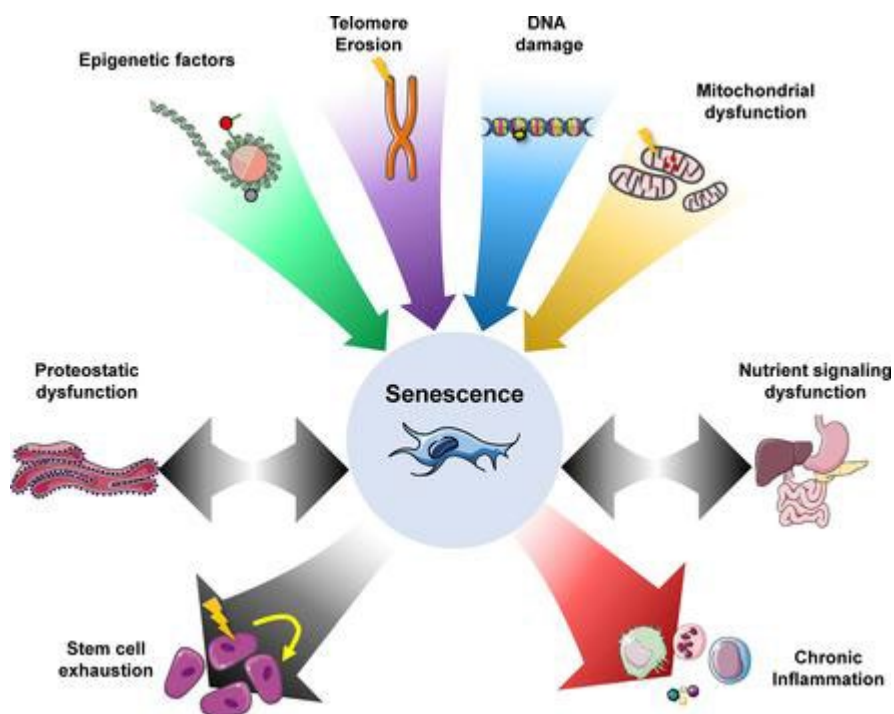
Diet, and environmental factors-calory restriction

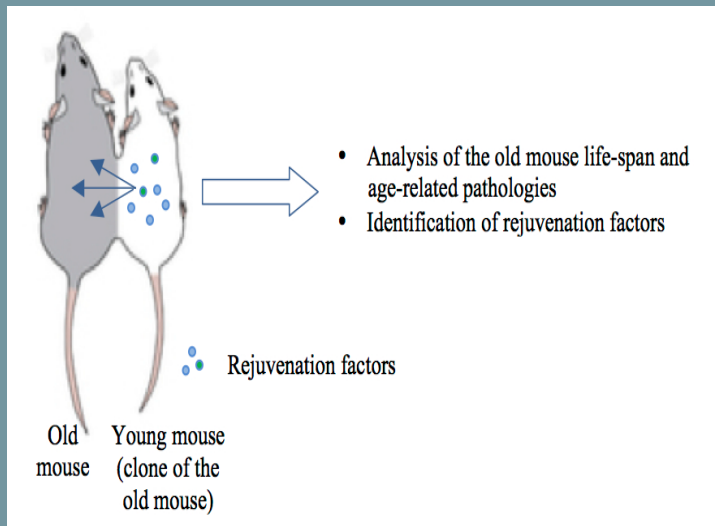
03

researching new tissue and blood-microbiota transplantation

04

Reprogramming senescent cells-hormone replacing therapy





The general hypothesis is that young molecules help repair damage and detoxify old mice.

Using parabiosis with blood exchange between old and young mice that new brain cells form in the hippocampus of young mice with young blood, but when young mice received old blood, brain cell formation slows demonstrating that old blood in mice contains substances that can cause health decline



ARTICLE

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OPEN

A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood

Justin Rebo^{1,*}, Melod Mehdipour^{1,*}, Ranveer Gathwala¹, Keith Causey², Yan Liu¹, Michael J. Conboy¹ & Irina M. Conboy¹

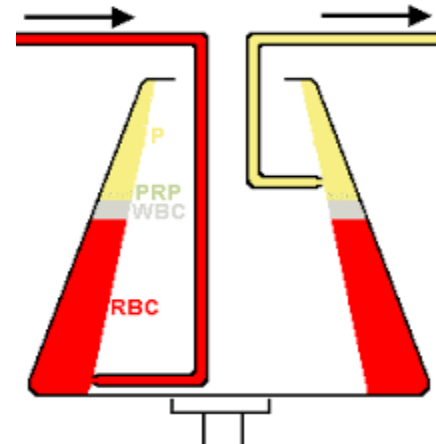
complex (7). Old mice were better able to recover from muscle tissue injury when given young blood, but the young blood did not improve neuron regeneration in the old mice. Neuron and liver cell



regeneration in the old mice. Neuron and liver cell regeneration were inhibited in young mice that received blood from elderly animals implying old blood contains substances that cause health decline. They go on to state that identifying the



decline. They go on to state that identifying the substances and figuring out ways to remove them from old blood may be a more successful approach to thwarting the aging process than a dose of young blood (7).



Editorial

Aging, Disease, and Therapeutic Apheresis



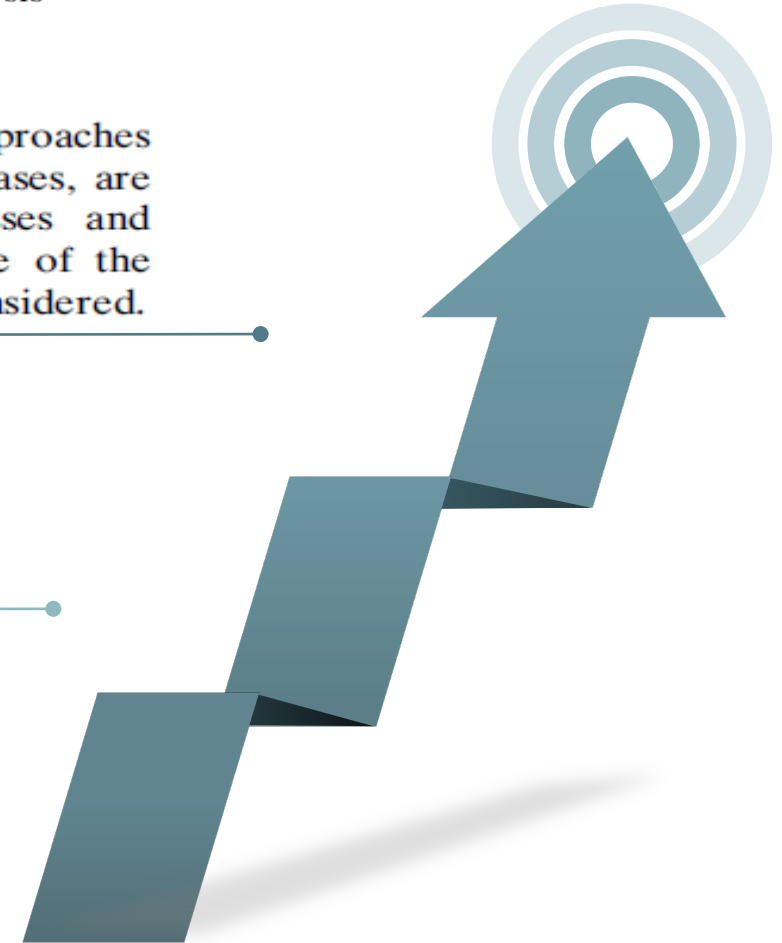
can spill over into the plasma. As one approaches the questions on aging and causes of diseases, are there ways to slow down these processes and improve the quality of life? The example of the treatment of Alzheimer's disease can be considered.



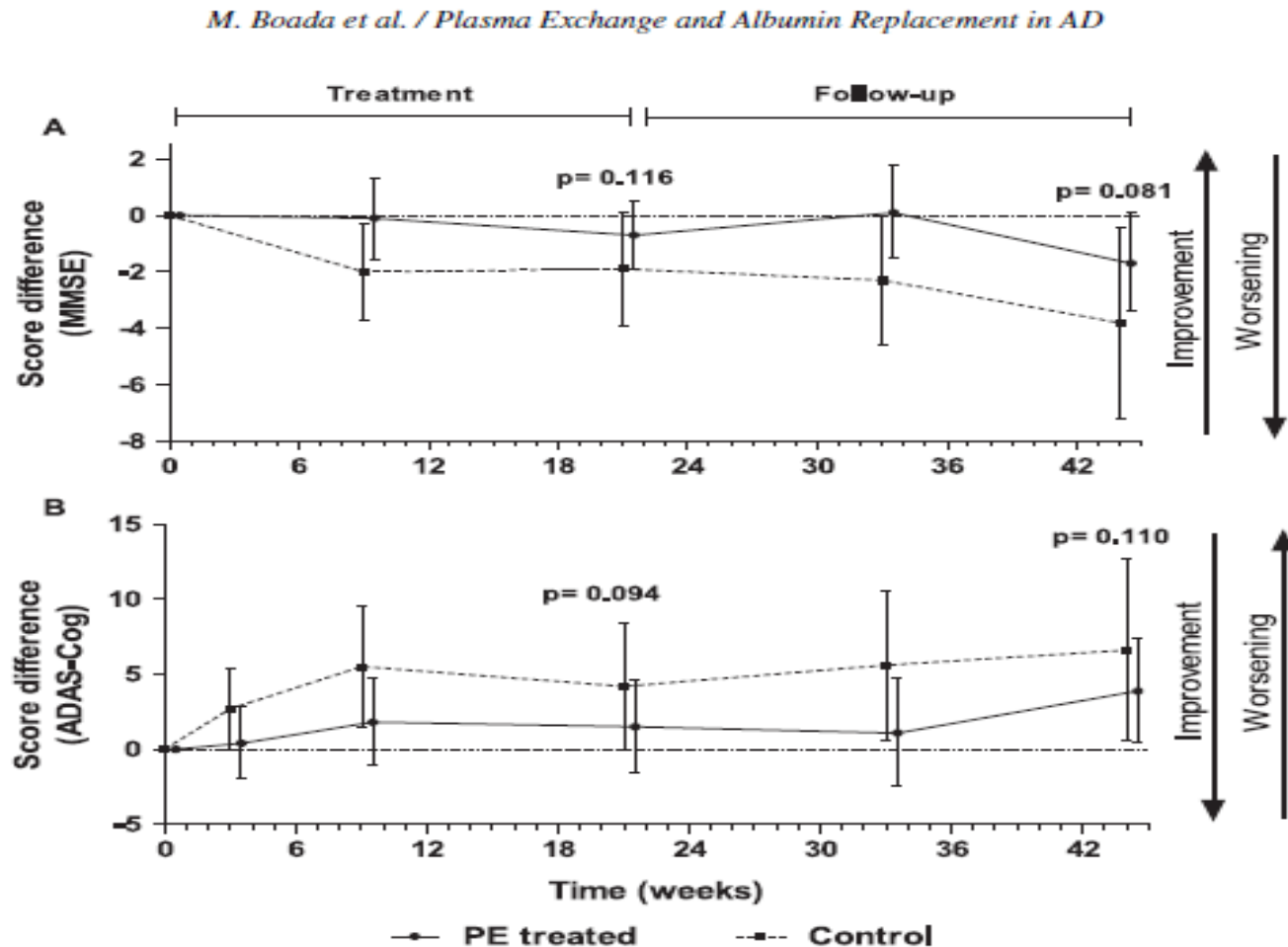
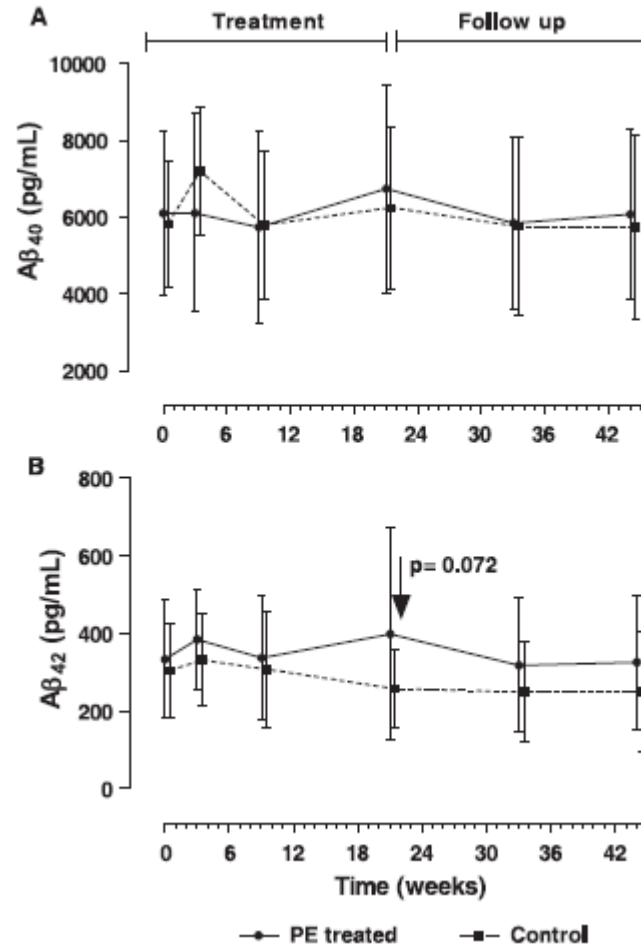
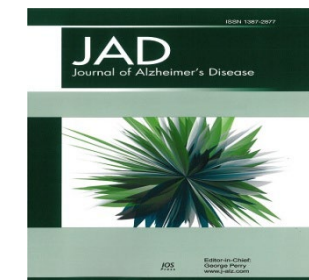
aggregation, and accumulation (8). Many diseases progress through protein aggregation (Parkinson's, Alzheimer's, Huntington's, ALS and others). Human cells intentionally collect aggregates to prevent other cellular damage (8) and these aggregates can spill over into the plasma. As one approaches



prevalent with aging. As noted, senescent cells accumulate abnormal proteins that impair cellular function. With age, protein accumulation results in impaired protein degradation as misfolding. Misfolding of proteins leads to their non-function, aggregation, and accumulation (8). Many diseases



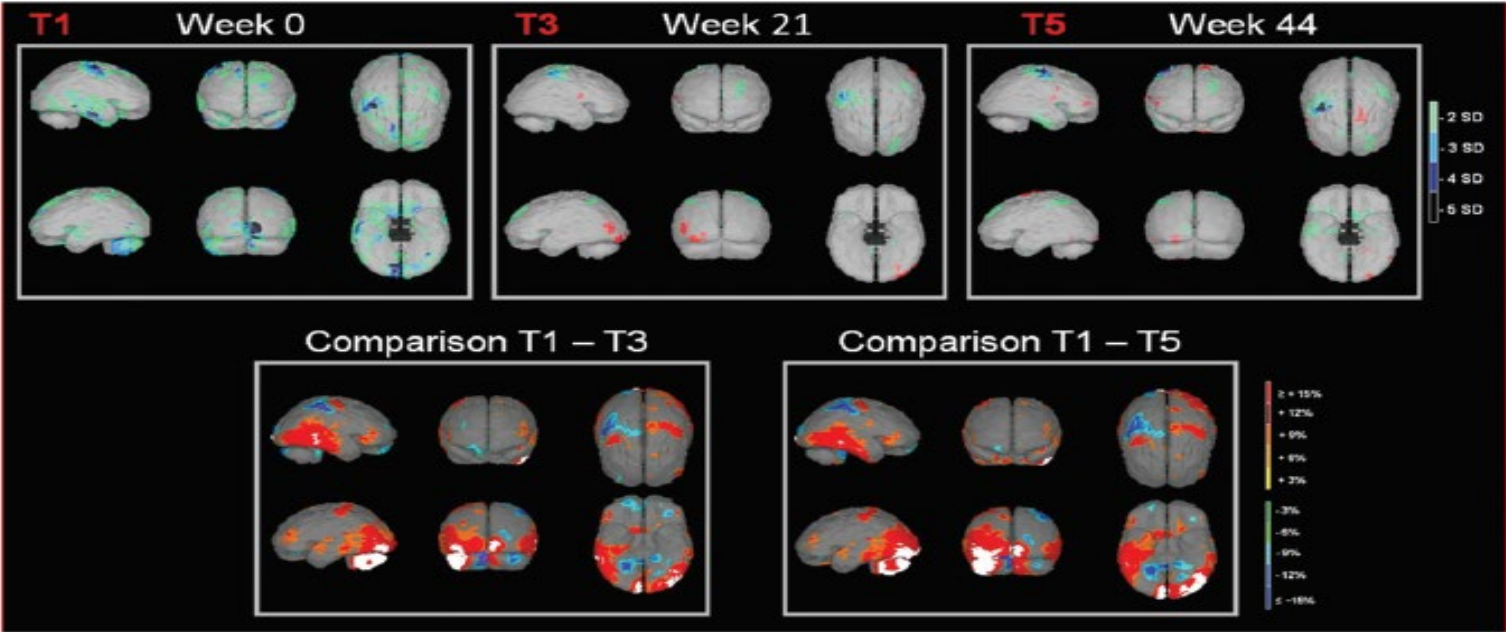
Efficacy and Safety of Plasma Exchange with 5% Albumin to Modify Cerebrospinal Fluid and Plasma Amyloid- β Concentrations and Cognition Outcomes in Alzheimer's Disease Patients: A Multicenter, Randomized, Controlled Clinical Trial



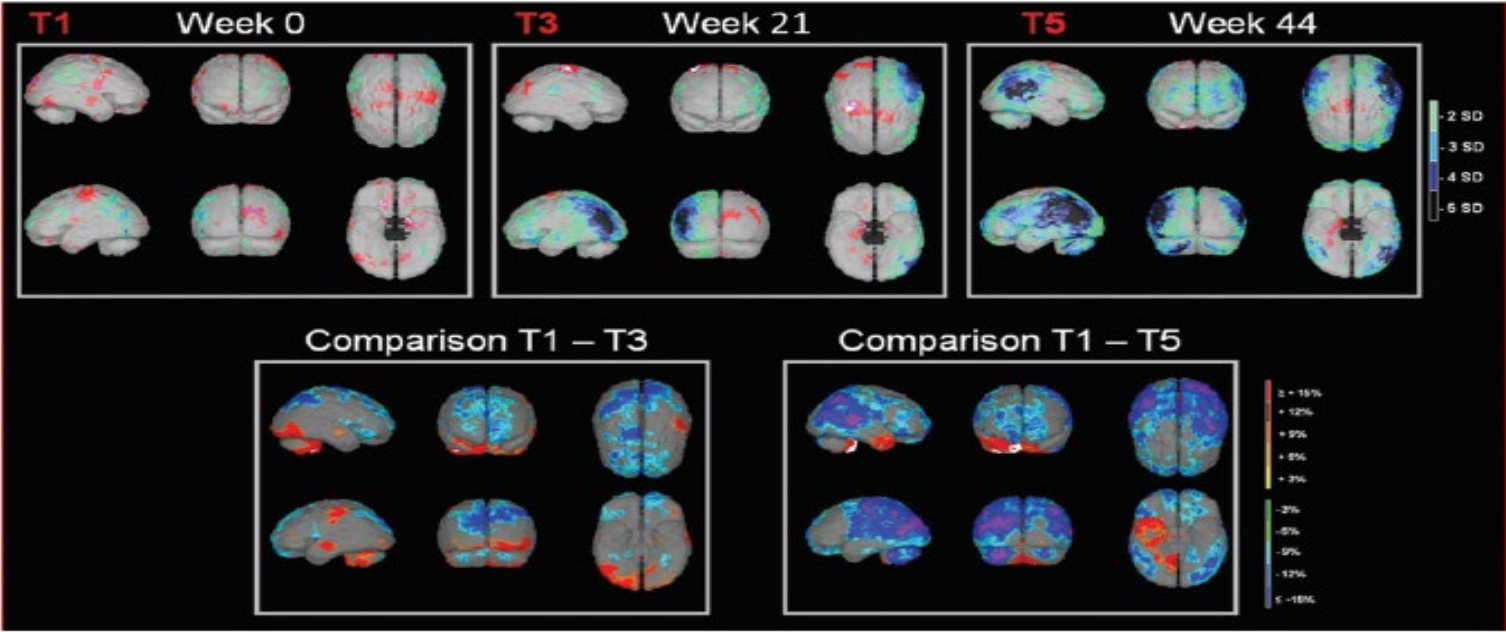
Longitudinal Neuroimaging Analysis
in Mild-Moderate Alzheimer's Disease
Patients Treated with Plasma Exchange
with 5% Human Albumin

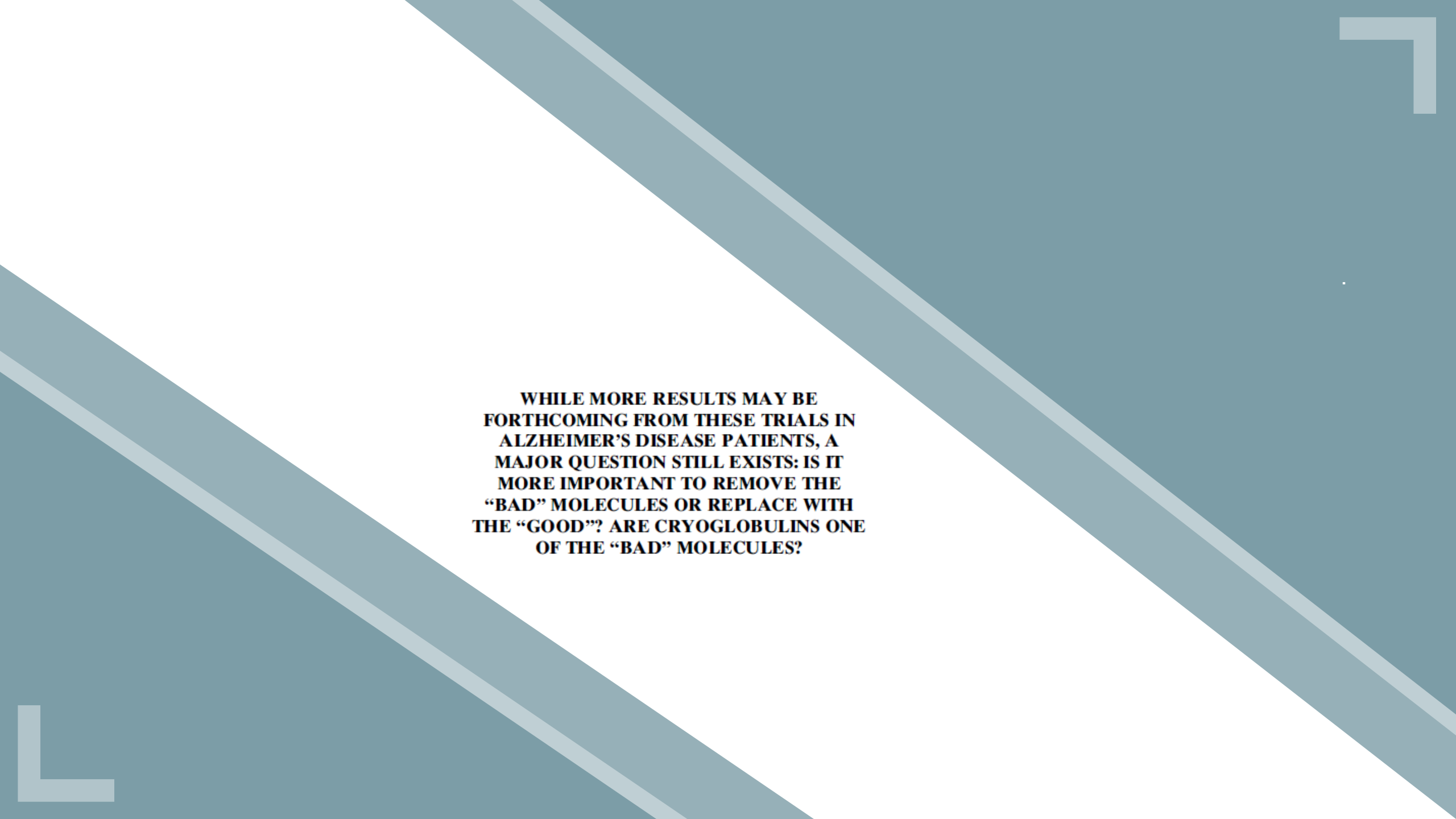


PE-treated



Control





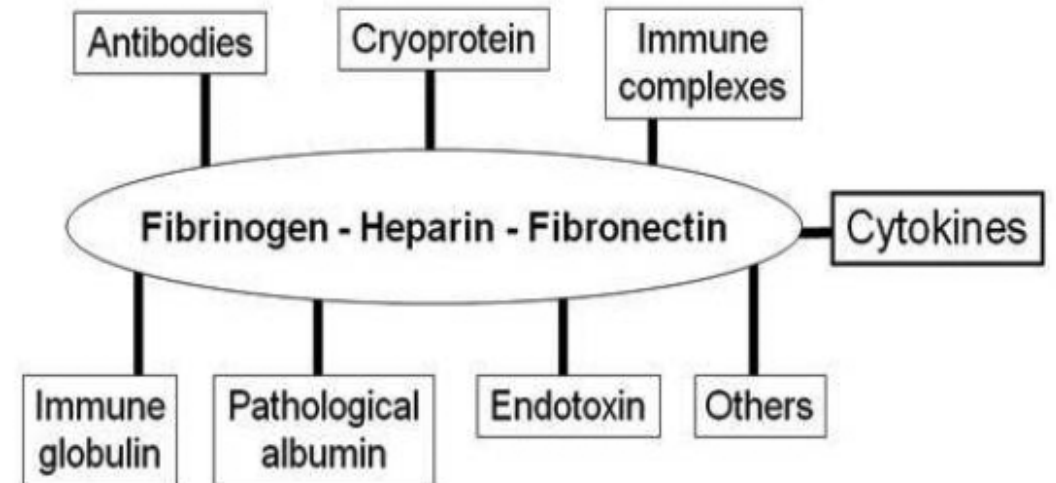
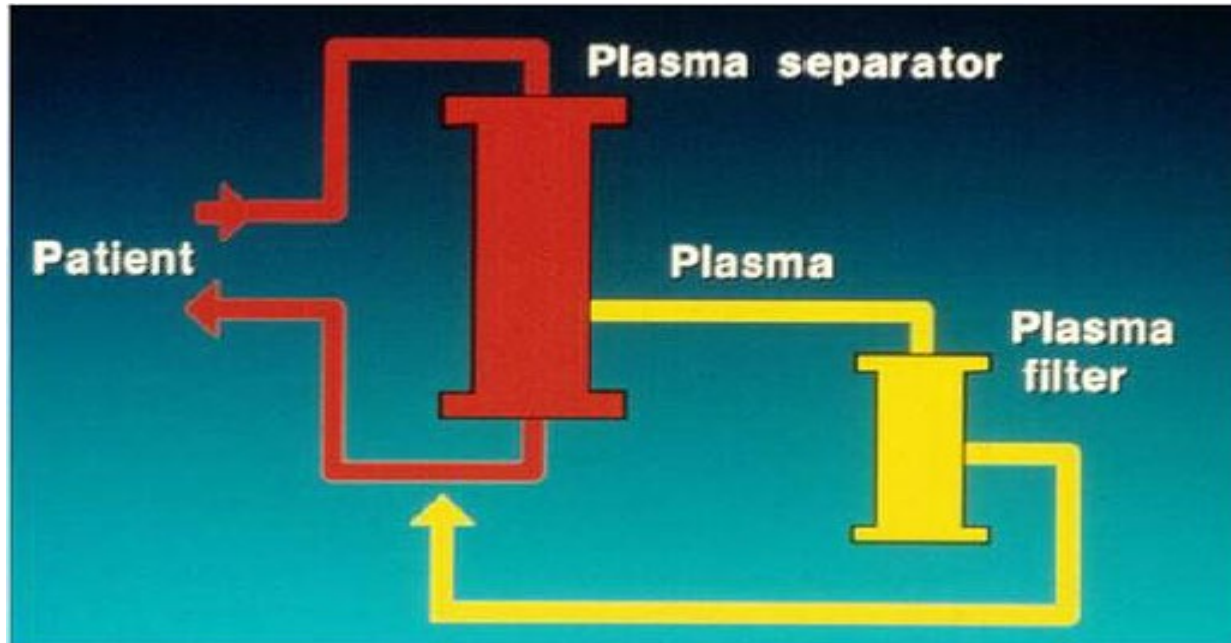
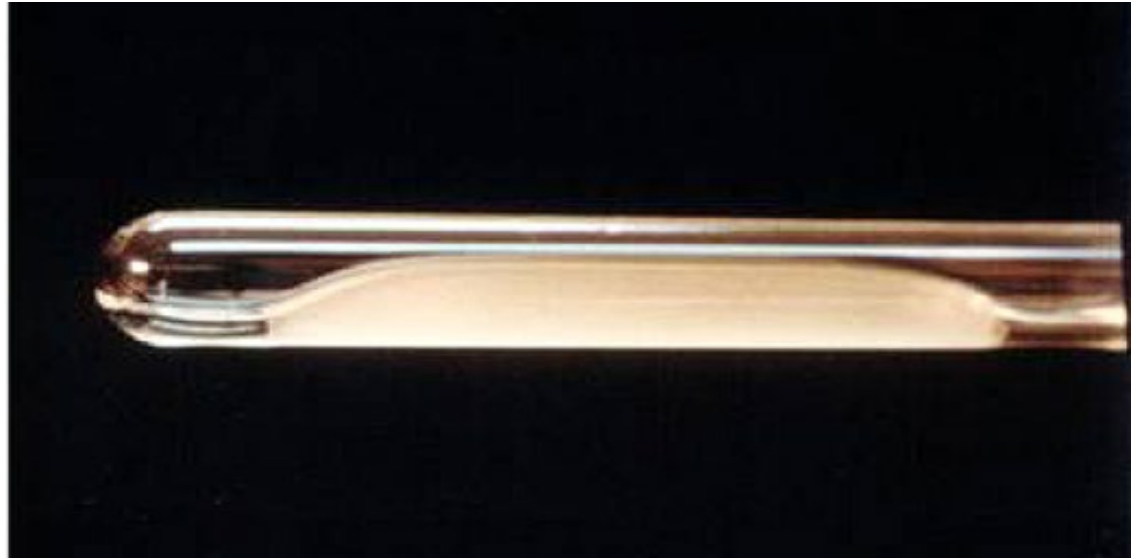
**WHILE MORE RESULTS MAY BE
FORTHCOMING FROM THESE TRIALS IN
ALZHEIMER'S DISEASE PATIENTS, A
MAJOR QUESTION STILL EXISTS: IS IT
MORE IMPORTANT TO REMOVE THE
“BAD” MOLECULES OR REPLACE WITH
THE “GOOD”? ARE CRYOGLOBULINS ONE
OF THE “BAD” MOLECULES?**

Review Article

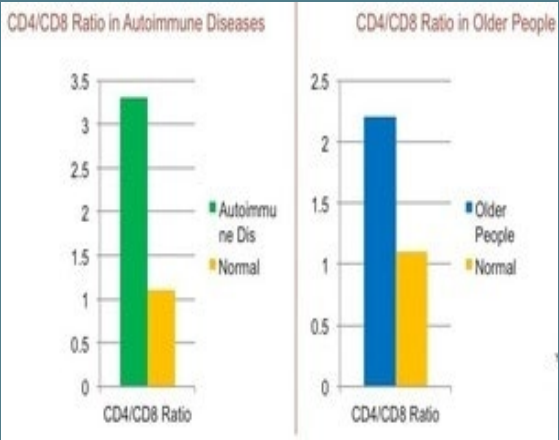
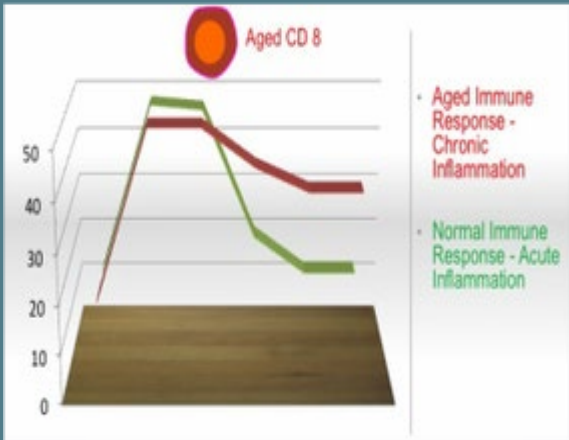
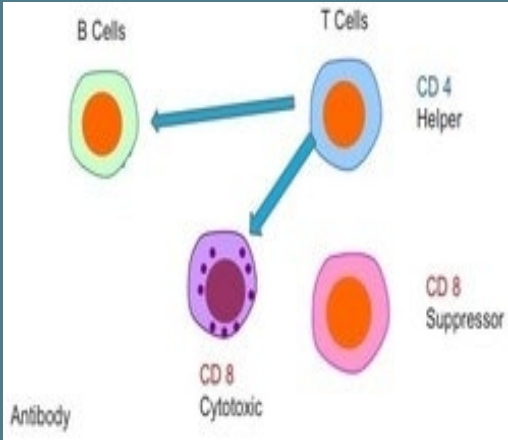
Can an Apheresis Therapy become an Effective Method for Anti-Aging Medicine?

Hiroshi Miyamoto, Yukihiro Nosé

Michael E. DeBakey Department of Surgery, Baylor College of Medicine



Factors Present	Autoimmune Disease	Chronic Inflammation	Older People
1. Autoantibodies	✖	✖	✖
2. Pro-inflammatory factors	✖	✖	✖
3. T cell abnormalities	✖	✖	✖



Review Article

Can an Apheresis Therapy become an Effective Method for Anti-Aging Medicine?

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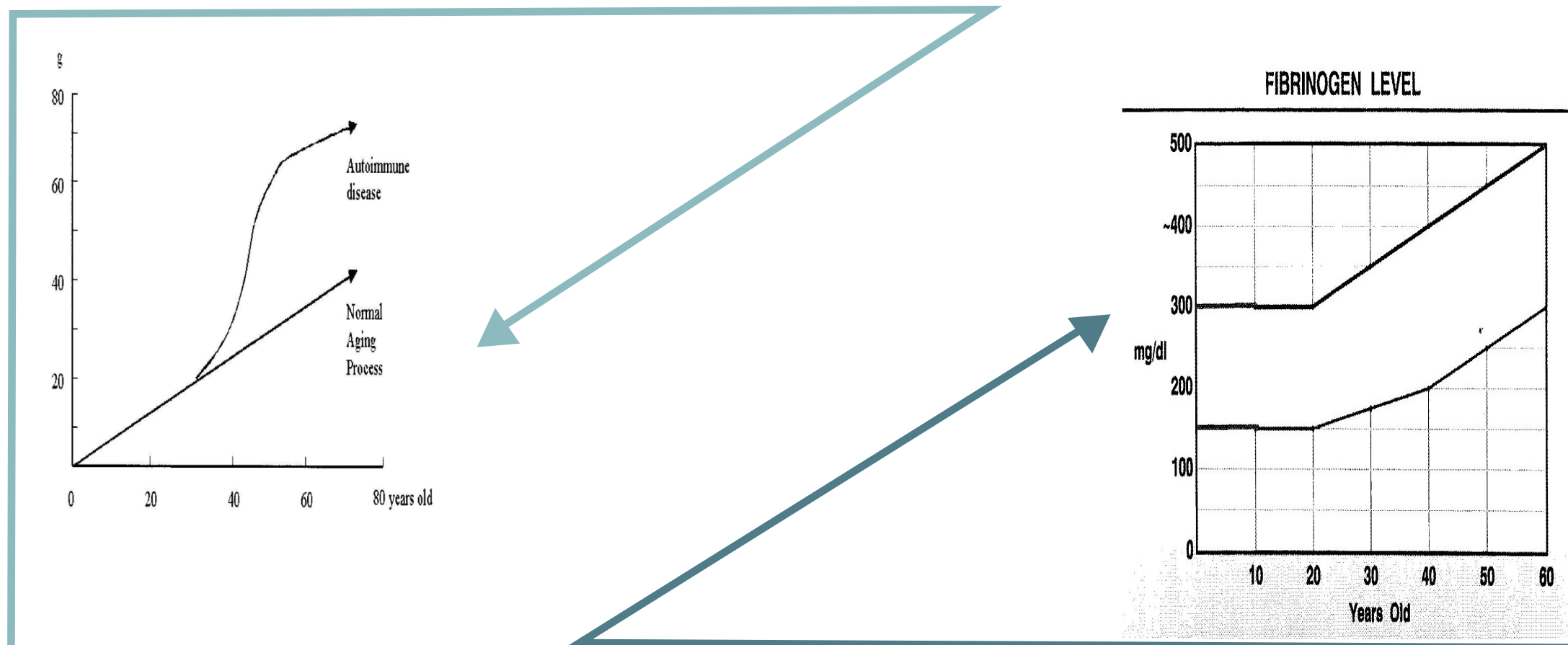


Fig. 4. Kinetics of cryogel in aged and diseased individuals (graphic display).

Fig. 5. Aging and increased level of fibrinogen in plasma.

CAN THERAPEUTIC APHERESIS SUPPORT OUR BIOLOGY TO IMPROVE LONGEVITY OR DECREASE MORBIDITY AND MORTALITY?

100

diseases of varying types affecting the major organ systems are associated with abnormal or high concentrations of macromolecular proteins and other chemistries in plasma that would lend themselves to therapeutic apheresis.

Many diseases state (metabolic and immunologic) exhibit abnormalities of higher molecular weight solutes or protein-bound solutes.

The identification of the most appropriate and cost-effective separation/removal means is important.

It is also critically important that the effectiveness and long term safety of apheresis be tested in randomized clinical trials.

Plasma membrane filtration technologies

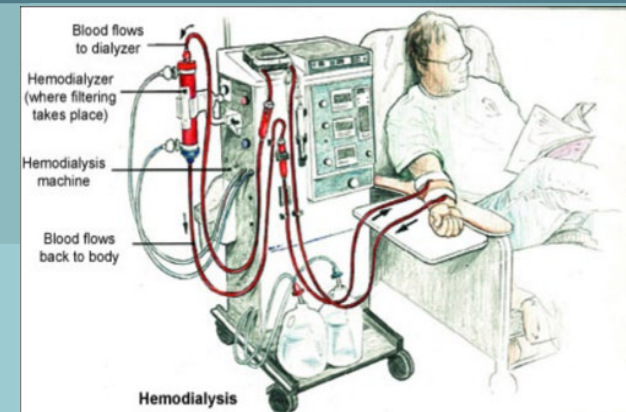
Cryofiltration

Thermofiltration

Cryoprecipitable proteins are suppressive to the immunological system such as inhibiting the blastogenesis of normal mononuclear cells and inhibiting neutrophil phagocytosis in a concentration-dependent manner

Cryoprecipitable proteins had a suppressive effect on normal lymphocyte proliferation

Patient plasmas with cryoglobulinemia were inhibitory to normal granulocyte chemotaxis .



Accumulation of pathological
macromolecules and subsequent
cellular malfunction



Autoimmune
diseases

Atherosclerosis

Malignancies

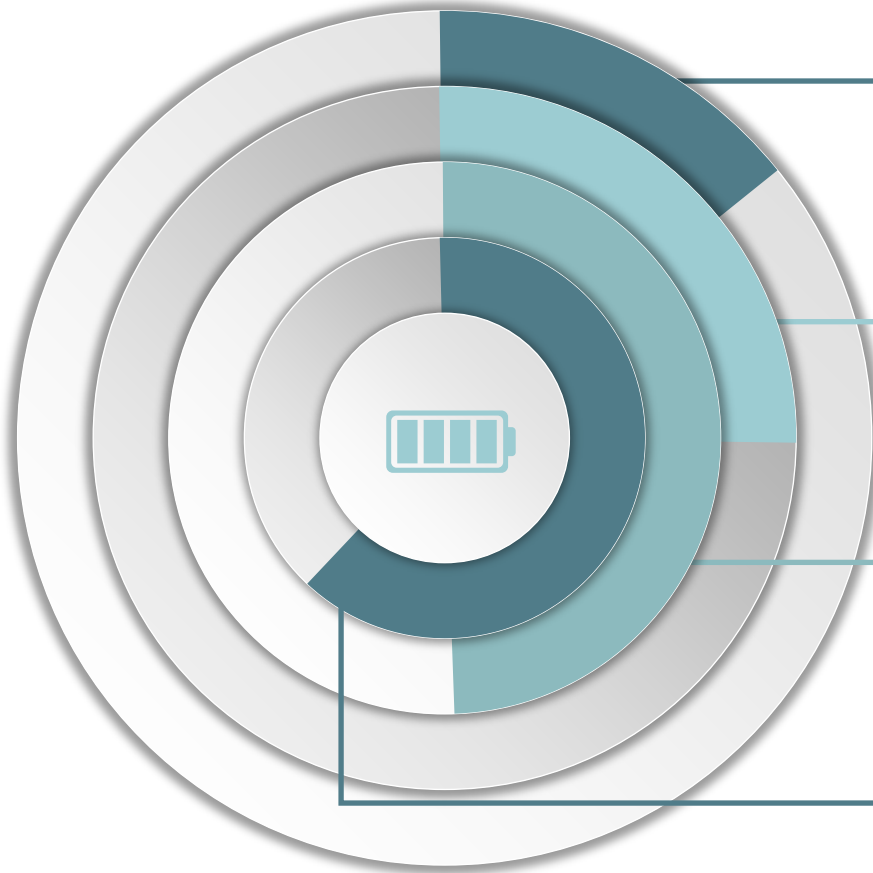
Editorial

Aging, Disease, and Therapeutic Apheresis

X-Effect Hypothesis

ing of the “biological smoke”, those abnormally high concentration and toxic macromolecules, can activate the biological system to return to normalcy and allow pharmaceutical agents to work more effectively. The normal detoxification processes are lacking in disease states and in aging. “Factors”

“Juzo” as an Anti-Aging Artificial Organ



functions. This situation produced either immunoactivation or immunodeficiencies depending upon what kinds of cellular and humoral immune systems were activated or suppressed. If the immunoactivation status was dominant, then the patient suffers various types of autoimmune diseases. However, in general the aging process produced immunodeficiency status. In this situation,

infection and malignant tumors had a tendency to occur more frequency. If the hypothesis described above was the actual aging processes taking place in our body, certainly we knew medically how to prevent it with our apheresis technologies. We would like

creator intentionally forgot to install the age preventing organ inside of the body. So our life was limited in less than 100 years old. In order to keep us alive longer, it should be necessary to install the so called aging preventing artificial organ or “Juzo” into the patient. So we believed we could slow down the physical aging process by apheresis technologies. This new contribution of

We began to initiate for the development of the second generation apheresis therapy five years ago. Heart diseases, diabetes mellitus and cancer were selected as target diseases. These diseases were strongly related with aging. Therefore, to

2007-2008

Cryoaggregate Filtration

At lower temperatures (4°C ~ 30°C), the diseased heparinized plasma developed cryoaggregates. This method is to remove cryoaggregates formed at lower temperatures from the plasma. Almost all of cryoaggregates existed between 0.1 and 0.01 µm under below 20°C¹³⁾. Therefore, cryoaggregates would be removable by the plasma fractionator having its pore structures between 0.01 ~ 0.1 µm (10 ~ 100 nm). We developed 2 kinds of cryoaggregate filtration systems which could be performed online as PAT CAT (Pressure and temperature controlled apheresis therapy)¹⁴⁾ and offline as Off-LAPPET (Off-line automatic plasma purifier for exchange transfusion)¹⁵⁾.

This procedure (Off-LAPPET) was approved by the US FDA and currently this pilot study on non-ischemic cardiomyopathy patients was under way. For this patient population effective removal of pathological globulin would be considered to be clinically beneficial. The initial preliminary results revealed

2008-2009-2010

Cryoreactive Albumin Removal Apheresis (CRARA) Therapy

Currently diabetic complications (nephropathy, retinopathy and neuropathy) were considered to be generated by increased plasma levels of glycated albumin (GA) and other glycated proteins¹⁶⁻¹⁹⁾. These glycated proteins, increased in diabetic patients, caused heart and vascular diseases and complication^{20,21)}. We investigated whether cryofiltration removed GA from cooled heparinized plasma of the hemodialysis patients due to type 2 diabetic nephropathy by using filter²²⁾. The plasma was cooled down at 4 °C and filtration was made *in vitro* through 0.2 µm filter. The plasma samples from 5 diabetic patients with 5 non-diabetic patients were subjected for cryofiltration. The increased GA was effectively removed as the cryoreactive albumin by cryofiltration, but non-glycated albumin was not removed. Namely, it showed that cryofiltration could remove selectively only cryoreactive GA from the patient's plasma as pathological molecules.

The size of albumin molecules (68,000 daltons) were smaller than the size of globulin molecules (150,000 daltons), so in order to remove albumin cryoaggregates effectively, lower temperatures ($5 \pm 5^{\circ}\text{C}$) than the removal of cryoaggregated globulin ($15 \pm 10^{\circ}\text{C}$) were necessary to be employed. With these filtration pore sizes and temperatures, the CRARA therapy removed not only cryogel but also cryoaggregates. It demonstrated more effective than the simple cryofiltration. Effective removal of pathological albumin from diabetic patients' plasma by CRARA therapy should be able to reduce or eliminate microvascular complications occurred for the end stage diabetic patients.

2010

Bioincompatible Apheresis System for Cancer Therapy

“The immunological control shock might have had therapeutic effects on the cancer patients”. Thus, it is expected that a bioincompatible apheresis system may be effective for immunostimulation or immunoactivation. We will be able to report about this project in near future.

2010

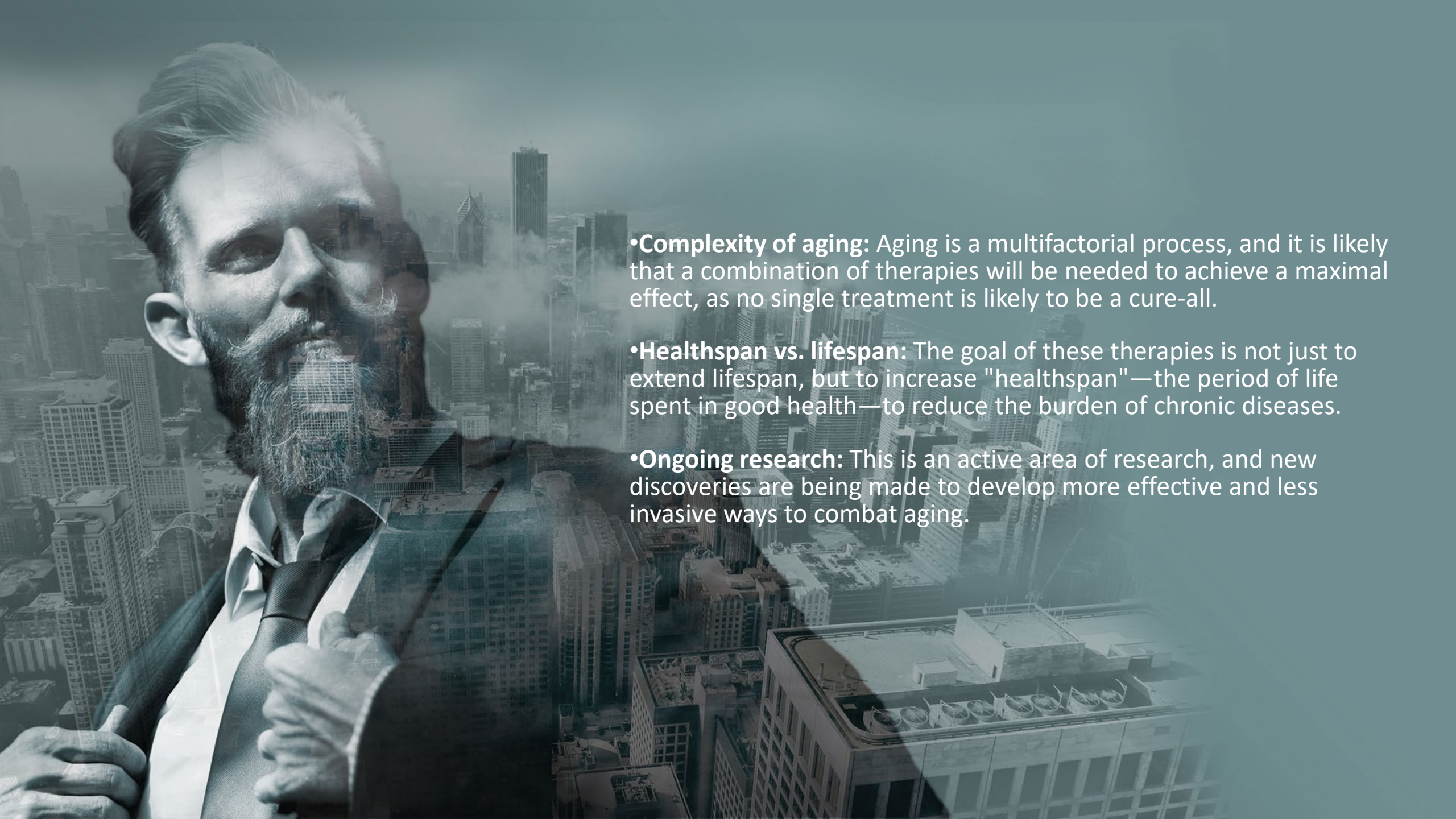
Heparin Cryoprecipitation , Atherosclerosis

At first, Meilin *et al.* in Israel reported whether heparin cryoprecipitation which was an *in vitro* method of plasma purification using centrifuge removed non-traditional risk factors for atherosclerosis from cooled heparinized plasma of the patients with hemodialysis²⁵⁾. Their method was based upon a cryofiltration method²⁶⁾. Since cryogel was formed by heparin and fibrinogen under cooled temperature, they tried to remove the cryoprecipitation by centrifuge. Their result showed that treatment of hemodialysis plasma with heparin cryoprecipitation (freezing -20°C , thawing 4°C , centrifugation 800g, 4°C) significantly reduced fibrinogen, carbonylated fibrinogen, carbonylated albumin and TNF- α to control levels which were simultaneously found in the cryogel. They also compared differences of removal effect between albumin and carbonylated albumin or fibrinogen and carbonylated fibrinogen by their method. Interestingly, it was revealed that carbonylated albumin and carbonylated fibrinogen were selectively removed from patient's plasma. These carbonylated proteins were produced as a result of strong influences of the oxidative stress^{27,28)}. This oxidative stress is strongly related with not only atherosclerosis but also aging²⁹⁻³¹⁾. Therefore, their removal method could remove not all plasma molecules non-selectively but undesirable plasma molecules for living body selectively.

A person in a dark suit and white shirt is holding a tablet computer. The background is a hazy, high-angle view of a city skyline with many skyscrapers. The overall color palette is muted, with greys, blues, and a hint of orange from the sky.

Generally, aging process should be a physiological phenomenon. However, if its speed is too fast or too strong, it might produce a disease. If this hypothesis would be true, “apheresis therapy”, which could remove pathological molecules accumulated excessively inside of the living old body and could return them to the normal level, should be an effective interventional method as an Anti-Aging medical therapy. For such purpose, the cryofiltration or cryoaggregate filtration should be an ideal therapeutic artificial organ called “Juzo” or to prevent aging processes of patients. We should proceed to investigate and expand the possibility of “apheresis therapy” as an active Anti-Aging medical therapy in the future.

Conclusion



- **Complexity of aging:** Aging is a multifactorial process, and it is likely that a combination of therapies will be needed to achieve a maximal effect, as no single treatment is likely to be a cure-all.
- **Healthspan vs. lifespan:** The goal of these therapies is not just to extend lifespan, but to increase "healthspan"—the period of life spent in good health—to reduce the burden of chronic diseases.
- **Ongoing research:** This is an active area of research, and new discoveries are being made to develop more effective and less invasive ways to combat aging.