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REVIEW

Plasma-Based Strategies for Therapeutic Modulation of Brain Aging

Viktoria Kheifets[1] · Steven P. Braithwaite[1]

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Abstract

Age is the primary risk factor for the vast majority of disorders, including neurodegenerative diseases impacting brain function. Whether the consequences of aging at the biological level can be reversed, or age-related changes prevented, to change the trajectory of such disorders is thus of extreme interest and value. Studies using young plasma, the acellular component of blood, have demonstrated that aging is malleable, with the ability to restore functions in old animals. Fascinatingly, this functional improvement is even observed in the brain, despite the blood-brain barrier, indicating that peripheral sources can effectively impact central sites leading to clinically relevant changes such as enhancement of cognitive function. A plasma-based approach is also attractive as aging is inherently complex, with an array of mechanisms dysregulated in diverse cells and organs throughout the body leading to disturbed function. Plasma, containing a natural mixture of components, has the ability to act multimodally, modulating diverse mechanisms that can converge to change the trajectory of age-related diseases. Here we review the evidence that plasma modulates aging processes in the brain and consider the therapeutic applications that derive from these observations. Plasma and plasma-derived therapeutics are an attractive translation of this concept, requiring critical consideration of benefits, risks, and ethics. Ultimately, knowledge derived from this science will drive a comprehensive molecular understanding to deliver optimized therapeutics. The potential of highly differentiated, multimodal therapeutics for treatment of age-related brain disorders provides an exciting new clinical approach to address the complex etiology of aging.

Key Words Blood · plasma · plasma fractions · parabiosis · heterochronic parabiosis · chronokine · transfusion

Introduction

Aging is a multifactorial progression of dysfunction throughout the body, reflected in a range of biological processes that go awry, having far-reaching consequences for health and quality of life. Age is also the major risk factor for virtually all disorders, notably cardiovascular disease, diabetes, and dementias including Alzheimer's disease (AD). Therefore, if the mechanisms of aging can be understood, and therapeutics developed to change the underlying aging mechanisms, there is potential to tremendously impact health of the world's increasing elderly population. Mechanisms relevant in aging have been considered to fall into eight categories as reviewed in [1]: mitochondrial dysfunction, cellular senescence, proteostasis deficits, chromosomal instability, epigenetic changes, inflammation, metabolic deficit, and stem cell exhaustion. Whether just modulating one of these underlying dysfunctions is sufficient to be efficacious in age-related disease, or if there is requirement to impact multiple of these critical mechanisms, is fundamental in developing therapeutic strategies. Positing that the greatest impact will be gained from the broadest intervention provides the conundrum of how this could be therapeutically achieved. The presence of a single molecular target hub that underlies all these mechanisms is highly unlikely, and therefore, a polypharmacy approach would have to be invoked. The difficulty in developing combination products makes a cocktail of individual therapeutics also very complicated, and thus, the application of natural cocktails is appealing. In recent times, consideration of plasma, the acellular component of blood, and its potential to rejuvenate processes throughout the body, including the brain, has become resurgent and fulfills many criteria which may be beneficial for efficacy in age-related diseases [2]. Plasma is a

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✉ Steven P. Braithwaite
sbraithwaite@alkahest.com

[1] Alkahest Inc., 125 Shoreway Road, Suite D, San Carlos, CA 94070, USA

natural mixture of thousands of components, proteins, peptides, RNA, lipids, etc. and can subserve the role as a natural cocktail. The concept of manipulations of blood being of benefit is also not new: bloodletting has gone back hundreds of years, and blood's healing properties abound in folklore and early medical practices [3]. The use of blood transfusions to rejuvenate has roots going back to the 1900s (reviewed in [4, 5]). The history of this approach has however been littered with dire consequences, particularly in times preceding the understanding of blood groups and infective agents, with death from adverse reactions or acquired infection being not uncommon. Now is a time to revisit the potential of plasma or plasma-derived products as therapeutics with a true scientific basis, with a requirement to be intensely aware of safety and ethical considerations, towards developing a wholly new paradigm for treating age-related disorders.

The Science of Plasma in Aging

The modern science that has led to a renewed interest in plasma as a therapeutic source came from the technique of parabiosis, the surgical connection of two animals leading to their shared blood supply, first reported in the mid-nineteenth century [6]. Heterochronic parabiosis, in which animals of different ages are subject to parabiotic pairing, advanced the field to the first scientific demonstration of beneficial properties of young blood to extend life span [7]. Advances in animal strain inbreeding and surgical optimization have allowed for more robust parabiosis techniques and the expanded use of this methodology to study a wide range of biological processes [8, 9]. Benefits of a young circulatory system have been demonstrated in muscle and liver [10], pancreas [11], heart [12], and other organs through heterochronic parabiosis. The intimate connection of blood with these peripheral organs makes the appreciation of efficacy rational. However, it was unexpected for heterochronic parabiosis to have effects in the brain due to the blood-brain barrier (BBB) which is understood to restrict blood's access to the central nervous system [13–15]. Despite this apparent limitation, studies using heterochronic parabiosis demonstrated that the older heterochronic parabiont can detrimentally affect centrally mediated behaviors and associated histology in the brain of the younger parabiont [16]. Notably, these effects included a reduction in neurogenesis in the dentate gyrus of the young parabiont, as measured by doublecortin immunostaining, and an increase in neurogenesis in the older parabiont indicating that the connected circulatory systems can induce bidirectional changes in important central processes dependent on the age of the animal [16]. Similarly, investigating the subventricular zone in heterochronic parabionts has demonstrated bidirectional modulation of stem cell populations (detected by Sox2 and Ki67 immunoreactivity), leading to subsequent changes in olfactory neurogenesis [17]. Further effects have been identified including electrophysiological deficits in long-term potentiation in the young parabiont [16] and enhancement in the old parabiont [18], increases in blood vessel volume and branching in old parabionts [17], and increases in spine density and immediate early gene activation in old parabionts [18]. These multiple mechanistic changes observed in heterochronic parabiosis experiments impact a number of the hallmarks of biological aging indicating broad antiaging biology [1]. Assessing behaviors in parabiotically connected animals is difficult, but studies have shown effects on olfactory discrimination [17]. Further understanding has been achieved using heterochronic blood administration, rather than parabiotic connection, in order to focus on purely the circulatory component and separate potential confounding factors of additionally shared organ functions between animals [19]. In the shorter temporal profile of these studies (days compared to weeks), profound effects of old blood are observed in the young brain, with marked reduction in neurogenesis in the subventricular zone, but less profound positive effects are seen in old animals with blood from the young animals [19]. These findings, although from limited sample sizes, are intriguing on the rapid ability to modify proliferative processes in the brain and present the possibility of differential relative effects of detrimental and beneficial blood components with temporal complexity.

Parabiosis and blood exchange experiments demonstrated the potential to modulate central processes through connected circulation, but there is a potential for both cells and plasma to be shared with an additional complication of limited behavioral assessment being possible due to physical joining of the two animals. This complex interplay and biology in the parabiotic pairing makes conclusive determination of mechanisms underlying efficacy difficult to interpret and therefore additional studies were needed. In-depth studies demonstrated that there is likely no stem cell contribution from the young parabiont to the old animal [10, 16] despite sharing of the circulation between the pair [20], suggesting that circulating soluble factors are responsible for both beneficial and detrimental effects in these animals. To further assess the contribution of soluble plasma factors separately from the whole organism, injections into young animals of plasma derived from old mice were performed and showed that equivalent histological effects to those observed in the young parabiont were produced [16]. Conversely, young plasma injections into old animals also showed similar cellular effects in the brain to those seen in old parabionts [18]. These experiments demonstrate that plasma contains detrimental and beneficial components which alter aging processes, thus decoupling the effect of cellular and organ contribution of the other parabiont from the effect of transferred plasma factors. Nonetheless, circulating plasma factors may be either directly or indirectly affecting the brain; for example, action on peripheral organs may have subsequent effects centrally. Furthermore, the infusion of plasma allows exploration in multiple behavioral paradigms. Old plasma infused into

young mice inhibits performance in the radial arm water maze (RAWM) [16], and young plasma infusion into old mice enhances performance in the RAWM, contextual fear conditioning, and novel object recognition tests [18, 21]. This has been extended further using human plasma derived from the umbilical cord which also imparts cognitive benefits including in the Barnes maze test [22]. Many of the histological correlates observed in parabiosis experiments were also observed in these studies [16, 18], although neurogenesis enhancements in the old brain after young plasma infusions have thus far not been reported.

The basic finding of cognitive and histological improvements with young plasma in aging has been extended further into disease models. As AD is the major age-related disorder of cognitive function, the use of parabiosis and young plasma infusions into amyloid precursor protein (APP) overexpressing mice was investigated [23]. In APP mouse parabionts joined to young wild-type animals, there was no effect on amyloid-β levels, but the APP animals' synaptophysin and calbindin levels were normalized to nontransgenic levels indicative of synaptic improvements, coupled with normalization of important signaling pathways [23]. The presence of soluble plasma factors responsible for the efficacy in heterochronic parabiosis was confirmed with young plasma infusions into APP transgenic mice, which produced comparable synaptic effects as seen in parabiosis, as well as improvement in cognitive function assessed with Y maze and contextual fear conditioning (CFC) tests [23], demonstrating that young plasma can also enhance CNS function in a severely dysregulated disease environment. These data build on prior studies of the peripheral sink hypothesis [24] and with isochronic parabiosis pairing of APP/PS1 transgenic mice with wild-type animals showing reduction in amyloid-related pathology in the brain [25], which together demonstrate that plasma is an important compartment for modulating AD-relevant biology. Importantly, the plasma infusion experiments further show that there are soluble factors that can act from the periphery for functional benefit in the CNS.

How plasma proteins can exert central effects remains an open question. A few proteins may efficiently cross the BBB directly [22], although many others are also detected in central compartments from peripheral sources at low levels [26]. Breakdown of the BBB is associated with neurological diseases, cognitive impairment, and processes of aging, and it is possible that due to this impairment [27], further proteins found in blood can find their way into the CNS and exert their effect. However, to date, there have been no studies directly addressing this hypothesis and at least some of the biological effects seen in the brain may be due to peripheral sites of action [28].

Chronokines—Key Drivers of Function That Change with Age

The findings of young plasma efficacy in aging are enticing but raise the question of the molecular basis—presumably, individual plasma factors can be identified that underlie beneficial and detrimental activity observed in heterochronic parabiosis and plasma infusion experiments. This molecular understanding can provide the scientific underpinnings for therapeutic modulation of aging processes and diseases of aging.

Many studies have shown that levels of plasma proteins change over aging [29–32], though their identification does not discern whether they are merely biomarkers, passive indicators of change, versus critical drivers of the aging process and thus biologically highly relevant in the search for bioactive components in parabiosis or plasma infusion experiments. Furthermore, specific proteoforms may change over aging with altered post-translational modifications or other factors that can affect their activity. We have termed these proteins chronokines—biological drivers of function that change with aging. In a reductionist simplification of age-related proteomic changes in plasma, aged blood contains increased levels of negative, detrimental chronokines, while young blood contains positive, beneficial chronokines which decrease during the course of aging (Fig. 1). The balance of these factors determines systemic functions and biological aging processes. There are of course exceptions to this simplified view with some chronokines serving compensatory functions, and thus, the increase in their levels over aging may be viewed as beneficial [12, 33, 34]; nonetheless, the balance of chronokine activity can determine biological state.

Multiple groups have undertaken proteomic analysis of plasma across the life span, and several candidate chronokines that impact the brain neurogenic niche and central functions have been characterized (Table 1). The identification of these chronokines and examination of their functional implications is helping to further elucidate mechanisms of relevance driving cognitive benefit by young plasma and deficits in brain function mediated by old plasma. The detrimental chronokines β2 microglobulin (B2M) and eotaxin have been described to increase with aging, and their delivery or overexpression in young animals can lead to cognitive dysfunction [16, 35]. Importantly, the removal or neutralization of these proteins can have beneficial effects on central processes—knockout of B2M improves cognitive function in aged animals in the RAWM test and results in increased neurogenesis, specifically in aged animals [35]. Antibody-mediated neutralization of eotaxin can also reverse the eotaxin-mediated detrimental effects on neurogenesis. Further negative chronokines are likely to be identified as many proteins change with age and there are strong effects of old plasma on function in young animals. Beneficial chronokines present in young plasma have also been described, notably tissue inhibitor of metalloproteinases 2 (TIMP2), colony-stimulating factor 2 (CSF2), growth differentiation factor 11 (GDF11), and osteocalcin (OCN). TIMP2 has a canonical function as an inhibitor of matrix metalloproteins and is found at higher concentration in

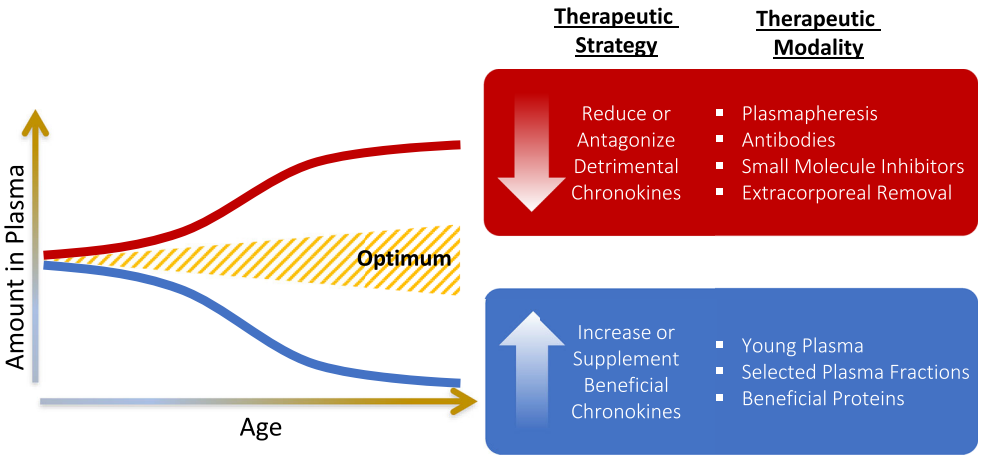

Fig. 1 From chronokines to therapeutics: a molecular understanding of the changes in the chronokines—plasma protein components that drive function over aging—leads to therapeutic targeting strategies

umbilical cord plasma relative to adult plasma. Its injection into old animals resulted in enhanced cognitive performance in the Barnes maze and enhancement in the neurogenic niche [22]. GDF11, a member of the BMP/TGF-β family, has been shown to have regenerative effects in hypertrophic cardiac muscle [12] and increased brain blood vessel volume and the number of Sox2+ cells in the subventricular zone [17], although there has been notable debate about this factor due to the tools used for its identification and for interrogating its effects in aging processes [33, 36–38]. OCN is a bone-derived hormone which also drives beneficial effects on central processes. OCN, signaling through Gpr158, has been demonstrated to be of benefit through complementary studies using knockout mice, antibody depletion, and protein infusion on endpoints of memory using novel object recognition, Morris water maze tests, and anxiety behaviors using the elevated plus maze [21]. Further beneficial chronokines will be identified with the use of innovative technologies. Insights are already being gained into potential positive chronokines in peripheral tissues, for example, using bio-orthogonal proteome labeling in heterochronic parabiosis which identified multiple factors in old muscle exposed to young blood [26].

The study of individual proteins with diverse known functions, such as growth factors, cytokines, and protease modulators, affecting centrally relevant processes suggests their importance in the regulation of downstream mechanisms. While there is considerable discussion regarding the presence and implications of human adult neurogenesis [39–42], the effect of aging on the rodent neurogenic niche is well established [43–46]. Stem cell harboring neurogenic niches in the subventricular zone and the dentate gyrus are most susceptible to acute and chronic stressors such as inflammation, aging, and metabolic dysregulation [47], and therefore, changes in neurogenesis are a sensitive biomarker of aging and rejuvenation mechanisms. The regenerative capacity of the neurogenic niche is impacted by complex physical interactions with the surrounding vasculature and it is greatly influenced by changes in peripheral and CNS inflammation, intercellular signaling via exosomes, organismal stress, and the relatively recently discovered glymphatic system [47–54]. Neurogenesis decline with aging is further exacerbated in disease states such as AD and depression [55, 56], and modulation of neurogenesis is at least in part responsible for the therapeutic effect of selective serotonin reuptake inhibitors (SSRIs) and exercise (reviewed in [57]). Findings that several individual chronokines could impact the neurogenic niche and modulate age-dependent levels of neurogenesis, regardless of their primary biological mechanism of action, further highlight the multifactorial complexity of aging processes. Whether these factors directly impact the brain neurogenic niche, or have an indirect peripheral effect, is yet to be determined. It is likely that both mechanisms are physiologically relevant, and possibly necessary, to reset the clock of aging. The role of plasma and systemic factors in relation to neurogenesis has been thoroughly reviewed recently, indicating the importance of this biology [58]. Although *in vivo* effects of single chronokines in isolation have been demonstrated, it is doubtful that one central protein can account for all the activity observed with plasma manipulations or be driving detriment or benefit alone, as these are highly multifactorial processes that involve many cell types, environments, and tissues. Nonetheless, the identification of individual chronokines is a critical step in understanding the molecular basis of plasma-mediated biology and can provide strong rationale for therapeutic strategies.

Therapeutic Translation

The above described studies in mice have clearly demonstrated great potential of plasma, and individual chronokines, to reverse age-related processes resulting in cognitive improvements. However, it is well known that many therapeutics have demonstrated cognitive benefit in mouse models yet not translated to efficacy in man, particularly in the field of AD [59]. The potential reasons for this are multiple, including different biological mechanisms between species, only partial modeling of complex human biology in mice, and basic lack of

Table 1 Identified chronokines that impact the brain neurogenic niche and central functions

Chronokine	Protein ID	Function	References
β2-Microglobulin	B2M	Detrimental	[35]
Eotaxin	CCL11	Detrimental	[16]
Growth differentiation factor 11	GDF11	Beneficial	[17]
Granulocyte-macrophage colony-stimulating factor	CSF2	Beneficial	[22]
Growth hormone-releasing hormone	GHRH	Beneficial	[77]
Insulin-like growth factor 1	IGF1	Beneficial/detrimental	[78–80]
Osteocalcin	OCN	Beneficial	[21]
Oxytocin	OXT	Beneficial	[81]
Tissue inhibitor of metalloproteinases 2	TIMP2	Beneficial	[22]

understanding of disease pathogenesis. Considering aging as a therapeutic target obviates some of these translational components, as there is no bias towards a predetermined mechanistic hypothesis and many fundamental aging mechanisms are common between species [1]. Animal studies have begun to show that the human plasma proteome can act on murine biology to have procognitive efficacy through the ability of human umbilical cord plasma to exert effects in immunocompromised mice [22]. Nonetheless, the ultimate goal is to reverse aging processes and improve function via human plasma in man. Due to concerns of umbilical cord plasma being limited and hence not feasible for widespread utility, as well as potentially inducing biological changes more consistent with developmental processes, utilization of adult plasma is preferred. The amounts of cord blood collected (average of 60 mL per donation, 1 time event) compared to adult plasma donations (~800 mL for plasmapheresis donations, twice a week) [60] make adult plasma a more feasible source for beneficial chronokines. Controlled clinical trials are a necessary step to ensure that this is truly safe and effective. Consideration of such approaches is certainly warranted as there are multiple conceivable ways to achieve the multimodality that may be necessary in the complex biology of aging. The preclinical science driving the appreciation of chronokines leads to several potential therapeutic approaches to return the plasma proteomic composition towards an optimal state and stop, or ameliorate, age-related disorders (Fig. 1).

Plasma as a Therapeutic

The simplest translation of parabiosis and plasma transfusion experiments is to administer young plasma on a regular basis to elderly subjects. Plasma is readily available, and the blood donation system is well developed throughout the world, providing multiple components—red blood cells, plasma, and platelets—that fulfill a critical need for a range of patients. Plasma can be stored frozen for up to 1 year; categorized for clinical use are fresh frozen plasma (FFP), frozen within 8 h of collection, and plasma frozen within 24 h of phlebotomy (PF24). However, the routine use of these plasma units in the clinical setting is limited to only acute needs. Current evidence-based clinical practice only supports plasma utilization for large-volume transfusion and reversal of warfarin anticoagulation in the presence of intracranial hemorrhage [61]. These limitations relate to the potential risks of plasma, particularly with respect to immunological reactions. It is well established that plasma and blood transfusions require cross-matching of blood types for the ABO and rhesus (Rh) epitopes to avoid life-threatening complications [62]. However, a range of additional histocompatibility components must also be considered, and it is not possible to screen for all components. At this time, even the routine screening of Rh types is limited only to the D-antigen, yet over 50 Rh epitopes have been characterized. Beyond ABO/Rh antigens, the H-system, Lewis antigens, and many others exist and over 300 antigens are recognized [63]. The antibodies to these antigens present in plasma are often only relevant in a subset of patients, and thus, their consideration in the current plasma transfusion setting is minor; however, as potential extension of plasma infusions into a broader aging population is considered with frequent doses, even rare epitopes may cause issues and the well-being of all individuals must be considered. Other risks of transfusions relate to transmission of infectious diseases—hepatitis B, hepatitis C, and human immunodeficiency virus (HIV) having all been major issues historically in the blood and plasma transfusion industry [64]. In current practice, these risks have been reduced to near negligibility through donor screening, donation testing, and pathogen inactivation procedures, but there is a residual risk and new viruses coming into the donation stream can be of concern as exemplified by Zika [65]. Other noninfectious complications of concern include, among others, transfusion-related acute lung injury (TRALI) and transfusion-associated coronary overload (TACO) [66], as well as allergic reactions. The potential benefits of whole plasma need to be carefully weighed against the potential risks

(Fig. 2). Recognizing these risks, the Federal Drug Administration (FDA) issued a statement cautioning the use of young plasma infusions for unapproved conditions outside of controlled clinical trials [67]. Safety and ethical considerations must be paramount as we consider translating the potential of the biology observed in mouse models to man.

As an initial step towards understanding potential viability of plasma transfusions in aging, and to learn the scope of such approaches, a controlled clinical study has been performed to assess the safety and tolerability of repeated plasma infusions into mild-moderate AD patients [68]. In this study, 250-mL units of FFP from 18- to 30-year-old male donors were infused into elderly AD patients, once per week for 4 weeks. The infusions were ABO and Rh matched and primary endpoints demonstrated no serious adverse events, a few mild-moderate adverse events including hypertension, dizziness, and headache, but generally good adherence to the protocol. Intriguing exploratory endpoint analysis indicated a functional improvement in activities of daily living for treated subjects even in the short timeframe of this investigation, although it must be recognized that this is a small, low-powered study [68]. These results encourage that there can be translation from animal studies to man, but still caution is required to extend these findings more widely.

Plasma Fractions as Therapeutics

Considering the safety concerns of repeated whole plasma infusions and the logistical complexities required in cross-matching blood type and use of frozen plasma units, alternative approaches also need to be considered. The preclinical studies reported to date infer that multiple proteins drive various mechanisms, but this certainly does not mean that all the proteins present in plasma are required for a therapeutic effect. Therefore, a subset of proteins that drive efficacy could be isolated by removing additional proteins that can lead to safety concerns. The blood product industry has indeed developed methodologies to take source plasma donations and fractionate pooled plasma into more defined components to provide a series of plasma-derived therapeutic products—giving the advantage of industrial processes that can scale, introduce additional safety measures, and provide multiple therapeutics from each donation. This is exemplified by the generation of clotting factors such as factor VIII for hemophiliacs, immunoglobulins such as intravenous immunoglobulin (IVIG) for a range of disorders, and albumin for volume replacement. It is appealing that a procognitive, antiaging fraction may be isolated in addition to these other critical products, while also removing factors which may be counterindicated in aged subjects, such as those involved in coagulation.

The collection of source plasma for fractionation generally uses plasmapheresis, a process allowing the collection of relatively large plasma volumes per donor (600–900 mL) and with a higher frequency than whole blood donations (up to twice per week for plasmapheresis compared to once every 56 days recommended by the Red Cross for whole blood). The profile of plasma donors in the USA leads to an average age of donors in the early 30s and hence young compared to the recipients considered for age-related disorders. Plasma collected from thousands of donors can be pooled to generate a relatively homogeneous profile that then undergoes fractionation, primarily driven by proteins having differential precipitation properties with temperature, ethanol concentration, and pH. The most utilized methodology involves the basic principles of the Cohn process [69], but alternative precipitations and processing can also be utilized [70]. Importantly, as products are generated from pools, and when immunoglobulins are removed, immunogenic properties are significantly reduced leading to many plasma products being universally applicable without the need for blood type matching. Screening for infectious agents at multiple points, along with incorporation of dedicated steps in the manufacturing processes to inactivate or remove potential pathogens, generates products with a safer profile for patients than FFP. Transfusion-related risks of plasma fractions remain due to the potential to drive immunological reactions through the large number of human proteins remaining in the mixture, and hence, TRALI and TACO remain as potential issues. However, as the complexity of the mixture is simplified to identify the drivers of antiaging efficacy, those risks should significantly diminish (Fig. 2).

Our understanding of potential plasma fractions that would be beneficial for aging and age-related disorders is evolving and will continue to develop as more is understood of the molecular drivers of efficacy. The most studied plasma fraction for age-related cognitive disorders to date has been IVIG. Studies with IVIG have progressed from preclinical animal models to phase III clinical testing in AD. A primary hypothesis behind the use of IVIG is that there are natural antibodies to detrimental proteins, such as Aβ, a key protein in AD, and thus, IVIG use may neutralize and remove these for therapeutic benefit. The concept of IVIG administration has been tested in a phase 3 trial in mild to moderate AD but demonstrated no beneficial effects in the coprimary endpoints of Alzheimer's Disease Assessment Scale-cognitive subscale (ADASCog) and Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) [71, 72]. Identification of additional detrimental factors and defining optimal IVIG dosing paradigms may be required for successful application in disorders of aging.

Albumin, the most abundant protein in plasma, is generated as an important plasma fraction for volume replacement applications. Albumin has been demonstrated to bind specific forms of Aβ, and thus approaches to actively remove Aβ-bound albumin and supplement with fresh albumin-containing plasma fractions could have therapeutic benefit in AD [73]. One study has investigated this concept, using

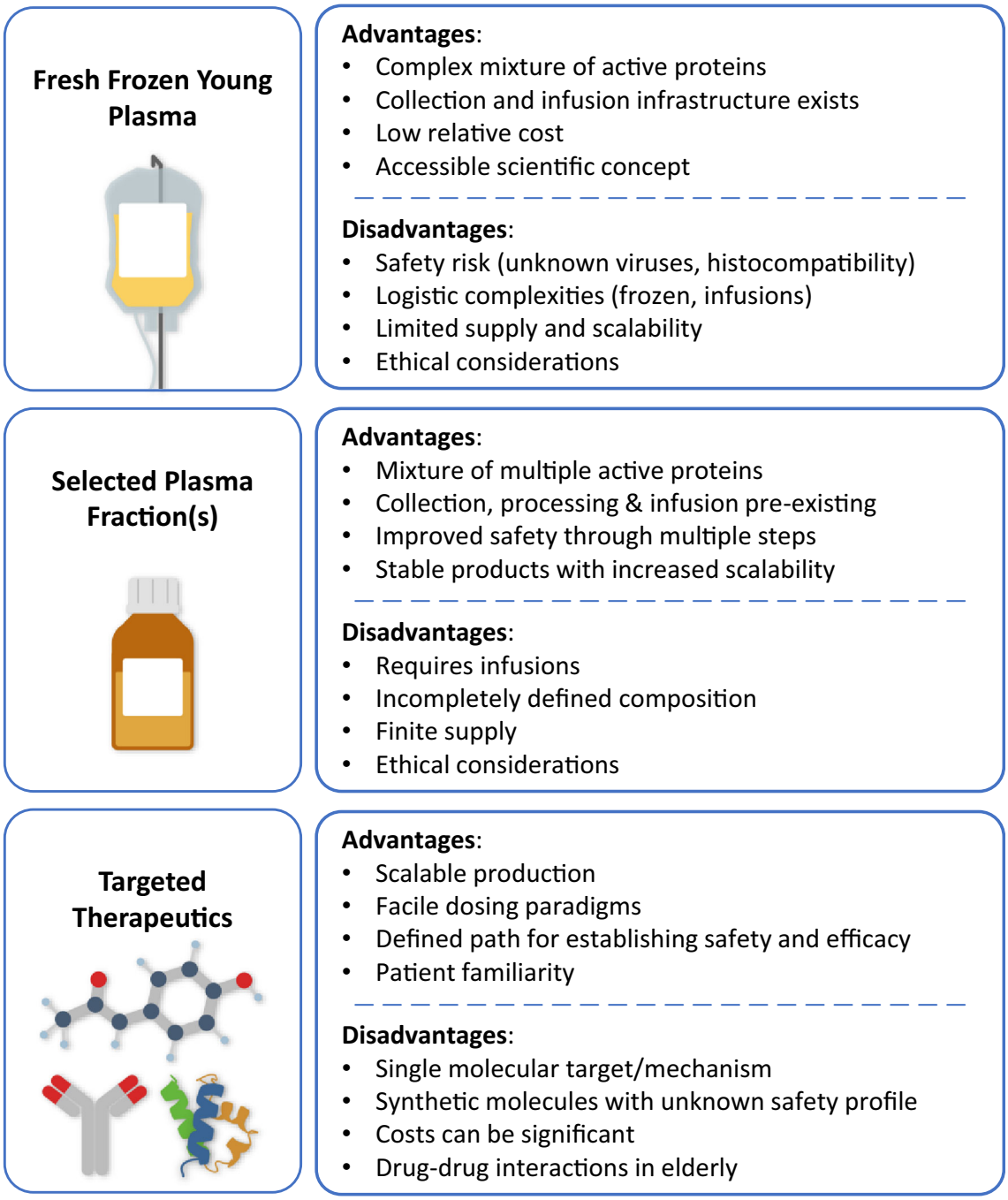

Fig. 2 Therapeutic approaches from plasma: advantages and disadvantages of opportunities to be considered in advancement of therapeutics for age-related disorders

plasmapheresis to remove detrimental proteins and replacing with fresh albumin [74]. This concept builds on the amyloid sink hypothesis capitalizing on the equilibrium between Aβ in central and peripheral compartments [75]. Encouragingly, patients undergoing plasmapheresis and albumin supplementation showed improvement in both the Boston Naming Test and Semantic Verbal Fluency through treatment [74, 76]. In addition to supplementing albumin, the plasmapheresis procedure also depletes other plasma proteins, including detrimental chronokines, thus reducing their negative effects on the disease state. The impact of their removal cannot be deconvoluted from the removal of Aβ-bound albumin due to the nature of plasmapheresis exchange, but this study provides the basis for further investigation of both the removal of detrimental and replenishment of beneficial chronokines.

As the key drivers of function and dysfunction, especially those that change with aging, are identified, the potential exists to develop new plasma fractions optimizing this beneficial

activity. Such procognitive, or generally beneficial plasma fractions, would be more defined than whole young plasma and, through their reduced complexity, will be safer and more universally applicable.

Individual Proteins as Therapeutic Targets

The ultimate goal is to achieve a molecular understanding of positive effects observed in preclinical experiments with young plasma to translate these results to the clinic and human conditions. With these molecular understandings, a number of chronokines, functional drivers that change with aging, will be identified that are critical to modulate the aging process. Both positive beneficial factors from young plasma and negative detrimental factors from old plasma can be explored for therapeutic intervention with either supplementation, inhibition, or depletion. Initial target identification has highlighted some of these chronokines as discussed previously [17, 18, 21, 35]. How these can be capitalized as therapeutics is already being considered. Individual positive proteins may be generated recombinantly and delivered directly, although issues of protein half-life, post-translational modifications, and stability need to be considered. Bioactive peptides or peptidomimetics may also be viable therapeutic options. Negative chronokines can be removed or inhibited with antibodies, or small molecules can be used to inhibit these proteins or their downstream signaling cascades. Nonetheless, a perceived challenge of these approaches is how many proteins will be required to achieve sufficient efficacy. Presumably, there are a select number of key chronokines and whether just modulating one individually will be sufficient is unknown. It is possible that a cocktail of multiple proteins may be required, or a combination of supplementing positive factors and inhibiting one or more detrimental factors will be key to reversing the processes of aging and age-related diseases. The complexity of regulatory pathways for cocktail therapeutics makes this currently a difficult proposition, but an openness to the need for such a broader approach and evolution in our ability to generate such combination therapeutics should be considered for the future (Fig. 2).

Conclusions

Discoveries from the technique of parabiosis have led rapidly to the consideration and application of the concepts of young plasma components to treating aging and age-related disorders. The prospect of such therapies, being differentiated from how the pharmaceutical industry currently thinks about highly specific molecular targeting, provides innovative ways to treat age-related disorders. Nonetheless, care needs to be taken in the application of these therapies from a number of perspectives. Individual young plasma donations have several safety-related challenges, but, moreover, the ethical considerations and limitations of appropriate donors for a potentially huge target population cannot be underestimated. Extreme caution needs to be taken in navigating these aspects, and therapies which are based on rigorous science, deep understanding of mechanistic targets, and biological processes of aging need to be developed to ensure an appropriate and ethical application. In the future, the advancement of plasma fractions can overcome some, but not all, of these considerations, being inherently safer and more scalable, but still relying on primary plasma donations. In the long term, consideration of recombinant protein therapies, small molecules, or other modalities which evolve from the molecular understandings we glean from plasma-based approaches, can guide us towards a sustainable future. Exciting opportunities from plasma understandings, coupled with a strong awareness of ethical considerations, can lead to highly novel therapeutic approaches transforming how we consider biological aging and the ability to modulate age-related process for a range of disorders whose prevalence is rapidly increasing.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

Compliance with Ethical Standards

Disclosures The authors are employees of Alkahest, Inc.

References

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The Hallmarks of Aging. Cell 2013;153:1194–217.
2. Castellano JM. Blood-Based Therapies to Combat Aging. Gerontology 2019;65:84–9.
3. Meletis J, Konstantopoulos K. The beliefs, myths, and reality surrounding the word hema (blood) from homer to the present. Anemia 2010;2010:857657.
4. Huestis DW. Alexander Bogdanov: The Forgotten Pioneer of Blood Transfusion. Transfus Med Rev 2007;21:337–40.
5. Heselhaus H. The temporality of anti-aging: a short history of the fight against time. Area Studies Tsukuba 2018;39:109–22.
6. Bert P. Expériences et Considérations Sur la Greffe Animale. J Anat Physiol 1864;1:69–87.
7. Ludwig FC, Elashoff RM. Mortality in syngeneic rat parabionts of different chronological age. Trans N Y Acad Sci 1972;34:582–7.
8. Conboy MJ, Conboy IM, Rando TA. Heterochronic parabiosis: Historical perspective and methodological considerations for studies of aging and longevity. Aging Cell 2013;12:525–30.
9. Eggel A, Wyss-Coray T. A revival of parabiosis in biomedical research. Swiss Med Wkly 2014;144:w13914.
10. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando T A. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005;433:760–4.
11. Salpeter SJ, Khalaileh A, Weinberg-Corem N, Ziv O, Glaser B, Dor Y. Systemic regulation of the age-related decline of pancreatic β-cell replication. Diabetes 2013;62:2843–8.

12. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, et al. Growth Differentiation Factor 11 Is a Circulating Factor that Reverses Age-Related Cardiac Hypertrophy. Cell 2013;153:828–39.
13. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron 2015;85:296–302.
14. Erdő F, Denes L, de Lange E. Age-associated physiological and pathological changes at the blood-brain barrier: A review. J Cereb Blood Flow Metab 2017;37:4–24.
15. Sharif Y, Jumah F, Coplan L, Krosser A, Sharif K, Tubbs RS. Blood brain barrier: A review of its anatomy and physiology in health and disease. Clin Anat 2018;31:812–23.
16. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 2011;477:90–4.
17. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science 2014;344: 630–4.
18. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med 2014;20:659–63.
19. Rebo J, Mehdipour M, Gathwala R, Causey K, Liu Y, Conboy MJ, et al. A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood. Nat Commun 2016;7:13363.
20. Gibney BC, Chamoto K, Lee GS, Simpson DC, Miele LF, Tsuda A, et al. Cross-circulation and cell distribution kinetics in parabiotic mice. J Cell Physiol 2012;227:821–8.
21. Khrimian L, Obri A, Ramos-Brossier M, Rousseaud A, Moriceau S, Nicot A-S, et al. Gpr158 mediates osteocalcin's regulation of cognition. J Exp Med 2017;214:2859–73.
22. Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, et al. Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature 2017;544:488–92.
23. Middeldorp J, Lehallier B, Villeda SA, Miedema SSM, Evans E, Czirr E, et al. Preclinical Assessment of Young Blood Plasma for Alzheimer Disease. JAMA Neurol 2016;73:1325.
24. Liu Y-H, Wang Y-R, Xiang Y, Zhou H-D, Giunta B, Mañucat-Tan NB, et al. Clearance of Amyloid-Beta in Alzheimer's Disease: Shifting the Action Site from Center to Periphery. Mol Neurobiol 2015;51:1–7.
25. Xiang Y, Bu X-L, Liu Y-H, Zhu C, Shen L-L, Jiao S-S, et al. Physiological amyloid-beta clearance in the periphery and its therapeutic potential for Alzheimer's disease. Acta Neuropathol 2015;130:487–99.
26. Liu Y, Conboy MJ, Mehdipour M, Liu Y, Tran TP, Blotnick A, et al. Application of bio-orthogonal proteome labeling to cell transplantation and heterochronic parabiosis. Nat Commun 2017;8:643.
27. Elahy M, Jackaman C, Mamo JC, Lam V, Dhaliwal SS, Giles C, et al. Blood-brain barrier dysfunction developed during normal aging is associated with inflammation and loss of tight junctions but not with leukocyte recruitment. Immun Ageing 2015;12:2.
28. Ozek C, Krolewski RC, Buchanan SM, Rubin LL. Growth Differentiation Factor 11 treatment leads to neuronal and vascular improvements in the hippocampus of aged mice. Sci Rep 2018;8: 17293.
29. Tanaka T, Biancotto A, Moaddel R, Moore AZ, Gonzalez-Freire M, Aon MA, et al. Plasma proteomic signature of age in healthy humans. Aging Cell 2018;17:e12799.
30. Schöneich C. Mass spectrometry in aging research. Mass Spectrom Rev 2005;24:701–18.
31. Hoffman JM, Lyu Y, Pletcher SD, Promislow DEL. Proteomics and metabolomics in ageing research: from biomarkers to systems biology. Essays Biochem 2017;61:379–88.
32. Moreno-Villanueva M, Bernhard J, Blasco M, Zondag G, Hoeijmakers JHJ, Toussaint O, et al. MARK-AGE biomarkers of ageing. Mech Ageing Dev 2015;151:2–12.
33. Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, et al. GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. Cell Metab 2015;22:164–74.
34. Corre J, Hébraud B, Bourin P. Concise review: growth differentiation factor 15 in pathology: a clinical role? Stem Cells Transl Med 2013;2:946–52.
35. Smith LK, He Y, Park J-S, Bieri G, Snethlage CE, Lin K, et al. B2-Microglobulin Is a Systemic Pro-Aging Factor That Impairs Cognitive Function and Neurogenesis. Nat Med 2015;21:932–7.
36. Harper SC, Brack A, MacDonnell S, Franti M, Olwin BB, Bailey BA, et al. Is Growth Differentiation Factor 11 a Realistic Therapeutic for Aging-Dependent Muscle Defects? Circ Res 2016;118:1143–50; discussion 1150.
37. McNally EM. Questions and Answers About Myostatin, GDF11, and the Aging Heart. Circ Res 2016;118:6–8.
38. Walker RG, Poggioli T, Katsimpardi L, Buchanan SM, Oh J, Wattrus S, et al. Biochemistry and Biology of GDF11 and Myostatin: Similarities, Differences, and Questions for Future Investigation. Circ Res 2016;118:1125–41; discussion 1142
39. Baptista P, Andrade JP. Adult Hippocampal Neurogenesis: Regulation and Possible Functional and Clinical Correlates. Front Neuroanat 2018;12:44.
40. Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. Nat Med 1998;4:1313–7.
41. Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 2018.
42. Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, et al. Human Hippocampal Neurogenesis Persists throughout Aging. Cell Stem Cell 2018:589–99.
43. Galvan V, Jin K. Neurogenesis in the aging brain. Clin Interv Aging 2007;2:605–10.
44. Yang T-T, Lo C-P, Tsai P-S, Wu S-Y, Wang T-F, Chen Y-W, et al. Aging and Exercise Affect Hippocampal Neurogenesis via Different Mechanisms. PLoS One 2015;10:e0132152.
45. Bordiuk OL, Smith K, Morin PJ, Semënov M V. Cell Proliferation and Neurogenesis in Adult Mouse Brain. PLoS One 2014;9: e111453.
46. Ming G-L, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011;70: 687–702.
47. Delgado-Garcia LM, Amorim RM International Journal of Stem Cell Research & Therapy Adult Brain Neurogenesis, Neural Stem Cells and Neurogenic Niches. Int J Stem Cell Res Ther 2016;3:39.
48. Benveniste H, Liu X, Koundal S, Sanggaard S, Lee H, Wardlaw J. The Glymphatic System and Waste Clearance with Brain Aging: A Review. Gerontology 2018:1–14.
49. Bátiz LF, Castro MA, Burgos P V, Velásquez ZD, Muñoz RI, Lafourcade CA, et al. Exosomes as Novel Regulators of Adult Neurogenic Niches. Front Cell Neurosci 2015;9:501.
50. Mirescu C, Gould E. Stress and adult neurogenesis. Hippocampus 2006;16:233–8.
51. Bjornsson CS, Apostolopoulou M, Tian Y, Temple S. It takes a village: constructing the neurogenic niche. Dev Cell 2015;32: 435–46.
52. Chesnokova V, Pechnick RN, Wawrowsky K. Chronic peripheral inflammation, hippocampal neurogenesis, and behavior. Brain Behav Immun 2016;58:1–8.
53. Solano Fonseca R, Mahesula S, Apple DM, Raghunathan R, Dugan A, Cardona A, et al. Neurogenic Niche Microglia Undergo Positional Remodeling and Progressive Activation Contributing to

Age-Associated Reductions in Neurogenesis. Stem Cells Dev 2016;25:542–55.
54. Licht T, Keshet E. The vascular niche in adult neurogenesis. Mech Dev 2015;138:56–62.
55. Moreno-jiménez EP, Flor-garcía M, Terreros-roncal J, Rábano A, Cafini F, Pallas-bazarra N, et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer ' s disease. Nat Med 2019.
56. Boldrini M, Galfalvy H, Dwork AJ, Rosoklija GB, Trencevska-Ivanovska I, Pavlovski G, et al. Resilience Is Associated With Larger Dentate Gyrus, While Suicide Decedents With Major Depressive Disorder Have Fewer Granule Neurons. Biol Psychiatry 2019;85:850–62.
57. El-Hage W, Leman S, Camus V, Belzung C. Mechanisms of antidepressant resistance. Front Pharmacol 2013;4:146.
58. Smith LK, White CW, Villeda SA, Villeda SA. The systemic environment: at the interface of aging and adult neurogenesis. Cell Tissue Res 2018;371:105–13.
59. Windisch M. We Can Treat Alzheimer's Disease Successfully in Mice but Not in Men: Failure in Translation? A Perspective. Neurodegener Dis 2014;13:147–50.
60. Laub R, Baurin S, Timmerman D, Branckaert T, Strengers P. Specific protein content of pools of plasma for fractionation from different sources: impact of frequency of donations. Vox Sang 2010;99:220–31.
61. Roback JD, Caldwell S, Carson J, Davenport R, Drew MJ, Eder A, et al. Evidence-based practice guidelines for plasma transfusion. Transfusion 2010;50:1227–39.
62. Cooling L. ABO, H and Lewis Blood Groups. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. Tech. Man. 18th ed., AABB; 2014, p. 291–315.
63. Storry JR. Other Blood Group Systems and Antigens. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. Tech. Man. 18th ed., AABB; 2014, p. 337–52.
64. Velthove KJ, Over J, Abbink K, Janssen MP. Viral Safety of Human Plasma–Derived Medicinal Products: Impact of Regulation Requirements. Transfus Med Rev 2013;27:179–83.
65. Katz LM, Rossmann SN. Zika and the blood supply: A work in progress. Arch Pathol Lab Med 2017;141:85–92.
66. Pandey S, Vyas GN. Adverse effects of plasma transfusion. Transfusion 2012;52:65S–79S.
67. Gottlieb S. Statement from FDA Commissioner Scott Gottlieb, M.D., and Director of FDA's Center for Biologics Evaluation and Research Peter Marks, M.D., Ph.D., cautioning consumers against receiving young donor plasma infusions that are promoted as unproven treatment for varying conditions 2019. https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-director-fdas-center-biologics-evaluation-and-0 (accessed May 6, 2019).
68. Sha SJ, Deutsch GK, Tian L, Richardson K, Coburn M, Gaudioso JL, et al. Safety, Tolerability, and Feasibility of Young Plasma Infusion in the Plasma for Alzheimer Symptom Amelioration Study. JAMA Neurol 2018.
69. Cohn EJ, Strong LE, Hughes WL, Mulford DJ, Ashworth JN, Melin M, et al. Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids. J Am Chem Soc 1946;68:459–75.
70. Schneider W, Wolter D, McCarty LJ. Alternatives for Plasma Fractionation. Vox Sang 1976;31:141–51.
71. Relkin NR, Szabo P, Adamiak B, Burgut T, Monthe C, Lent RW, et al. 18-Month study of intravenous immunoglobulin for treatment of mild Alzheimer disease. Neurobiol Aging 2009;30:1728–36.
72. Relkin N. Clinical Trials of Intravenous Immunoglobulin for Alzheimer's Disease. J Clin Immunol 2014;34:74–9.
73. Milojevic J, Costa M, Ortiz AM, Jorquera JI, Melacini G. In Vitro Amyloid-β Binding and Inhibition of Amyloid-β Self-Association by Therapeutic Albumin. J Alzheimers Dis 2013;38:753–65.
74. Boada M, Anaya F, Ortiz P, Olazarán J, Shua-Haim JR, Obisesan TO, et al. 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. J Alzheimers Dis 2017;56:129–43.
75. Zhang Y, Lee DHS. Sink Hypothesis and Therapeutic Strategies for Attenuating Aβ Levels. Neurosci 2011;17:163–73.
76. Boada M, López O, Núñez L, Szczepiorkowski ZM, Torres M, Grifols C, et al. Plasma exchange for Alzheimer's disease Management by Albumin Replacement (AMBAR) trial: Study design and progress. Alzheimers Dement (New York, N Y) 2019;5:61–9.
77. Thornton PL, Ingram RL, Sonntag WE. Chronic [D-Ala 2]-Growth Hormone-Releasing Hormone Administration Attenuates Age-Related Deficits in Spatial Memory. vol. 55. 2000.
78. Mariño G, Ugalde AP, Fernández AF, Osorio FG, Fueyo A, Freije JMP, et al. Insulin-like growth factor 1 treatment extends longevity in a mouse model of human premature aging by restoring somatotroph axis function. Proc Natl Acad Sci U S A 2010;107:16268–73.
79. Junnila RK, List EO, Berryman DE, Murrey JW, Kopchick JJ. The GH/IGF-1 axis in ageing and longevity. Nat Rev Endocrinol 2013;9:366–76.
80. Mao K, Quipildor GF, Tabrizian T, Novaj A, Guan F, Walters RO, et al. Late-life targeting of the IGF-1 receptor improves healthspan and lifespan in female mice. Nat Commun 2018;9:2394.
81. Elabd C, Cousin W, Upadhyayula P, Chen RY, Chooljian MS, Li J, et al. Oxytocin is an age-specific circulating hormone that is necessary for muscle maintenance and regeneration. Nat Commun 2014;5:4082.