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(54) Title: BLOOD PLASMA FRACTIONS AS A TREATMENT FOR CHEMOTHERAPY INDUCED COGNITIVE DISORDERS

(57) Abstract: Methods and compositions for treating chemotherapy induced cognitive disorders, e.g., cognitive impairment, are provided. The compositions used in the methods include blood plasma and blood plasma fractions derived from blood plasma with efficacy in treating chemotherapy induced cognitive disorders.

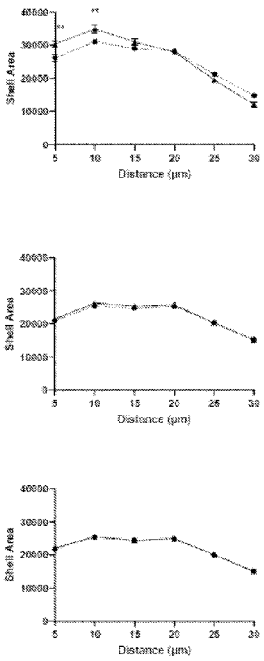

FIG. 5A

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BLOOD PLASMA FRACTIONS AS A TREATMENT FOR CHEMOTHERAPY INDUCED COGNITIVE DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

Pursuant to 35 U.S.C. § 119(e), this application claims priority to the filing dates of: U.S. Provisional Application Serial No. 63/559,401 filed on February 29, 2024, U.S. Provisional Application Serial No. 63/548,302 filed on November 13, 2023, and U.S. Provisional Application Serial No. 63/472,737 filed on June 13, 2023; the disclosures of these applications are herein incorporated by reference.

FIELD OF THE INVENTION

This invention pertains to the prevention and treatment of chemotherapy induced cognitive disorders, such as chemotherapy induced cognitive impairment (CICI). The invention relates to the use of blood products, such as blood plasma fractions, to treat and/or prevent cognitive disorders, e.g., impairment associated with chemotherapy, such as CICI.

BACKGROUND

The following is offered as background information only and is not admitted to be prior art to the present invention.

Cisplatin (CisPt) and other platinum (Pt)-based antineoplastic drugs (e.g., carboplatin (CarboPt), oxaliplatin (OxaliPt)) are highly effective and widely used in the treatment of solid tumors in both children and adult patients. The antineoplastic mechanism of CisPt involves formation of intrastrand cross-links that disrupt the DNA helical structure necessary for transcription. This initiates apoptotic cell death through DNA damage-recognition pathways.

While cisplatin is used extensively to treat solid-state tumors, 35-85% of patients suffer from side effects including long term cognitive dysfunction, impaired memory and attention, and decreased executive function. Cisplatin administration in juvenile rats and adult mice induces blood-brain barrier dysfunction, resulting in cisplatin crossing into the brain parenchyma,

inhibition of neuronal stem cell proliferation and neurogenesis, changes in white matter, increased reactive oxygen species (ROS), accelerated biological aging, increased neuroinflammatory state and reduction in cognitive function.

SUMMARY

Therefore, therapeutics are desired to treat or prevent these neurotoxic effects resulting from a chemotherapy, such as cisplatin treatment.

The present disclosure is based on the production and use of blood products for treating and/or preventing chemotherapy induced cognitive disorders, such as CICI. Derived from blood and blood plasma, the compositions described in this disclosure relate to a solution for the failures and shortcomings of current therapies. Particularly, blood plasma fractions disclosed herein exhibit efficacy in the treatment and/or prevention of chemotherapy induced cognitive disorders, such as CICI. Additionally, the disclosure relates to proteins identified in blood plasma fractions that either may exhibit efficacy as treatments or preventative agents for chemotherapy induced cognitive disorders, such as CICI.

The disclosure also describes that differences in protein content between different blood plasma fractions (e.g., fractions, effluents, "Plasma Fractions," Plasma Protein Fraction, Human Albumin Solution) can be responsible for preventing and/or improving certain cognitive impairments associated with chemotherapy.

Certain aspects of the disclosure provide, methods of treating or preventing chemotherapy induced cognitive disorder, e.g., CICI. Certain aspects of the methods disclosed herein include administering a blood plasma fraction to an individual suffering from or at risk of developing a chemotherapy induced cognitive disorder, e.g., CICI. Additional aspects of the methods include administering a blood plasma fraction derived from a pool of donors of a specific age range to an individual suffering from or at risk of developing a chemotherapy induced cognitive disorder, such as CICI. Also provided are reagents, devices, and kits thereof that find use in practicing the subject methods.

In an embodiment, the blood plasma fraction may be, for example, one of several blood plasma fractions obtained from a blood fractionation process, such as the Cohn fractionation process described below. In another embodiment, the blood plasma fraction may be of the type, herein referred to as "Plasma Fraction," which is a solution comprised of one or more of human

albumin, alpha and beta globulins, gamma globulin, and other proteins, either individually or as complexes. In another embodiment, the blood plasma fraction may be a type of blood plasma fraction known to those having skill in the art as a "Plasma Protein Fraction" (PPF). In another embodiment, the blood plasma fraction may be a "Human Albumin Solution" (HAS) fraction. In yet another embodiment, the blood plasma fraction may one in which substantially all of the clotting factors are removed to retain the efficacy of the fraction with reduced risk of thromboses. Embodiments of the invention may also include administering, for example, a fraction derived from a young donor or pools of young donors. Another embodiment of the invention may include the monitoring of cognitive improvement in a subject treated with a blood plasma fraction.

INCORPORATION BY REFERENCE

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF DRAWINGS

The patent or application file contains a least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1. Certain examples of symptoms of a CICI. CICI Internal Validation Significant Findings in a mouse model tested with 2 dosing paradigms: Cisplatin injection (1 mg/kg italicized) and (2.3 mg/kg underlined).

FIGS. 2A-2C. Certain molecular effects involved in the development of CICI. Effects of chemotherapy on proliferation (A), neurogenesis (B), and BBB permeability (C) are shown. For the graphs in A and B: for each time point, bars from left to right: vehicle, 1 mg/kg cisplatin, 1 mg/kg cisplatin + 100 mg/kg metformin, 2.3 mg/kg cisplatin, 2.3 mg/kg cisplatin + 100 mg/kg metformin. For the graph in C: for each timepoint, bars from left to right: 1 mg/kg cisplatin, 1 mg/kg cisplatin + 100 mg/kg metformin, 2.3 mg/kg cisplatin, 2.3 mg/kg cisplatin + 100 mg/kg metformin. Data was normalized to the average of vehicle treated mice at 100%.

FIGS. 3A-3B. Experimental design and core concept. A. Timeline of dosing and subsequent experiments for 24-hr (n = 4/group), 2 wk (n = 11-12), and 6 wk (n = 10-14) time points. B. Comparison of conventional line scan approach and segmentation detection.

FIGS. 4A-4C. Histological analysis approach. A. 20X images taken with Zeiss Axioscan of entire rostral hippocampus for Hoechst-33342 or nuclear stain (blue), AQP4-AF647 (white), lectin-AF594 (green). B. 100% zoomed region of representative image taken from CA1. BV, blood vessel. C. Example of shell analysis used as an alternative to the conventional line scan. Rings denote radii from nearest vascular object in 5 μm steps from which target protein AQP4 is measured.

FIGS. 5A-5D show total extravascular AQP4 signal by cisplatin treatment. For each A to D, the top panel shows the data for 24 weeks, the middle panel shows the data for 2 weeks, and the bottom panel shows the data for 6 weeks. Also, the dotted line graph represents vehicle treated and solid line represents 2.3 mg/kg cisplatin treated mice. Moreover, the lighter bars show vehicle treated and darker bars show 2.3 mg/kg cisplatin treated mice. A. The area in pixels contained in each shell starting at 5 μm to 30 μm from nearest vascular (lectin+) segment at 24 hours, 2 weeks, and 6 weeks post cisplatin treatment. B. The area under the curve (AUC) generated from line graphs in A. C. The cumulative AQP4-AF647 signal across distance, showing a linear increase in total AF647 signal as distance from the nearest vascular signal increases. D. The AUC generated from cumulative AQP4-AF647 signal shown in panel C. For A and C: ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$ *$p < 0.05$, n = 4-12. For B and D: Analysis of XY plot performed with Two-way ANOVA with Sidak post-hoc test for multiple comparisons. Analysis of AUC performed with t-test. Data are mean ± SEM.

FIGS. 6A-6B. Cisplatin-induced BBB leakage of sodium fluorescein correlates with extravascular AQP4 signal. For each graph, the top panel shows the data for 24 weeks and the bottom panel shows the data for 6 weeks. A shows sodium fluorescein (NaF) signal (RFU) in cortex homogenate from 24 hour and 6 week samples as a measurement of extravasation. The lighter bars show vehicle treated and darker bars show 2.3 mg/kg cisplatin treated mice. B. Sodium fluorescein strongly correlated with 20 μm shell AQP4 signal at 24 hours but not 6 weeks. The circles show the data for vehicle treated and triangles show the data for 2.3 mg/kg cisplatin treated mice. *$p < 0.05$, n = 4-12, analysis of NaF fluorescent intensity performed with t-test. Data are mean ± SEM.

FIGS. 7A-7B show that the segmentation method detects AQP4 mislocalization up to 2 weeks post-cisplatin treatment. For each A and B, the top panel shows the data from 24 weeks, the middle panel shows the data from 2 weeks, and the bottom panel shows the data from 6 weeks. A. AQP4-AF647 fluorescence (RFU) measured in each 5 μm shell from nearest vascular segment normalized to total area (pixels) contained in the shell (as measured in FIG. 5A). The circles show the data for vehicle treated and triangles show the data for 2.3 mg/kg cisplatin treated mice. B. The area under the curve generated from A. The lighter bars show vehicle treated and darker bars show 2.3 mg/kg cisplatin treated mice. $^{**}p < 0.01$, $^{*}p < 0.05$, n = 4-12. Analysis of XY plot performed with Two-way ANOVA with Sidak post-hoc test for multiple comparisons and analysis of AUC performed with t-test. Data are mean ± SEM.

FIGS. 8A-8C. Changes in Oligodendrocyte Lineage Cell Population. A. Study design. B. Representative hippocampal image demonstrating OLIG2 (white) and DAPI (blue). C. Effects of cisplatin treatment on total oligodendrocyte counts in the hippocampus measured by OLIG2+ cells at 3, 14, or 42 days following the last cisplatin treatment. Cisplatin treated group was normalized to vehicle treated group and analyzed by Mann-Whitney tests.

FIGS. 9A-9D. Cisplatin impact on oligodendrocyte is stage-specific. A and B. Representative 200 μm by 200 μm CA1 images demonstrating oligodendrocytes (OL) (white), oligodendrocyte precursor cells (OPCs) (red), mature OLs (green), and DAPI (blue). Yellow arrow indicates a colocalizing cell. C. Effects of cisplatin treatment on OPC population measured by OLIG2+ GPR17+ colocalized cell counts in the hippocampus at 3, 14, or 42 days following the last cisplatin treatment. Cisplatin treated group was normalized to vehicle treated group and analyzed by Mann-Whitney tests. $p < 0.05$. D. Effects of cisplatin treatment on mature oligodendrocyte population measured by OLIG2+ GSTπ+ colocalized cell counts in the hippocampus at 3, 14, or 42 days following the last cisplatin treatment. Cisplatin treated group was normalized to vehicle treated group and analyzed by Mann-Whitney tests.

FIGS. 10A-10B. Metformin attenuates cisplatin impact on OPC. A. Fold change from vehicle treatment group of count per mm^2 quantification of OLIG2+, GPR17+, or GSTπ+ cells at 3 days post cisplatin and Metformin treatment. Cisplatin treated group was normalized to vehicle treated group and analyzed by Kruskal-Wallis tests with Dunn's multiple comparisons. B. Fold change from vehicle treatment group of count per mm2 quantification of OLIG2+, GPR17+, or GSTπ+ cells at 14 days post cisplatin and Metformin treatment. Cisplatin treated group was

normalized to vehicle treated group and analyzed by Kruskal-Wallis tests with Dunn's multiple comparisons.

FIG. 11. SOD plasma level is increased with cisplatin treatment. Data was analyzed by Kruskal-Wallis tests with Dunn's multiple comparisons. *** $p < 0.001$, **$p < 0.01$, *$p < 0.05$.

FIGS. 12A-12B. Relative pattern changes and association of oligodendrocyte population and oxidative stress. A. General changes in oligodendrocyte lineage cell population pattern with cisplatin treatment. B. Relationship between oligodendrocyte population and oxidative stress level with and without prophylactic Metformin treatment. Left panel illustrates general changes in oligodendrocyte lineage cell population pattern with cisplatin treatment. Right panel shows relationship between oligodendrocyte population and oxidative stress level with and without prophylactic metformin treatment.

FIGS. 13A-13F. PPF shows modest improvement in spatial cognition in cisplatin treated mice as estimated in a Y-maze assay. A. Experimental design. B. % Body weight change in cisplatin and PPF (identified as GRF6020) + cisplatin treated mice as compared to vehicle treatment. ****$P < 0.0001$, 2-Way ANOVA with Sidak's Post Hoc. C. Distance traveled in Open Field test (PPF identified as PF in this Figure). D. 2-trial Y-maze layout, training and testing time, and ITI (intertrial interval) obtained from Sithisarn *et al.* (2013), Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine Volume 2013, Article ID 701956, 9 pages). E. Y-maze % Novel arm time represented as (novel arm time / (novel arm time + familiar arm time) x 100. PPF (identified as PF in this Figure) + cisplatin (mean = 58.07 ± S.E.M.) **$P < 0.01$ vs. null hypothesis of 50%, Wilcoxon ranked sign test. Cisplatin (mean = 50.21 ± S.E.M.). F. Novel Object Recognition (NOR) test. % Novel interaction time in nonsignificant (NS) increase in PPF (PPF identified as PF in this Figure) + cisplatin (mean=55.26 ± S.E.M.) vs. null hypothesis of 50%. Cisplatin (mean = 49.71 ± S.E.M.).

FIGS. 14A-14G. PPF (identified as PF in this Figure) moderately attenuates neurogenesis and reductions in proliferation in cisplatin treated mice. A. Neurogenesis as determined by DCX positive labeled cells within the granule layer of the dentate gyrus in cisplatin, cisplatin + PF and vehicle treated animals. Cisplatin treatment (mean=1639.92 ± S.E.M.), vs. vehicle (mean=2371.32 ± S.E.M.), n = 12 per group, *$P < 0.05$, One Way ANOVA with Dunnett's Post Hoc. B. Proliferation as measured by Ki67 expressing cells within the primary granule cell layer of the dentate gyrus. Cisplatin treatment (mean=368.5 ± S.E.M.), vs. vehicle (mean=576.9 ± S.E.M.), n

= 12 per group, *P < 0.05, One Way ANOVA with Dunnett's Post Hoc. C. Plasma cytokine TNFα in cisplatin and cisplatin + PPF treated mice. D. Plasma cytokine IFNγ in cisplatin and cisplatin + PPF treated mice. E. DCX positive cells (white) within the hippocampus of vehicle treated mice. F. DCX positive cells (white) within the hippocampus of PPF + cisplatin treated mice. G. DCX positive cells (white) within the hippocampus of cisplatin treated mice.

DETAILED DESCRIPTION

Introduction

The present disclosure relates to the identification and discovery of methods and compositions for the treatment and/or prevention of chemotherapy induced cognitive disorders, e.g., CICI. Described herein in some embodiments are methods and compositions for the treatment of subjects suffering from such disorders. In some cases, the methods and compositions described herein are useful in: preventing chemotherapy induced cognitive disorders, e.g., CICI; ameliorating the symptoms of chemotherapy induced cognitive disorders, e.g., CICI; slowing progression of chemotherapy induced cognitive disorders, e.g., CICI; and/or reversing the progression of chemotherapy induced cognitive disorders, e.g., CICI.

Certain nervous system related symptoms of chemotherapy induced cognitive disorders are provided in FIG. 1. Moreover, certain molecular effects of chemotherapy induced neurological changes are described in FIGS. 2A-2C.

Certain embodiments of the disclosure include using in the methods disclosed herein certain blood plasma fractions, such as one or more fractions or effluents obtained from blood fractionation processes, e.g., the Cohn fractionation process described below. An embodiment of the invention includes using a Plasma Fraction (a solution comprised of normal human albumin, alpha and beta globulins, gamma globulin, and other proteins either individually or as complexes, hereinafter referred to as "Plasma Fraction"). Another embodiment of the invention includes using in the methods disclosed herein Plasma Protein Fraction (PPF). A further embodiment of the invention includes using in the methods disclosed herein Human Albumin Solution (HAS) fraction. Yet another embodiment includes using in the methods disclosed herein effluents from blood fractionation processes such as Effluent I or Effluent II/III. An additional embodiment

includes using in the methods disclosed herein a blood plasma fraction from which substantially all the clotting factors have been removed to retain efficacy while reducing the risk of thromboses.

Before describing the present invention in detail, it is to be understood that this invention is not limited to a particular method or composition described, as such may, of course, vary. It is also understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

It is noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein have discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or the spirit of the present invention. Any recited

method can be carried out in the order of events recited or in any other order which is logically possible.

Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one having ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure supersedes any disclosure of an incorporated publication to the extent there is a contradiction.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those having skill in the art, and so forth.

In describing methods of the present invention, the terms "host," "subject," "individual," and "patient" are used interchangeably and refer to any mammal in need of such treatment according to the disclosed methods. Such mammals include, e.g., humans, ovines, bovines, equines, porcines, canines, felines, non-human primate, mice, and rats. In certain embodiments, the subject is a non-human mammal. In some embodiments, the subject is a farm animal. In other embodiments, the subject is a pet. In some embodiments, the subject is mammalian. In certain instances, the subject is human. Other subjects can include domestic pets (e.g., dogs and cats), livestock (e.g., cows, pigs, goats, horses, and the like), rodents (e.g., mice, guinea pigs, and rats, e.g., as in animal models of disease), as well as non-human primates (e.g., chimpanzees, and monkeys). As such, subjects of the invention, include but are not limited to mammals, e.g., humans and other primates, such as chimpanzees and other apes and monkey species; and the like, where in certain embodiments the subject are humans. The term subject is also meant to include a person or organism of any age, weight or other physical characteristic, where the subjects may be an adult, a child, an infant or a newborn.

The term “young” or "young individual" as it relates humans, refers to an individual human that is of chronological age of 40 years old or younger, e.g., 35 years old or younger, including 30 years old or younger, e.g., 25 years old or younger or 22 years old or younger. In some instances, the individual human that serves as the source of the young plasma-comprising blood product is one that is 10 years old or younger, e.g., 5 years old or younger, including 1-year-old or younger. In some instances, the subject is a newborn human and the source of the plasma product is the umbilical cord, where the plasma product is harvested from the umbilical cord of the newborn human. As such, “young” and "young individual" may refer to a human that is between the ages of 0 and 40, e.g., 0, 1, 5, 10, 15, 20, 25, 30, 35, or 40 years old. In other instances, “young” and “young individual” may refer to a biological (as opposed to chronological) age such as an individual who has not exhibited the levels of inflammatory cytokines in the plasma exhibited in comparatively older individuals. Conversely, these “young” and “young individual” may refer to a biological (as opposed to chronological) age such as an individual who exhibits greater levels of anti-inflammatory cytokines in the plasma compared to levels in comparatively older individuals. By way of example, and not limitation, the inflammatory cytokine is Eotaxin, and the fold difference between a young subject or young individual and older individuals is at least 20%. Similarly, the fold difference between older and younger individuals in other inflammatory cytokines may be used to refer to a biological age. (*See* U.S. Pat. Application No. 13/575,437 published as US 20130040844 A1 which is herein incorporated by reference). Usually, the individual is healthy, e.g., the individual has no hematological malignancy or autoimmune disease at the time of harvest.

The term “young” or “young individual” as it may relate to a non-human animal species refers to an animal considered “young” in the corresponding animal species depending on the life-span/life-cycle of the individuals of the animal species.

As used herein, “treatment” refers to any of (i) the prevention of the disease or disorder, or (ii) the reduction or elimination of symptoms of the disease or disorder. Treatment may be effected prophylactically (prior to the onset of disease) or therapeutically (following the onset of the disease). The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. Thus, the term “treatment” as used herein covers any treatment of a chemotherapy induced cognitive disorder, e.g., CICI, in a mammal, and

includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. Treatment may result in a variety of different physical manifestations, e.g., modulation in gene expression, rejuvenation of tissue or organs, etc. The therapeutic agent may be administered before, during or after the onset of disease. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment may be performed prior to complete loss of function in the affected tissues. The subject therapy may be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease.

In some embodiments, the disorder that is treated is chemotherapy induced cognitive disorder, e.g., CICI. By cognitive ability, or "cognition," it is meant the mental processes that include attention and concentration, learning complex tasks and concepts, memory (acquiring, retaining, and retrieving new information in the short and/or long term), information processing (dealing with information gathered by the five senses), visuospatial function (visual perception, depth perception, using mental imagery, copying drawings, constructing objects or shapes), producing and understanding language, verbal fluency (word-finding), solving problems, making decisions, and executive functions (planning and prioritizing). By "cognitive decline." it is meant a progressive decrease in one or more of these abilities, e.g., a decline in memory, language, thinking, judgment, etc. By "an impairment in cognitive ability" and "cognitive impairment," it is meant a reduction in cognitive ability relative to a healthy individual, e.g., an age-matched healthy individual, or relative to the ability of the individual at an earlier point in time, e.g., 2 weeks, 1 month, 2 months, 3 months, 6 months, 1 year, 2 years, 5 years, or 10 years or more previously. By "chemotherapy induced cognitive disorder, e.g., chemotherapy induced cognitive impairment," it is meant an impairment in cognitive ability that is typically associated with chemotherapy, e.g., platinum based chemotherapy, e.g., cisplatin therapy.

Blood Products Comprising Plasma Components

In practicing the subject methods, a blood product comprising plasma components is administered to an individual in need thereof, e.g., an individual suffering or at risk of suffering from a chemotherapy induced cognitive disorder, e.g., CICI. As such, methods according to

embodiments of the invention including administering a blood product comprising plasma components from an individual (the "donor individual", or "donor") to an individual at least at risk of suffering or suffering from chemotherapy induced cognitive disorder, e.g., CICI (the "recipient individual" or "recipient"). By a "blood product comprising plasma components," it is meant any product derived from blood that comprises plasma (e.g. whole blood, blood plasma, or fractions thereof). The term "plasma" is used in its conventional sense to refer to the straw-colored/pale-yellow liquid component of blood composed of about 92% water, 7% proteins such as albumin, gamma globulin, anti-hemophilic factor, and other clotting factors, and 1 % mineral salts, sugars, fats, hormones and vitamins. Non-limiting examples of plasma-comprising blood products suitable for use in the subject methods include whole blood treated with anti-coagulant (e.g., EDTA, citrate, oxalate, heparin, etc.), blood products produced by filtering whole blood to remove white blood cells ("leukoreduction"), blood products consisting of plasmapheretically-derived or aphereticallyderived plasma, fresh-frozen plasma, blood products consisting essentially of purified plasma, and blood products consisting essentially of plasma fractions. In some instances, plasma product that is employed is a non-whole blood plasma product, by which is meant that the product is not whole blood, such that it lacks one or more components found in whole blood, such as erythrocytes, leukocytes, etc., at least to the extent that these components are present in whole blood. In some instances, the plasma product is substantially, if not completely, acellular, where in such instances the cellular content may be 5% by volume or less, such as 1 % or less, including 0.5% or less, where in some instances acellular plasma fractions are those compositions that completely lack cells, i.e., they include no cells.

Collection of blood products comprising plasma components.

Embodiments of the methods described herein include administration of blood products comprising plasma components which can be derived from donors, including human volunteers. The term, "human-derived" can refer to such products. Methods of collection of plasma comprising blood products from donors are well-known in the art. (See, e.g., AABB TECHNICAL MANUAL, (Mark A. Fung, et al., eds., 18th ed. 2014), herein incorporated by reference).

In one embodiment, donations are obtained by venipuncture. In another embodiment, the venipuncture is only a single venipuncture. In another embodiment, no saline volume replacement is employed. In an embodiment, the process of plasmapheresis is used to obtain the plasma

comprising blood products. Plasmapheresis can comprise the removal of a weight-adjusted volume of plasma with the return of cellular components to the donor. In the embodiment, sodium citrate is used during plasmapheresis in order to prevent cell clotting. The volume of plasma collected from a donor is preferably between 690 to 880 mL after citrate administration, and preferably coordinates with the donor's weight.

Blood Plasma Fractions

During the Second World War, there arose a need for a stable plasma expander which could be employed in the battlefield when soldiers lost large amounts of blood. As a result, methods of preparing freeze-dried plasma were developed. However, use of freeze-dried plasma was difficult in combat situations since reconstitution required sterile water. As an alternative, Dr. E.J. Cohn suggested that albumin could be used, and prepared a ready-to-use stable solution that could be introduced immediately for treatment of shock. (*See* JOHAN VANDERSANDE, CURRENT APPROACHES TO THE PREPARATION OF PLASMA FRACTIONS in (BIOTECHNOLOGY OF BLOOD) 165 (Jack Goldstein ed., 1st ed. 1991)). Dr. Cohn's procedure of purifying plasma fractions utilized cold ethanol for its denaturing effect, and employs changes in pH and temperature to achieve separation.

An embodiment of the methods described herein includes the administration of plasma fractions to a subject. Fractionation is the process by which certain protein subsets are separated from plasma. Fractionation technology is known in the art and relies on steps developed by Cohn *et al.* during the 1940s. (E. Cohn, *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.* 68 J Am Chem Soc 459 (1946), herein incorporated by reference). Several steps are involved in this process, each step involving specific ethanol concentrations as well as pH, temperature, and osmolality shifts which result in selective protein precipitation. Precipitates are also separated via centrifugation or precipitation. The original "Cohn fractionation process" involved separation of proteins through precipitates into five fractions, designated fraction I, fraction II+III, fraction IV-1, fraction IV-4 and fraction V. Albumin was the originally identified endpoint (fraction V) product of this process. In accordance with embodiments of the invention, each fraction (or effluent from a prior separation step) contains or potentially contains therapeutically-useful protein fractions. (*See* Thierry Burnouf, *Modern Plasma Fractionation*,

21(2) Transfusion Medicine Reviews 101 (2007); Adil Denizli, *Plasma fractionation: conventional and chromatographic methods for albumin purification*, 4 J. Biol. & Chem. 315, (2011); and T. Brodniewicz-Proba, *Human Plasma Fractionation and the Impact of New Technologies on the Use and Quality of Plasma-derived Products*, 5 Blood Reviews 245 (1991), and U.S. Patent Nos. 3869431, 5110907, 5219995, 7531513, and 8772461 which are herein incorporated by reference). Adjustment of the above experimental parameters can be made in order to obtain specific protein fractions.

More recently, fractionation has reached further complexity, and as such, comprises additional embodiments of the invention. This recent increase in complexity has occurred through: the introduction of chromatography resulting in isolation of new proteins from existing fractions like cryoprecipitate, cryo-poor plasma, and Cohn fractions; increasing IgG recovery by integrating chromatography and the ethanol fractionation process; and viral reduction/inactivation/removal. (*Id.*) In order to capture proteins at physiological pH and ionic strength, anion-exchange chromatography can be utilized. This preserves functional activity of proteins and/or protein fractions. Heparin and monoclonal antibodies are also used in affinity chromatography. One of ordinary skill in the art would recognize that the parameters described above may be adjusted to obtain specifically-desired plasma protein-containing fractions.

In an embodiment of the invention, blood plasma is fractionated in an industrial setting. Frozen plasma is thawed at 1°C to 4°C. Continuous refrigerated centrifugation is applied to the thawed plasma and cryoprecipitate isolated. Recovered cryoprecipitate is frozen at -30°C or lower and stored. The cryoprecipitate-poor ("cryo-poor") plasma is immediately processed for capture (via, for example, primary chromatography) of labile coagulation factors such as factor IX complex and its components as well as protease inhibitors such as antithrombin and C1 esterase inhibitor. Serial centrifugation and precipitate isolation can be applied in subsequent steps. Such techniques are known to one of ordinary skill in the art and are described, for example, in U.S. patent nos. 4624780, 5219995, 5288853, and U.S. patent application nos. 20140343255 and 20150343025, which disclosures are incorporated by reference in their entirety herein.

In an embodiment of the invention, the plasma fraction may comprise a plasma fraction containing a substantial concentration of albumin. In another embodiment of the invention, the plasma fraction may comprise a plasma fraction containing a substantial concentration of IgG or intravenous immune globulin (IGIV) (e.g. Gamunex-C®). In another embodiment of the invention

the plasma fraction may comprise an IGIV plasma fraction, such as Gamunex-C® which has been substantially depleted of immune globulin (IgG) by methods well-known by one of ordinary skill in the art, such as for example, Protein A-mediated depletion. (See Keshishian, H., *et al.*, *Multiplexed, Quantitative Workflow for Sensitive Biomarker Discovery in Plasma Yields Novel Candidates for Early Myocardial Injury*, Molecular & Cellular Proteomics, 14 at 2375-93 (2015)). In an additional embodiment, the blood plasma fraction may be one in which substantially all the clotting factors are removed in order to retain the efficacy of the fraction with reduced risk of thromboses. For example, the plasma fraction may be a plasma fraction as described in United States Patent No. 62/376,529 filed on August 18, 2016; the disclosure of which is incorporated by reference in its entirety herein.

Albumin Products

To those having ordinary skill in the art, there are two general categories of Albumin Plasma Products ("APP"): plasma protein fraction (PPF) and human albumin solution (HAS). PPF is derived from a process with a higher yield than HAS, but has a lower minimum albumin purity than HAS (>83% for PPF and > 95% for HAS). (*Production of human albumin solution: a continually developing colloid*, P. Matejtschuk *et al.*, British J. of Anaesthesia 85(6): 887-95, at 888 (2000)). Additionally, some have noted that PPF has a disadvantage because of the presence of protein "contaminants" such as PKA. *Id.* As a consequence, PPF preparations have lost popularity as Albumin Plasma Products, and have even been delisted from certain countries' Pharmacopoeias. *Id.* Contrary to these concerns, the invention makes beneficial use of these "contaminants." Besides α, β, and γ globulins, as well as the aforementioned PKA, the methods of the invention utilize additional proteins or other factors within the "contaminants" that promote processes such as neurogenesis, neuronal cell survival, and improved cognition.

Those of skill in the art will recognize that there are, or have been, several commercial sources of PPF (the "Commercial PPF Preparations.") These include Plasma-Plex™ PPF (Armour Pharmaceutical Co., Tarrytown, NY), Plasmanate™ PPF (Grifols, Clayton, NC), Plasmatein™ (Alpha Therapeutics, Los Angeles, CA), and Protenate™ PPF (Baxter Labs, Inc. Deerfield, IL).

Those of skill in the art will also recognize that there are, or have been, several commercial sources of HAS (the "Commercial HAS Preparations.") These include Albuminar™ (CSL Behring), AlbuRx™ (CSL Behring), Albutein™ (Grifols, Clayton, NC), Buminate™ (Baxatla,

Inc., Bannockburn, IL), Flexbumin™ (Baxatla, Inc., Bannockburn, IL), and Plasbumin™ (Grifols, Clayton, NC).

PPF can be produced from a plasma using a manufacturing process that uses acetone. PPF can also be produced from plasma using a manufacturing process that does not use acetone.

A. Plasma Protein Fraction (Human) (PPF)

According to the United States Food and Drug Administration ("FDA"), "Plasma Protein Fraction (Human)," or PPF, is the proper name of the product defined as "a sterile solution of protein composed of albumin and globulin, derived from human plasma." (Code of Federal Regulations "CFR" 21 CFR 640.90 which is herein incorporated by reference). PPF's source material is plasma recovered from Whole Blood prepared as prescribed in 21 CFR 640.1 – 640.5 (incorporated by reference herein), or Source Plasma prepared as prescribed in 21 CFR 640.60 – 640.76 (incorporated by reference herein).

PPF is tested to determine it meets the following standards as per 21 CFR 640.92 (incorporated by reference herein):

(a) The final product shall be a 5.0 +/- 0.30 percent solution of protein; and

(b) The total protein in the final product shall consist of at least 83 percent albumin, and no more than 17 percent globulins. No more than 1 percent of the total protein shall be gamma globulin. The protein composition is determined by a method that has been approved for each manufacturer by the Director, Center for Biologics Evaluation and Research, Food and Drug Administration.

As used herein, "Plasma Protein Fraction" or "PPF" refers to a sterile solution of protein composed of albumin and globulin, derived from human plasma, with an albumin content of at least 83% with no more than 17% globulins (including α1, α2, β, and γ globulins) and other plasma proteins, and no more than 1% gamma globulin as determined by electrophoresis. (Hink, J.H., Jr., *et al.*, Preparation and Properties of a Heat-Treated Human Plasma Protein Fraction, VOX SANGUINIS 2(174) (1957)). PPF can also refer to a solid form, which when suspended in solvent, has similar composition. The total globulin fraction can be determined through subtracting the albumin from the total protein. (Busher, J., *Serum Albumin and Globulin*, CLINICAL METHODS: THE HISTORY, PHYSICAL, AND LABORATORY EXAMINATIONS, Chapter 10, Walker HK, Hall WD, Hurst JD, eds. (1990)).

B. Albumin (Human) (HAS)

According to the FDA, "Albumin (Human)" (also referred to herein as "HAS") is the proper name of the product defined as "sterile solution of the albumin derived from human plasma." (Code of Federal Regulations "CFR" 21 CFR 640.80 which is herein incorporated by reference.) The source material for Albumin (Human) is plasma recovered from Whole Blood prepared as prescribed in 21 CFR 640.1-640.5 (incorporated by reference herein), or Source Plasma prepared as prescribed in 21 CFR 640.60-640.76 (incorporated by reference herein). Other requirements for Albumin (Human) are listed in 21 CFR 640.80 – 640.84 (incorporated by reference herein).

Albumin (Human) is tested to determine if it meets the following standards as per 21 CFR 640.82:

(a) *Protein concentration.* Final product shall conform to one of the following concentrations: 4.0 +/-0.25 percent; 5.0 +/-0.30 percent; 20.0 +/-1.2 percent; and 25.0 +/-1.5 percent solution of protein.

(b) *Protein composition.* At least 96 percent of the total protein in the final product shall be albumin, as determined by a method that has been approved for each manufacturer by the Director, Center for Biologics Evaluation and Research, Food and Drug Administration.

As used herein, "Albumin (Human)" or "HAS" refers to a to a sterile solution of protein composed of albumin and globulin, derived from human plasma, with an albumin content of at least 95%, with no more than 5% globulins (including $\alpha 1$, $\alpha 2$, β, and γ globulins) and other plasma proteins. HAS can also refer to a solid form, which when suspended in solvent, has similar composition. The total globulin fraction can be determined through subtracting the albumin from the total protein.

As can be recognized by one having ordinary skill in the art, PPF and HAS fractions can also be freeze-dried or in other solid form. Such preparations, with appropriate additives, can be used to make tablets, powders, granules, or capsules, for example. The solid form can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or non-aqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

Clotting Factor-Reduced Fractions

Another embodiment of the invention uses a blood plasma fraction from which substantially all of the clotting factors are removed in order to retain the efficacy of the fraction with reduced risk of thromboses. Conveniently, the blood product can be derived from a young donor or pool of young donors, and can be rendered devoid of IgM in order to provide a young blood product that is ABO compatible. Currently, plasma that is transfused is matched for ABO blood type, as the presence of naturally occurring antibodies to the A and B antigens can result in transfusion reactions. IgM appears to be responsible for transfusion reactions when patients are given plasma that is not ABO matched. Removal of IgM from blood products or fractions helps eliminate transfusion reactions in subjects who are administered the blood products and blood plasma fractions of the invention.

Accordingly, in one embodiment, the invention is directed to a method of treating or preventing a chemotherapy induced cognitive disorder, e.g., chemotherapy induced cognitive impairment or neurodegeneration in a subject. The method comprises: administering to the subject a blood product or blood fraction derived from whole-blood from an individual or pool of individuals, wherein the blood product or blood fraction is substantially devoid of (a) at least one clotting factor and/or (b) IgM. In some embodiments, the individual(s) from whom the blood product or blood fraction is derived are young individuals. In some embodiments, the blood product is substantially devoid of at least one clotting factor and IgM. In certain embodiments, the blood product is substantially devoid of fibrinogen (Factor I). In additional embodiments, the blood product substantially lacks erythrocytes and/or leukocytes. In further embodiments, the blood product is substantially acellular. In other embodiments, the blood product is derived from plasma. Such embodiments of the invention are further supported by U.S. Patent Application No. 62/376,529 filed on August 18, 2016, which is incorporated by reference in its entirety herein.

Protein-Enriched Plasma Protein Products

Additional embodiments of the invention use plasma fractions with reduced albumin concentration compared to PPF, but with increased amounts of globulins and other plasma proteins (what have been referred to by some as "contaminants"). The embodiments, as with PPF, HAS, Effluent I, and Effluent II/III are all effectively devoid of clotting factors. Such plasma fractions are hereinafter referred to as "protein-enriched plasma protein products." For example, an

embodiment of the invention may use a protein-enriched plasma protein product comprised of 82% albumin and 18% α, β, and γ globulins and other plasma proteins. Another embodiment of the invention may use a protein-enriched plasma protein product comprised of 81% albumin and 19% of α, β, and γ globulins and/or other plasma proteins. Another embodiment of the invention may use a protein-enriched plasma protein product comprised of 80% albumin and 20% of α, β, and γ globulins and/or other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 70-79% albumin and a corresponding 21-30% of α, β, and γ globulins and other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 60-69% albumin and a corresponding 31-40% of α, β, and γ globulins and other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 50-59% albumin and a corresponding 41-50% of α, β, and γ globulins and other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 40-49% albumin and a corresponding 51-60% of α, β, and γ globulins and other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 30-39% albumin and a corresponding 61-70% of α, β, and γ globulins and other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 20-29% albumin and a corresponding 71-80% of α, β, and γ globulins and other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 10-19% albumin and a corresponding 81-90% of α, β, and γ globulins and other plasma proteins. Additional embodiments of the invention may use protein-enriched plasma protein products comprised of 1-9% albumin and a corresponding 91-99% of α, β, and γ globulins and other plasma proteins. A further embodiment of the invention may use protein-enriched plasma protein products comprised of 0-1% albumin and 99-100% of α, β, and γ globulins and other plasma proteins

Embodiments of the invention described above may also have total gamma globulin concentrations of 0-5%.

The specific concentrations of proteins in a plasma fraction may be determined using techniques well-known to a person having ordinary skill in the relevant art. By way of example, and not limitation, such techniques include electrophoresis, mass spectrometry, ELISA analysis, and Western blot analysis.

Preparation of Blood Plasma Fractions

Methods of preparing PPF and other plasma fractions are well-known to those having ordinary skill in the art. An embodiment of the invention allows for blood used in the preparation of human plasma protein fraction to be collected in flasks with citrate or anticoagulant citrate dextrose solution for inhibition of coagulation, with further separation of Fractions I, II + III, IV, and PPF as per the method disclosed in Hink *et al.* (*See* Hink, J.H., Jr., *et al.*, Preparation and Properties of a Heat-Treated Human Plasma Protein Fraction, VOX SANGUINIS 2(174) (1957), herein incorporated by reference.) According to this method, the mixture can be collected to 2 – 8 °C. The plasma can then subsequently be separated by centrifugation at 7°C, removed, and stored at -20°C. The plasma can then be thawed at 37°C and fractionated, preferably within eight hours after removal from -20°C storage.

Plasma can be separated from Fraction I using 8% ethanol at pH 7.2 and a temperature at -2 to -2.5°C with protein concentration of 5.1 to 5.6 percent. Cold 53.3 percent ethanol (176 mL/L of plasma) with acetate buffer (200 mL 4M sodium acetate, 230 mL glacial acetic acid *quantum satis* to 1 L with H_2O) can be added using jets at a rate, for example, of 450 mL/minute during the lowering the plasma temperature to -2°C. Fraction I can be separated and removed from the effluent (Effluent I) through ultracentrifugation. Fibrinogen can be obtained from Fraction I as per methods well-known to those having ordinary skill in the art.

Fraction II + III can be separated from Effluent I through adjustment of the effluent to 21 percent ethanol at pH 6.8, temperature at -6°C, with protein concentration of 4.3 percent. Cold 95 percent ethanol (176 mL/L of Effluent I) with 10 M acetic acid used for pH adjustment can be added using jets at a rate, for example, of 500 mL/minute during the lowering of the temperature of Effluent I to -6°C. The resulting precipitate (Fraction II + III) can be removed by centrifugation at -6°C. Gamma globulin can be obtained from Fraction II + III using methods well-known to those having ordinary skill in the art.

Fraction IV-1 can be separated from Effluent II + III ("Effluent II/III") through adjustment of the effluent to 19 percent ethanol at pH 5.2, temperature at -6°C, and protein concentration of 3 percent. H_20 and 10 M acetic acid used for pH adjustment can be added using jets while maintaining Effluent II/III at -6°C for 6 hours. Precipitated Fraction VI-1 can be settled at -6°C for 6 hours and subsequently separated from the effluent by centrifugation at the same temperature. Stable plasma protein fraction can be recovered from Effluent IV-1 through adjustment of the

ethanol concentration to 30 percent at pH 4.65, temperature -7°C and protein concentration of 2.5 percent. This can be accomplished by adjusting the pH of Effluent IV-1 with cold acid-alcohol (two parts 2 M acetic acid and one part 95 percent ethanol). While maintaining a temperature of -7°C, to every liter of adjusted Effluent IV-1 170 mL cold ethanol (95%) is added. Proteins that precipitate can be allowed to settle for 36 hours and subsequently removed by centrifugation at -7°C.

The recovered proteins (stable plasma protein fraction) can be dried (e.g. by freeze drying) to remove alcohol and H_20. The resulting dried powder can be dissolved in sterile distilled water, for example using 15 liters of water/kg of powder, with the solution adjusted to pH 7.0 with 1 M NaOH. A final concentration of 5 per cent protein can be achieved by adding sterile distilled water containing sodium acetyl tryptophanate, sodium caprylate, and NaCl, adjusting to final concentrations of 0.004 M acetyl tryptophanate, 0.004 M caprylate, and 0.112 M sodium. Finally, the solution can be filtered at 10°C to obtain a clear solution and subsequently heat-treated for inactivation of pathogens at 60°C for at least 10 hours.

One having ordinary skill in the art would recognize that each of the different fractions and effluents described above could be used with the methods of the invention to treat disease. For example, and not by way of limitation, Effluents I or Effluent II/III may be utilized to treat such diseases as cognitive and neurodegenerative disorders and are embodiments of the invention.

The preceding methods of preparing blood plasma fractions and plasma protein fraction (PPF) are only exemplary and involves merely embodiments of the invention. One having ordinary skill in the art would recognize that these methods can vary. For example, pH, temperature, and ethanol concentration, among other things can be adjusted to produce different variations of plasma fractions and plasma protein fraction in the different embodiments and methods of the invention. In another example, additional embodiments of the invention contemplate the use of nanofiltration for the removal/inactivation of pathogens from plasma fractions and plasma protein fraction.

An additional embodiment of the invention contemplates methods and composition using and/or comprising additional blood plasma fractions. For example, the invention, among other things, demonstrates that specific concentrations of albumin are not critical for improving cognitive activity. Hence, fractions with reduced albumin concentration, such as those fractions having below 83% albumin, are contemplated by the invention.

Treatment

Aspects of the methods described herein include treatment of a subject with a plasma comprising blood product, such as a blood plasma fraction, e.g., fractions as described above. An embodiment includes treatment of a human subject with a plasma comprising blood product. One of skill in the art would recognize that methods of treatment of subjects with plasma comprising blood products are recognized in the art. By way of example, and not limitation, one embodiment of the methods of the inventions described herein is comprised of administering fresh frozen plasma to a subject for treatment and/or prevention of chemotherapy induced cognitive disorder, e.g., CICI. In one embodiment, the plasma comprising blood product is administered immediately, e.g., within about 12-48 hours of collection from a donor, to the individual suffering or at risk from chemotherapy induced cognitive disorder, e.g., CICI. In such instances, the product may be stored under refrigeration, e.g., 0-10°C. In another embodiment, fresh frozen plasma is one that has been stored frozen (cryopreserved) at -18°C or colder. Prior to administration, the fresh frozen plasma is thawed and once thawed, administered to a subject 60-75 minutes after the thawing process has begun. Each subject preferably receives a single unit of fresh frozen plasma (200-250 mL), the fresh frozen plasma preferably derived from donors of a pre-determined age range. In one embodiment of the invention, the fresh frozen plasma is donated by (derived from) young individuals. In another embodiment of the invention, the fresh frozen plasma is donated by (derived from) donors of the same gender. In another embodiment of the invention, the fresh frozen plasma is donated by (derived from) donors of the age range between 18-22 years old. In one embodiment, subjects are treated twice per week with 3-4 days between infusions. In an embodiment of the invention, treatment persists until a specific endpoint is reached.

In an embodiment of the invention, the plasma comprising blood products are screened after donation by blood type. In another embodiment of the invention, the plasma comprising blood products are screened for infectious disease agents such as HIV I & II, HBV, HCV, HTLV I & II, anti-HBc per the requirements of 21 CFR 640.33 and recommendations contained in FDA guidance documents.

In yet another embodiment of the invention, the subject is treated with a "Plasma Fraction." In an embodiment of the invention, the Plasma Fraction is PPF or HAS. In a further embodiment of the invention, the Plasma Fraction is one of the Commercial PPF Preparations of the Commercial HAS Preparations. In another embodiment of the invention the Plasma Fraction is a

PPF or HAS derived from a pool of individuals of a specific age range, such as young individuals, or is a modified PPF or HAS fraction which has been subjected to additional fractionation or processing (e.g. PPF or HAS with one or more specific proteins partially or substantially removed). In another embodiment of the invention, the Plasma Fraction is an IGIV plasma fraction which has been substantially depleted of immune globulin (IgG). A blood fraction which is "substantially depleted" or which has specific proteins "substantially removed," such as IgG, refers to a blood fraction containing less than about 50% of the amount that occurs in the reference product or whole blood plasma, such as less than 45%, 40%, 35%, 30%, 25%, 20%, 15%, 5%, 4%, 3%, 2%, 1%, 0.5%, .25%, .1%, undetectable levels, or any integer between these values, as measured using standard assays well known in the art.

Monitoring

Another aspect of the present invention relates to methods of monitoring the effect of a medication on a subject for treating chemotherapy induced cognitive disorder, e.g., CICI, the method comprising comparing cognitive function before and after treatment. Those having ordinary skill in the art recognize that there are well-known methods of evaluating cognitive function. For example, and not by way of limitation, the method may comprise evaluation of cognitive function based on medical history, family history, physical and neurological examinations by clinicians who specialize dementia and cognitive function, laboratory tests, and neuropsychological assessment. Additional embodiments which are contemplated by the invention include: the assessment of consciousness, such as using the Glasgow Coma Scale (EMV); mental status examination, including the abbreviated mental test score (AMTS) or mini-mental state examination (MMSE) (Folstein et al., J. Psychiatr. Res 1975; 12:1289-198); global assessment of higher functions; estimation of intracranial pressure such as by fundoscopy.

In one embodiment, examinations of peripheral nervous system may be used to evaluate cognitive function, including any one of the followings: sense of smell, visual fields and acuity, eye movements and pupils (sympathetic and parasympathetic), sensory function of face, strength of facial and shoulder girdle muscles, hearing, taste, pharyngeal movement and reflex, tongue movements, which can be tested individually (e.g. the visual acuity can be tested by a Snellen chart; a reflex hammer used testing reflexes including masseter, biceps and triceps tendon, knee

tendon, ankle jerk and plantar (i.e. Babinski sign); Muscle strength often on the MRC scale 1 to 5; Muscle tone and signs of rigidity.

Administration

In practicing methods of the invention, a blood plasma fraction is administered to the subject. In an embodiment, the blood plasma fraction is administered by intravenous infusion. The rate of infusion may vary, but in one embodiment of the invention, the infusion rate is 5-8 mL/minute. Those having ordinary skill in the art will recognize that the infusion rate can depend upon the subject's condition and response to administration.

In those embodiments where an effective amount of an active agent is administered to the adult mammal, the amount or dosage is effective when administered for a suitable period of time, such as one week or longer, including two weeks or longer, such as 3 weeks or longer, one month or longer, 2 months or longer, 3 months or longer, 4 months or longer, 5 months or longer, 6 months or longer, 1 year or longer etc., so as to evidence a reduction in the condition, e.g., cognitive impairment, or delay of cognitive impairment and/or cognitive improvement in the adult mammal. For example, an effective dose is the dose that, when administered for a suitable period of time, will slow e.g., by about 20% or more, e.g., by 30% or more, by 40% or more, or by 50% or more, in some instances by 60% or more, by 70% or more, by 80% or more, or by 90% or more, or will halt, cognitive decline in a patient undergoing or having undergone chemotherapy. In some instances, an effective amount or dose of blood product will not only slow or halt the progression of the chemotherapy induced cognitive disorder, e.g., CICI but will also induce the reversal of the condition, i.e., will cause an improvement in cognitive ability. For example, in some instances, an effective amount is the amount that when administered for a suitable period of time, usually at least about one week, and maybe about two weeks, or more, up to a person of about 3 weeks, 4 weeks, 8 weeks, or longer will improve the cognitive abilities of an individual suffering from a chemotherapy induced cognitive disorder, e.g., CICI, by, for example, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, in some instances 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold or more relative to cognition prior to administration of the blood product or fraction. In some instances, an effective amount or dose of active agent will not only slow or halt the progression of the disease condition but will also induce the reversal of the condition, i.e., will cause an improvement in cognitive function. For example, in some instances, an effective amount is the amount that when administered for a

suitable period of time, will improve the symptoms an individual suffering from chemotherapy induced cognitive disorder, e.g., CICI, for example 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, in some instances 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold or more relative to untreated individuals prior to administration of the agent.

In other embodiments, the blood plasma fraction or Plasma Fraction is administered in accordance with one or more dosing regimens described in U.S. Patent No. 10,357,513, which is herein incorporated by reference in its entirety. As such, an embodiment of the invention includes treating a subject diagnosed with a chemotherapy induced cognitive disorder, e.g., chemotherapy induced cognitive impairment, by administering to the subject an effective amount of blood plasma or Plasma Fraction wherein the blood plasma or Plasma Fraction is administered in a manner resulting in improved cognitive function or neurogenesis after the mean or median half-life of the blood plasma proteins or Plasma Fraction proteins been reached, relative to the most recent administered dose (referred to as "Pulsed Dosing" or "Pulse Dosed" herein). Another embodiment of the invention includes administering the blood plasma or Plasma Fraction via a dosing regimen of at least two consecutive days and monitoring the subject for improved cognitive function at least 3 days after the date of last administration. A further embodiment of the invention includes administering the blood plasma or Plasma Fraction via a dosing regimen of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive days and monitoring the subject for improved cognitive function at least 3 days after the date of last administration. Yet another embodiment of the invention includes administering the blood plasma or Plasma Fraction via a dosing regimen of at least 2 consecutive days and after the date of last administration, monitoring for cognitive improvement beyond when the average half-life of the proteins in the blood plasma or Plasma Fraction has been reached. Another embodiment of the invention includes administering the blood plasma or Plasma Fraction via a dosing regimen of 2 to 14 non-consecutive days wherein each gap between doses may be between 0-3 days each. In some instances, Pulsed Dosing in accordance with the invention includes administration of a first set of doses, e.g., as described above, followed by a period of no dosing, e.g., a "dosing-free period", which in turn is followed by administration of another dose or set of doses. The duration of this " dosing-free" period, may vary, but in some embodiments, is 7 days or longer, such as 10 days or longer, including 14 days or longer, wherein some instances the dosing-free period ranges from 15 to 365 days, such as 30 to 90 days and including 30 to 60 days. As such, embodiments of the methods include non-chronic (i.e., non-

continuous) dosing, e.g., non-chronic administration of a blood plasma product. In some embodiments, the pattern of Pulsed Dosing followed by a dosing-free period is repeated for a number of times, as desired, where in some instances this pattern is continued for 1 year or longer, such as 2 years or longer, up to and including the life of the subject. Another embodiment of the invention includes administering the blood plasma or Plasma Fraction via a dosing regimen of 5 consecutive days, with a dosing-free period of 2-3 days, followed by administration for 2-14 consecutive days.

Biochemically, by an "effective amount" or "effective dose" of active agent is meant an amount of active agent that will inhibit, antagonize, decrease, reduce, or suppress by about 20% or more, e.g., by 30% or more, by 40% or more, or by 50% or more, in some instances by 60% or more, by 70% or more, by 80% or more, or by 90% or more, in some cases by about 100%, i.e., to negligible amounts, and in some instances, reverse the progression of the chemotherapy induced cognitive disorder, e.g., CICI.

Plasma Protein Fraction

In practicing methods of the invention, a plasma fraction is administered to the subject. In an embodiment, the Plasma Fraction is plasma protein fraction (PPF). In additional embodiments, the PPF is selected from the Commercial PPF Preparations.

In another embodiment, the PPF is comprised of 88% normal human albumin, 12% alpha and beta globulins and not more than 1% gamma globulin as determined by electrophoresis. Embodiments of this embodiment used in practicing methods of the invention include, for example, this embodiment as a 5% solution of PPF buffered with sodium carbonate and stabilized with 0.004 M sodium caprylate and 0.004 M acetyltryptophan. Additional formulations, including those modifying the percentage of PPF (e.g. about 1% to about 10%, about 10% to about 20%, about 20% to 25%, about 25% to 30%) in solution as well as the concentrations of solvent and stabilizers may be utilized in practicing methods of the invention.

Plasma Fractions of Specific Donor Age

An embodiment of invention includes administering a blood plasma fraction or a Plasma Fraction derived from the plasma of individuals of certain age ranges. Additional embodiments include administering a plasma protein fraction derived from the plasma of individuals of certain

age ranges. An embodiment includes administering a PPF or a HAS which has been derived from the plasma of young individuals. In another embodiment of the invention the young individuals are of a single specific age or a specific age range. In yet another embodiment, the average age of the donors is less than that of the subject or less than the average age of the subjects being treated.

Certain embodiments of the invention include pooling blood or blood plasma from individuals of specific age ranges and fractionating the blood plasma as described above to attain a plasma protein fraction product such as PPF or HAS. In an alternate embodiment of the invention, the plasma protein fraction or specific plasma protein fraction is attained from specific individuals fitting a specified age range. In another embodiment of the invention, the blood plasma fraction, Plasma Fraction, or specific plasma protein fraction product is attained from a pool of young individuals, of which "young" may be determined by chronologic or biologic age as described above, and the age(s) of the individuals may be a specific age or age range.

Indications

The subject methods and plasma-comprising blood products and fractions find use in treating, including preventing, chemotherapy induced cognitive disorders, e.g., CICI.

Individuals suffering from or at risk of developing a chemotherapy induced cognitive disorder, e.g., impairment, that will benefit from treatment with the subject plasma-comprising blood product, e.g., by the methods disclosed herein, include individuals of any age that, where the individual are undergoing, or have undergone, a chemotherapeutic treatment regiment, where in some instances the individuals are about 0.5 years or older, e.g., 1 year or older, e.g., 2 years or older, e.g., 5 years or older, e.g., 10 years or older, e.g., 15 years or older, e.g., 20 years or older, e.g., 25 years or older, e.g., 30 years or older, e.g., 35 years or older, e.g., 40 years or older, e.g., 45 years or older, e.g., 50 years old or older, e.g., 60 years old or older, 70 years old or older, 80 years old or older, 90 years old or older, and 100 years old or older, and are suffering from cognitive impairment induced by the chemotherapeutic regime.

Subject treated in accordance with embodiments of the invention are those subjects undergoing, or having undergone, a chemotherapy regiment. Accordingly, the subject those that have been administered a chemotherapeutic agent one or more times, e.g., to treat the subject for a neoplastic disease condition, e.g., cancer. As such, subjects treated in accordance with

embodiments of the invention are those that have been diagnosed as having a neoplastic disease, e.g., cancer, and been administered a chemotherapeutic agent for the treatment thereof.

Chemotherapeutic agents are non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones.

Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (Cytoxan™), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol®), docetaxel (Taxotere®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, e.g. vincristine, vinblastine, vinorelbine, vindesine, etc.; podophyllotoxins, e.g. etoposide, teniposide, etc.; antibiotics, e.g. anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, etc.; phenoxizone biscyclopeptides, e.g. dactinomycin; basic glycopeptides, e.g. bleomycin; anthraquinone glycosides, e.g. plicamycin (mithramycin); anthracenediones, e.g. mitoxantrone; azirinopyrrolo indolediones, e.g. mitomycin; macrocyclic immunosuppressants, e.g. cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, etc.; and the like.

Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol®), Taxol® derivatives, docetaxel (Taxotere®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, epothilone B, discodermolide; estramustine, nocodazole, and the like.

Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, e.g. prednisone, dexamethasone, etc.; estrogens and pregestins, e.g. hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; etc.; and adrenocortical suppressants, e.g. aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex. Estrogens stimulate proliferation and differentiation, therefore compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

Other chemotherapeutic agents include metal complexes, e.g. cisplatin (cis-DDP), carboplatin, etc.; ureas, e.g. hydroxyurea; and hydrazines, e.g. N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc. Other anti-proliferative agents of interest include immunosuppressants, e.g. mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

"Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076;

U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from Taxus brevifolia; or T-1912 from Taxus yannanensis).

Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., Taxotere™ docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

In some embodiments, the methods comprise treating or preventing chemotherapy-induced cognitive disorder in a subject that has been administered a metal complex chemotherapeutic agent, such as a platinum complex chemotherapeutic agent, e.g., CisPt, CarboPt, and/or OxaliPt.

In some embodiments, the subject methods and compositions find use in slowing the progression of the chemotherapy induced cognitive disorder, e.g., CICI. In other words, cognitive abilities in the individual will decline more slowly following treatment by the disclosed methods than prior to or in the absence of treatment by the disclosed methods. In some such instances, the subject methods of treatment include measuring the progression of cognitive decline after treatment, and determining that the progression of cognitive decline is reduced. In some such instances, the determination is made by comparing to a reference, e.g., the rate of cognitive decline in the individual prior to treatment, e.g., as determined by measuring cognition prior at two or more time points prior to administration of the subject blood product.

The subject methods and compositions also find use in stabilizing the cognitive abilities of an individual, e.g., an individual suffering from chemotherapy induced cognitive disorder, e.g., CICI, or an individual at risk of suffering from chemotherapy induced cognitive disorder, e.g., CICI. For example, the individual may demonstrate some chemotherapy induced cognitive

disorder, e.g., CICI, and progression of cognitive impairment observed prior to treatment with the disclosed methods will be halted following treatment by the disclosed methods. As another example, the individual may be at risk for developing chemotherapy induced cognitive disorder, e.g., CICI (e.g., the individual may be undergoing and have undergone a chemotherapy regiment), and the cognitive abilities of the individual are substantially unchanged, i.e., no cognitive decline can be detected, following treatment by the disclosed methods as compared to prior to treatment with the disclosed methods.

The subject methods and compositions also find use in reducing cognitive impairment in an individual suffering from a chemotherapy induced cognitive disorder, e.g., chemotherapy induced cognitive impairment. In other words, cognitive ability is improved in the individual following treatment by the subject methods. For example, the cognitive ability in the individual is increased, e.g., by 2-fold or more, 5-fold or more, 10-fold or more, 15-fold or more, 20-fold or more, 30-fold or more, or 40-fold or more, including 50-fold or more, 60-fold or more, 70-fold or more, 80-fold or more, 90-fold or more, or 100-fold or more, following treatment by the subject methods relative to the cognitive ability that is observed in the individual prior to treatment by the subject methods. In some instances, treatment by the subject methods and compositions restores the cognitive ability in the individual suffering from a chemotherapy induced cognitive disorder, e.g., chemotherapy induced cognitive impairment.

Methods of Diagnosing and Monitoring for Improvement of Neurocognitive-Associated Disease

In some instances, among the variety of methods to diagnose and monitor disease progression and improvement in in the chemotherapy induced cognitive disorder, the following types of assessments are used alone or in combination with subjects suffering from a chemotherapy induced cognitive disorder, as desired. The following types of methods are presented as examples and are not limited to the recited methods. Any convenient methods to monitor disease may be used in practicing the invention, as desired. Those methods are also contemplated by the methods of the invention.

General Cognition

Embodiments of the methods of the invention further comprise methods of monitoring the effect of a medication or treatment on a subject for treating cognitive impairment and/or age-related dementia, the method comprising comparing cognitive function before and after treatment.

Those having ordinary skill in the art recognize that there are well-known methods of evaluating cognitive function. For example, and not by way of limitation, the method may comprise evaluation of cognitive function based on medical history, family history, physical and neurological examinations by clinicians who specialize dementia and cognitive function, laboratory tests, and neuropsychological assessment. Additional embodiments which are contemplated by the invention include: the assessment of consciousness, such as using the Glasgow Coma Scale (EMV); mental status examination, including the abbreviated mental test score (AMTS) or mini-mental state examination (MMSE) (Folstein et al., J. Psychiatr. Res 1975; 12:1289-198); global assessment of higher functions; estimation of intracranial pressure such as by fundoscopy.

In one embodiment, examinations of peripheral nervous system may be used to evaluate cognitive function, including any one of the followings: sense of smell, visual fields and acuity, eye movements and pupils (sympathetic and parasympathetic), sensory function of face, strength of facial and shoulder girdle muscles, hearing, taste, pharyngeal movement and reflex, tongue movements, which can be tested individually (e.g. the visual acuity can be tested by a Snellen chart; a reflex hammer used testing reflexes including masseter, biceps and triceps tendon, knee tendon, ankle jerk and plantar (i.e. Babinski sign); Muscle strength often on the MRC scale 1 to 5; Muscle tone and signs of rigidity.

Reagents, Devices, and Kits

Also provided are reagents, devices, and kits thereof for practicing one or more of the above-described methods. The subject reagents, devices, and kits thereof may vary greatly.

Reagents and devices of interest include those mentioned above with respect to the methods of preparing plasma-comprising blood product for transfusion into a subject in need hereof, for example, anti-coagulants, cryopreservatives, buffers, isotonic solutions, etc.

Kits may also comprise blood collection bags, tubing, needles, centrifugation tubes, and the like. In yet other embodiments, kits as described herein include two or more containers of blood plasma product such as plasma protein fraction, such as three or more, four or more, five or more, including six or more containers of blood plasma product. In some instances, the number of distinct containers of blood plasma product in the kit may be 9 or more, 12 or more, 15 or more, 18 or more, 21 or more, 24 or more 30 or more, including 36 or more, e.g., 48 or more. Each container

may have associated therewith identifying information which includes various data about the blood plasma product contained therein, which identifying information may include one or more of the age of the donor of the blood plasma product, processing details regarding the blood plasma product, e.g., whether the plasma product was processed to remove proteins above an average molecule weight (such as described above), blood type details, etc. In some instances, each container in the kit includes identifying information about the blood plasma contained therein, and the identifying information includes information about the donor age of the blood plasma product, e.g., the identifying information provides confirming age-related data of the blood plasma product donor (where such identifying information may be the age of the donor at the time of harvest). In some instances, each container of the kit contains a blood plasma product from a donor of substantially the same age, i.e., all of the containers include product from donors that are substantially the same, if not the same, age. By substantially the same age is meant that the various donors from which the blood plasma products of the kits are obtained differ in each, in some instances, by 5 years or less, such as 4 years or less, e.g., 3 years or less, including 2 years or less, such as 1 year or less, e.g., 9 months or less, 6 months or less, 3 months or less, including 1 month or less. The identifying information can be present on any convenient component of the container, such as a label, an RFID chip, etc. The identifying information may be human readable, computer readable, etc., as desired. The containers may have any convenient configuration. While the volume of the containers may vary, in some instances the volumes range from 10 ml to 5000 mL, such as 25 mL to 2500 mL, e.g., 50 ml to 1000 mL, including 100 mL to 500 mL. The containers may be rigid or flexible, and may be fabricated from any convenient material, e.g., polymeric materials, including medical grade plastic materials. In some instances, the containers have a bag or pouch configuration. In addition to the containers, such kits may further include administration devices, e.g., as described above. The components of such kits may be provided in any suitable packaging, e.g., a box or analogous structure, configured to hold the containers and other kit components.

In addition to the above components, the subject kits will further include instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a package insert, etc.

Yet another means would be a computer readable medium, e.g., diskette, CD, portable flash drive, etc., on which the information has been recorded. Yet another means that may be present is a website address which may be used via the internet to access the information at a removed site. Any convenient means may be present in the kits.

Experimental Procedures

The following examples are put forth to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is degrees Centigrade, and pressure is at near atmospheric.

General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., HaRBor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel et al. eds., John Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995; Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Precedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998), the disclosures of which are incorporated herein by reference. Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, Sigma-Aldrich, and Takara Bio USA.

Example 1 – Machine learning-based method of quantifying blood-brain barrier dysfunction in a mouse model of chemotherapy-induced cognitive impairment

Platinum-containing compounds such as oxaliplatin, carboplatin, and most notably cisplatin are common anti-cancer therapeutics targeting solid-state tumors. Cognitive impairment following the completion of a chemotherapy regimen is a chronic neurological condition affecting 20-33% of cancer survivors. Typically, patients report loss of cognitive processing speed, memory

impairment, and general "brain fog." Cisplatin induces dysfunction of the BBB and promotes DNA crosslinking in neuronal precursor cells. Ultimately, the effect of cisplatin induces the DNA-damage pathway, leading to apoptosis and the generation of reactive oxygen species. Therefore, proper characterization of dysfunctions occurring within the perivascular space and brain parenchyma, and correlating readouts to other CNS endpoints is crucial.

Characterizing severity of BBB dysfunction can be achieved with several approaches. This includes the quantification of fluorescein-conjugated molecules crossing the BBB in permeability assays; however, this approach requires the dedication of a fluorescent channel, and variability can be caused by incomplete or improper perfusion. An alternative is measuring serum proteins within the brain parenchyma. However, histological analysis is prone to inter-investigator variability, suffers from low sampling size, and is exceedingly time consuming.

Described herein is the use of a machine learning-based segmentation and quantification approach for aquaporin4 (AQP4) modified from published methodologies and applied to a model of CICI.

Mice: Thirty-week-old male C57BL/6J mice were acquired from The Jackson Labs and housed in accordance with IACUC. Animals were singly housed under 12-hour light/dark cycle with ad libitum access to food and water. Mice were treated with either cisplatin (MilliporeSigma) at 2.3 mg/kg or sterile PBS i.p. for 5 days in two series with a 9-day recovery period. Mice were also given subcutaneous injection of lactated Ringer's solution throughout dosing. Four hours prior to sac, mice were injected i.p. with 100 mg/kg sodium fluorescein (MilliporeSigma) for a secondary measurement of BBB permeability.

Tissue: After conclusion of treatment, mice were sacrificed, and brain tissue was harvested. The left hemisphere was isolated and drop fixed in 4% PFA for 48 hours at 4°C. After fixation, tissues were dehydrated in two 24-hour changes of 30% sucrose at 4°C. Brains were then sectioned on a sliding microtome at 30 μm and suspended in cryoprotectant solution. From the right hemisphere, the cortex was dissected and snap frozen in liquid nitrogen for sodium fluorescein (SF) assay.

Sodium fluorescein assay: Cerebellum tissue was homogenized in ice cold PBS and plated on a black bottom 96 well plate. Sodium fluorescein signal was then read using 488 nm excitation wavelength and 525 nm emission on a BMG CLARIOstar Plus plate reader.

Staining: Free floating sections were washed then blocked for 1 hour in 10% goat serum in PBST. After blocking, sections were stained in primary antibody solution [1:200 lectin-AF594 (ThermoFisher), 1:1000 rabbit anti-AQP4 (MilliporeSigma) in PBST containing 3% goat serum] overnight at 4°C. The following day, sections were stained with secondary antibody solution [goat antirabbit-AF647 (ThermoFisher) in PBST containing 3% goat serum] for 1 hour at room temperature. Cell nuclei were then stained (1:10000 Hoechst-33342; ThermoFisher) for 10 min before rinsing and placing onto slides.

Analysis: Slides were imaged on AxioScan.Z1 (CARL ZEISS), and rostral hippocampus was imaged at 20X. Fluorescent signal for AF647, AF594, and Hoechst-33342 was captured. Full size .czi files were exported for analysis in ZEN 3.7. Ten single channel images for lectin-AF594 were generated from each time point for training built in Zen Intellesis AI-based segmentation tool. Model was deployed on total image data set, and images were segmented into vascular (lectin-positive) and extravascular (lectin-negative) regions. From the extravascular segment, AQP4 signal was quantified in each 5 μm ring (shell) from the nearest vessel.

Statistics: Statistical testing was performed using GraphPad Prism v10.0.

FIGS. 3A-3B show the experimental design and core concept. FIG. 3A shows the timeline of dosing and subsequent experiments for 24-hr (n = 4 per group), 2 wk (n = 11-12), and 6 wk (n = 10-14) time points. Under healthy physiological conditions, most plasma proteins are contained within capillaries and excluded from brain parenchyma. With treatment of cisplatin, inflammation is induced, leading to the release of reactive oxygen species (ROS) and TNFα, causing endothelial damage. Pericytes become detached from the capillary wall, tight junctions break down, and astrocytes are damaged, leading to the mislocalization of AQP4 and the leakage of proteins such as IgGs and albumin. FIG. 3B shows the comparison of conventional line scan approach and segmentation detection. In line scan AQP4-AF647 signal (RFU) was detected across length of linear region of interest (ROI). To compare with the developed approach, 10 lines (FIG. 3B, top panel) were stacked over a 10 px wide, 40 μm long segmentation ROI (FIG. 3B, bottom panel).

FIGS. 4A-4C show the histological analysis approach. FIG. 4A shows the 20X images taken with Zeiss Axioscan of entire rostral hippocampus for Hoechst-33342 or nuclear stain (blue), AQP4-AF647 (white), lectin-AF594 (green). Segmentation was performed across the entire CA1 stratum radiatum with defined edges at the inferior stratum lacunosum-moleculare and superior pyramidal layer. White boxes denote location of 100% zoom representative images. (FIGS. 4B-

4C). FIG. 4B shows 100% zoomed region of representative image taken from CA1. Intellesis segmentation tool was trained off representative single channel images for lectin-AF594 to generate a model for detecting blood vessels (BV), segmenting image into vascular region (outlined) and extravascular region. FIG. 4C shows an example of shell analysis mimicking conventional line scan. Rings denote radii from nearest vascular object in 5 μm steps from which target protein AQP4 is measured.

FIGS. 5A-5D show total extravascular AQP4 signal by cisplatin treatment. FIG. 5A shows the area in pixels contained in each shell starting at 5 μm to 30 μm from nearest vascular (lectin+) segment at 24 hours, 2 weeks, and 6 weeks post cisplatin treatment. No change was observed in the total extravascular area in each shell. FIG. 5B shows the area under the curve (AUC) generated from line graphs in panel 5A, showing no difference in extravascular area between cisplatin and vehicle treated groups. FIG. 5C shows the cumulative AQP4-AF647 signal across distance, showing a linear increase in total AF647 signal as distance from the nearest vascular signal increases. FIG. 5D shows the AUC generated from cumulative AQP4-AF647 signal shown in panel C, showing an increase of extravascular AQP4 in cisplatin-treated group at 24 hours and 2 weeks that is attenuated at 6 weeks post treatment.

FIGS. 6A-6B show cisplatin-induced BBB leakage of sodium fluorescein correlates with extravascular AQP4 signal. FIG. 6A shows that sodium fluorescein (NaF) signal (RFU) was detected in cortex homogenate from 24 hour and 6 week samples as a measurement of extravasation. Increased sodium fluorescein extravasation observed at 24 hours but not 6 weeks post cisplatin treatment. FIG. 6B shows that sodium fluorescein strongly correlated with 20 μm shell AQP4 signal at 24 hours but not 6 weeks, suggesting both factors attenuate by 6 weeks post treatment.

FIGS. 7A-7B show that segmentation method detects AQP4 mislocalization up to 2 weeks post-cisplatin treatment. FIG. 7A shows AQP4-AF647 fluorescence (RFU) measured in each 5 μm shell from nearest vascular segment normalized to total area (pixels) contained in the shell (as measured in FIG. 5A). FIG. 7B shows the area under the curve generated from FIG. 7A, demonstrating difference in total extravascular AQP4-AF647 between cisplatin- and vehicle-treated mice across 30 μm. AQP4 mislocalization is observed at 24-hour and 2-week time points but is attenuated by 6 weeks.

This Example demonstrates a method of quantifying AQP4 mislocalization as a measurement of astrogliosis and BBB permeability. The approach has expanded the sampling region from a single line extending from the vessel to the total area around all blood vessels and condensing that information into 5 μm bins.

The developed approach was used to assess a model of CICI in which various endpoints are attenuated 2 weeks post cisplatin dosing. Across treatment time points, the area contained within each shell was consistent (FIGS. 5A-5D.) The current methodology is based off shells every 5 μm, where line scans can be done on a continuous scale. Granularity can be increased with more layers on a sub 5 μm interval. At time points with AQP4 mislocalization and sodium fluorescein leakage, the two outcomes may be correlated, showing that the BBB dysfunction identified form the methods disclosed herein can be validated using an established method. (FIGS. 6A-6B). The methods disclosed herein can detect the presence of AQP4 mislocalization acutely following cisplatin administration, and this effect is attenuated between 2 weeks and 6 weeks.

Example 2 – Altered Oligodendrocyte Lineage Contributes to Cisplatin-based Chemotherapy Induced Cognitive Impairment

Oligodendrocytes (OL) belong to a type of glia cells that undergo lineage progression from oligodendrocyte precursor cells (OPC) to pre- then mature OL. OL's main function is to produce and maintain myelin sheaths. This oligodendrocyte lineage dynamic is crucial for cognitive functions such as learning and memory and demonstrates central nervous system plasticity. OPC differentiation and adaptive myelination by mature OL are regulated by complex transcriptional network that is vital for cognition, as demonstrated by previous studies showing certain stage of lineage progression (such as formation of new mature OLs) to be necessary for certain cognitive tasks like the formation of new memories.

Cisplatin-based chemotherapy patients often experience induced cognitive deficit. This adverse neurological sequela, referenced herein as CICI, has previously been associated with various physiochemical changes in the brain. However, OL specific changes have not yet been characterized. With known implications of oligodendrocyte lineage on cognition, it is crucial to map the changes in population, differentiation, and myelination capacity of OL following chemotherapy.

Disclosed herein is the stage-specific impact of cisplatin treatment on oligodendrocyte lineage and its potential implications for CICI. To further investigate potential therapeutic reversal, metformin was prophylactically administered. Metformin is an AMPK activator that enhances OPC differentiation and myelination. Metformin is also known to prevent CICI.

Oxidative stress, known to disrupt oligodendrocyte differentiation, was measured to further identify which stage of oligodendrocyte lineage is impacted by cisplatin and metformin. Mice/Treatment: Thirty-week-old c57BL/6J male mice (Jackson Labs) were homogenized across treatment groups to home cage nestlet scoring. Mice were injected with 500 µL cisplatin (2.3 mg/kg, I.P.) or sterile PBS for 5-consecutive days, then a subsequent 5-consecutive days of cisplatin or PBS injection with 5 days recovery period between two cycles. An additional cisplatin treatment group received 500 µL Metformin (100 mg/kg, I.P.) for 7-consecutive days starting 1 day prior to cisplatin administration for two cycles while all other groups received an additional 500 µl sterile PBS. Mice were sacrificed 3 days, 14 days, or 42 days post last cisplatin administration. (FIG. 8A.)_At each timepoint (3, 14, and 42 days), a cohort of mice was tested alongside vehicle controls.

Histology: Heparin perfused hemibrains were collected then PFA drop-fixed. Free floating sections of 30 µm thickness were blocked with donkey serum before incubation with primary antibody solutions. Sections were stained in following dilutions: OLIG2 1:200 (R&D Systems), GPR17 1:200 (Cayman), GSTπ 1:200 (Bioss).

Image Analysis: Using Zen Blue 3.7 (ZEISS), manual ROI was drawn for hippocampus. Oligodendrocytes were counted as cells expressing above threshold OLIG2 using automated Three Sigma threshold method. OPC and mature OL density was calculated by counting OLIG2+ cells colocalizing with above threshold GPR17 or GSTπ puncta within the hippocampus ROI.

Superoxide Dismutase (SOD) Assay: SOD Colorimetric Activity Kit (Invitrogen) was used to conduct SOD assay with blood plasma samples as a measure of oxidative stress.

Statistics: For FIGS. 8A-8C and 9A-9C, cisplatin treated mice at each timepoint were normalized to their vehicle control and analyzed using a Mann-Whitney test. For FIGS. 10A-10B and 11, cisplatin and cisplatin + Metformin groups at each timepoint were normalized to their vehicle control and analyzed using a Kruskal-Wallis test with Dunn's post hoc multiple comparisons. All data are represented at mean ± S.E.M.

As shown in FIG. 8C, cisplatin treatment resulted in a reduction, but not to significant level, of oligodendrocyte population after 3 days that is recovered by 14 days measured by fold change of count per mm^2 quantification of OLIG2+ cells in hippocampus. Trending decrease from vehicle group (mean = 1.029 ± S.E.M, n = 7) at 3 days post treatment (mean = 0.8824 ± S.E.M, n = 7, P = 0.0530, Mann-Whitney test). Thus, cisplatin treatment induces a non-significant but trending reduction in hippocampal oligodendrocytes, although overall changes are restored over time.

FIGS. 9A-9C show that cisplatin impact on oligodendrocyte was stage-specific. FIGS. 9A and 9B provide representative 200 μm x 200 μm CA1 images demonstrating oligodendrocytes (white), OPCs (red), mature OLs (green), and DAPI (blue). Each arrow indicates a colocalizing cell. FIG. 9C shows that cisplatin treatment resulted in a decrease in OPC population after 3 days that is recovered by 14 days as measured by fold change from vehicle group of count per mm^2 quantification of OLIG2+ cells expressing above threshold GPR17 puncta. Significant decrease with cisplatin at 3 days post treatment (mean = 0.5466 ± S.E.M, n = 7, *P < 0.05, Mann-Whitney test) compared to the vehicle group (mean = 1.051 ± S.E.M, n = 7). FIG. 9D shows that no change was observed in mature oligodendrocyte population with cisplatin treatment. Fold change from vehicle group of count per mm^2 quantification of OLIG2+ cells expressing above threshold GSTπ+ puncta (Mann-Whitney test, n = 7).

FIGS. 10A-10B show that metformin attenuated cisplatin impact on OPC. FIG. 10A shows the fold change from vehicle treatment group of count per mm^2 quantification of OLIG2+, GPR17+, or GSTπ+ cells at 3 days post cisplatin and metformin treatment. Decrease in GPR17+ cells upon cisplatin treatment at 3 days post treatment (mean = 0.585 ± S.E.M, n = 7) that was attenuated by metformin treatment (mean = 0.929 ± S.E.M, n = 6). (Kruskal-Wallis test with Dunn's multiple comparisons, Vehicle group mean = 1 ± S.E.M, n = 7). FIG. 10B shows the fold change from vehicle treatment group of count per mm^2 quantification of OLIG2+, GPR17+, or GSTπ+ cells at 14 days post cisplatin and metformin treatment. (Kruskal-Wallis test, n = 7).

FIG. 11 shows that oxidative stress increased in cisplatin-treated mice. SOD plasma level also increased with cisplatin treatment. Oxidative stress increased with cisplatin treatment but returned to baseline level over time. Metformin attenuated oxidative stress level. Oxidative stress level was determined by measurement of SOD concentration in plasma samples. Oxidative stress was significantly increased with cisplatin at 3 days post treatment (**P < 0.005, Kruskal-Wallis

test with Dunn's post hoc) but not with additional metformin treatment. At 14 days post treatment, oxidative stress was significantly increased in both cisplatin treatment group with and without Metformin treatment (***P < 0.0005 and *P < 0.05, Kruskal-Wallis test with Dunn's post hoc) but there was less increase with Metformin treatment. There was no change in oxidative stress level 42 days post last cisplatin treatment.

FIGS. 12A-12B show relative pattern changes and association of oligodendrocyte population and oxidative stress. FIG. 12A illustrates general changes in oligodendrocyte lineage cell population pattern with cisplatin treatment and FIG. 12B shows the relationship between oligodendrocyte population and oxidative stress level with and without prophylactic metformin treatment.

These data suggest that cisplatin treatment induces a non-significant but trending reduction in hippocampal oligodendrocytes, although overall changes are restored over time. Also, OPCs are more vulnerable to cisplatin treatment and may take longer to fully recover compared to mature OLs. Moreover, prophylactic metformin attenuates cisplatin impact on oligodendrocytes, especially on OPCs. Oxidative stress is increased with cisplatin treatment but is restored over time. Further, oligodendrocytes are arrested at OPC stage upon cisplatin treatment, preventing differentiation of OPC to mature OL. Furthermore, the lack of OPC differentiation is a potential contributor to CICI, which could result in lack of myelin turnover or formation of new oligodendrocyte. Therefore, therapeutically promoting OPC differentiation may mitigate cognitive deficits in CICI.

Example 3 – Effects of Plasma Protein Fraction (PPF) Therapy on Chemotherapy Induced Cognitive Impairment

The data described herein is produced using Plasmanate™ PPF (Grifols, Clayton, NC), which is also referenced herein as "plasma protein fraction" or PPF. As noted above, PPF can be produced from a plasma using a manufacturing process that uses acetone. PPF can also be produced from plasma using a manufacturing process that does not use acetone. Additional details about PPF are described above, under Albumin products, "Plasma Protein Fraction (Human) (PPF)."

Approximately 74% of the 15.5 million cancer survivors are 60 years or older. Among this demographic, 35-85% suffer from long term cognitive dysfunction, decreased memory, attention,

and executive function. Cisplatin is a platinum-based chemotherapeutic used to treat solid-state tumors as part of the standard treatment for numerous malignancies including head and neck, testicular, gynecologic and non-small cell lung cancer. Nephrotoxicity is an acute concern when using platinum-based compounds, however, neurotoxicity present in both peripheral nerves as well as in the CNS have also been reported.

In conjunction with self-reported or assessed memory deficits, neuroimaging data indicate changes in white matter in addition to accelerated biological aging. These findings are further supported by functional neuroimaging studies specifically within the context of cisplatin-based therapeutics. Though most cisplatin binds to tissues and organs within the periphery, at very low doses, it does cross the BBB and inhibits neuronal stem cell proliferation. Cisplatin treatment reduces cognitive function in juvenile rats and adult mice. Therefore, therapeutics are desired for addressing these neurotoxic side-effects resulting from cisplatin treatment, namely, CICI.

Plasma contains many beneficial factors which have been shown in animal models to ameliorate multiple age-related deficits across varied organ systems, including the brain. Age-related hippocampal-dependent cognitive deficits are well studied. The hippocampus is well accessed by the vascular system, especially within the subgranular zone of the dentate gyrus (DG), an area of adult neurogenesis, making it an attractive target for evaluation of plasma-derived factors. The benefits are described for a fractionated plasma products, such as PPF to induce reversal of age-related cognitive decline, enhanced hippocampal neurogenesis, reduced neuroinflammation and cell survival in aged immunocompetent mice.

This Example shows that plasma fractions can therapeutically reverse the myriad of dysfunctions induced by cisplatin treatment within the CNS. The data disclosed herein also suggest that plasma fractions could be used for treating CICI.

Mice/Treatment: 24 month and 30-week-old C57BL/6J mice (Jackson Labs, strain #0664) were housed in accordance with IACUC. Animals were singly housed under 12-hour light/dark cycle with ad libitum access to food and water. Mice were homogenized across treatment groups according to home cage nestlet scoring, initial body weight, open field distance traveled and % center time. 5-bromo-2'-deoxyuridine (BrdU, Millipore-Sigma) (50 mg/kg) was administered I.P. (intraperitoneally). PPF was administered I.V. (intravenously) at 150 μL/day.

In CICI studies, cisplatin (Calbiochem) was dosed at 1 mg/kg in sterile-filtered PBS. Immunohistochemistry/Brightfield staining: Heparin/saline perfused brains were collected, PFA

drop-fixed hemibrains were sectioned at 30 μm thickness. Counts per animal consisted of average positive cells from 5 sections within the granule cell layer of the DG. Doublecortin (DCX) (EMD Millipore, AB2253) 1:2000, Ki67 (Abcam, ab15580) 1:500.

Behavioral assays: CleverSys (Reston, VA) TopScan V3.0 was used to track mouse behavior in Open Field, Novel Object Recognition and 2-Trial Y-maze.

FIGS. 13A-13F shows that 7-day I.V. pulse dosing of PPF partially improved spatial cognition deficit induced by cisplatin. As shown in FIG. 13A, in this experimental design: 30-week-old C57BL/6J mice were injected with cisplatin (1 mg/kg, I.P.) or PBS for 5-consecutive days, followed by 5 days recovery period. Then a subsequent 5-consecutive days of cisplatin or PBS dosing (10 mg/kg cisplatin total) was given. One week after cessation of cisplatin administration, mice were administered for 7 days (IV, pulse dosing) either vehicle or PPF. 3 weeks after the end of IV dosing, mice were tested in spatial cognitive assays. Following this, mice were sacrificed for histological endpoints. (n = 12/group).

FIG. 13B shows the percentage body weight change in cisplatin and PPF+ cisplatin treated mice as compared to vehicle treatment. FIG. 13C shows that the distance travelled in open field test indicates similar locomotor performance between all groups. FIG. 13D shows 2-trial Y-maze layout, training and testing time as well as intertrial interval. FIG. 13E shows the Y-maze % Novel arm time represented as (novel arm time / (novel arm time + familiar arm time) x 100. PPF + cisplatin (mean = 58.07 ± S.E.M.) **$P < 0.01$ vs. null hypothesis of 50%. Cisplatin (mean = 50.21 ± S.E.M.). FIG. 13F shows the results of the Novel Object Recognition (NOR) test. % Novel interaction time in nonsignificant (NS) increase in PPF + cisplatin (mean = 55.26 ± S.E.M.) vs. null hypothesis of 50%. Cisplatin (mean=49.71 ± S.E.M.).

FIGS. 14A-14G show that PPF moderately attenuated neurogenesis and reduction in proliferation in cisplatin treated mice. FIG. 14A shows that neurogenesis as determined by DCX positive labeled cells within the granule layer of the dentate gyrus in cisplatin, cisplatin + PPF and vehicle treated animals. Significant decrease was observed with cisplatin treatment vs. vehicle. FIG. 14B shows proliferation as measured by Ki67 expressing cells within the primary granule cell layer of the dentate gyrus. Significant decrease was observed with cisplatin treatment vs. vehicle. FIG. 14C shows that plasma cytokine TNFα displays a non-significant increase by cisplatin which is normalized to vehicle concentration by PPF treatment. FIG. 14D shows that plasma cytokine IFNγ is non-significantly elevated by cisplatin and normalized by PPF treatment.

FIG. 14E shows DCX positive cells (white) within the hippocampus of vehicle treated mice. FIG. 14F shows DCX positive cells (white) within the hippocampus of PPF + cisplatin treated mice and FIG. 14G shows DCX positive cells (white) within the hippocampus of cisplatin treated mice.

Increased hippocampal neuroinflammation, decreased cell survival and neurogenesis are hallmarks of aging and aging-associated neurological disorders. Cisplatin induces similar dysfunctions in adult mice, thereby allowing for a more robust dynamic range in therapeutic intervention studies. 7-day pulse dosing of PPF in aged WT mice have demonstrated increases in neurogenesis, cell survival and decreased inflammation compared to vehicle treated mice. Therapeutic administration of PPF partially improves spatial cognitive deficits induced by cisplatin administration. Cisplatin administration induces significant reductions in hippocampal neurogenesis and proliferation which therapeutic administration of PPF modestly improves. These data suggest the potential for plasma-derived therapeutics to ameliorate CCI or "brain fog," for example, CCI or brain fog associated with platinum-derived chemotherapeutic agents.

In this Example, it was investigated whether PPF can therapeutically reverse the concomitant CNS dysfunctions induced by cisplatin treatment, many of which parallel impacts occurring in biological aging. These presented data indicate the utility of PPF for treating brain fog or CICI.

At the molecular level, the goal of this study was to evaluate the effects of administration of cisplatin on chronic cognition, neurogenesis, inflammation, proliferation, BBB disfunction and other CNS endpoints in adult C57BL/6 mice and whether PPF can therapeutically attenuate any of these endpoints and the mechanisms involved in such therapeutic effects.

The results of these experiments showed that PPF therapeutically reversed a myriad of dysfunctions within the CNS induced by cisplatin treatment. Thus, these results suggest that PPF could be used for treating CICI.

The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without

limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of the present invention is embodied by the appended claims.

CLAIMS

What is claimed:

1. A method of treating a chemotherapy induced cognitive disorder, the method comprising:

administering an effective amount of a Plasma Fraction to a subject diagnosed with a chemotherapy induced cognitive disorder.

2. The method of claim 1, wherein the Plasma Fraction is a Plasma Protein Fraction.

3. The method of claim 2, wherein the Plasma Protein Fraction is a commercially available Plasma Protein Fraction.

4. The method of claim 1, wherein the Plasma Fraction is a Human Albumin Solution.

5. The method of claim 4, wherein the Human Albumin Solution is a commercially available Human Albumin Solution.

6. The method of claim 1, wherein the Plasma Fraction is a protein-enriched plasma protein product.

7. The method of claim 1, wherein the Plasma Fraction is Effluent I.

8. The method of claim 1, wherein the Plasma Fraction is Effluent II/III.

9. The method of claim 1, further comprising monitoring the subject for improved cognitive function.

10. The method of any of the preceding claims, wherein the Plasma Fraction is derived from plasma from a pool of young individuals.

11. The method of any of the preceding claims, wherein the Plasma Fraction is produced from a mammalian blood product.

12. The method of claim 11, wherein the mammalian blood product is a human blood product.

13. The method of any of the preceding claims, wherein the subject is a mammal.

14. The method of claim 13, wherein the mammal is a human.

15. The method of any of the preceding claims, wherein the chemotherapy induced cognitive disorder comprises chemotherapy induced cognitive impairment.

16. The method of any of the preceding claims, wherein the subject has been administered one or more dosages of a chemotherapeutic agent.

17. The method of claim 16, wherein the chemotherapeutic agent is cisplatin.

18. The method of any of the preceding claims, wherein the subject is or has been treated for a cancer.

19. A kit for use in treating a subject for a cognitive disorder, the kit comprising a containing comprising a Plasma Fraction as described in claim 1.

24hr
2wk
6wk
Cisplatin
2x 5d
Acute Reduction:
Body Weight (trend)
Neurogenesis
Proliferation
Increased:
BBB permeability
AQP4 Mislocalization
Cell adhesion proteins
Inflammatory cytokines
Semi-Chronic Reduction:
Body Weight
Locomotion Neurogenesis
Proliferation
Increased:
BBB permeability
Astrocytosis
AQP4 Mislocalization
Cell adhesion proteins
Inflammatory cytokines
Chronic Reduction:
Body Weight
Neurogenesis
Proliferation
Increased:
AQP4 Mislocalization
CNS Inflammation
Cell adhesion proteins
Inflammatory cytokines

FIG. 1

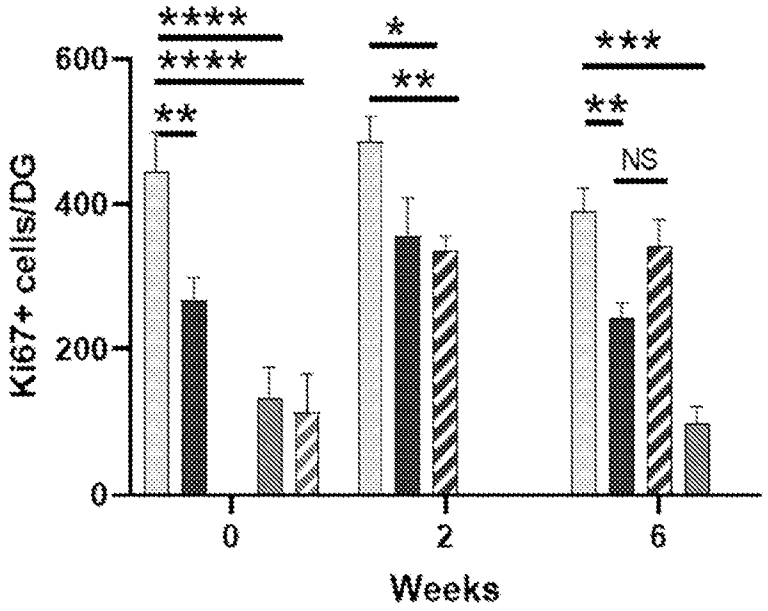
****
****
**
*
**
***
**
NS
600
400
200
0
Ki67+ cells/DG
0
2
6
Weeks

FIG. 2A

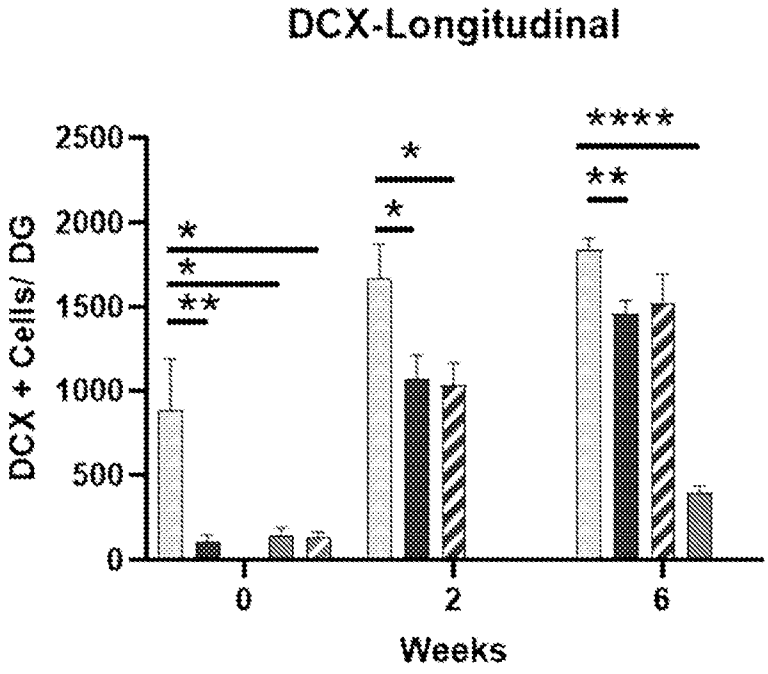
DCX-Longitudinal
2500
2000
1500
1000
500
0
DCX + Cells/ DG
*
*
**
*
*
****
**
0
2
6
Weeks

FIG. 2B

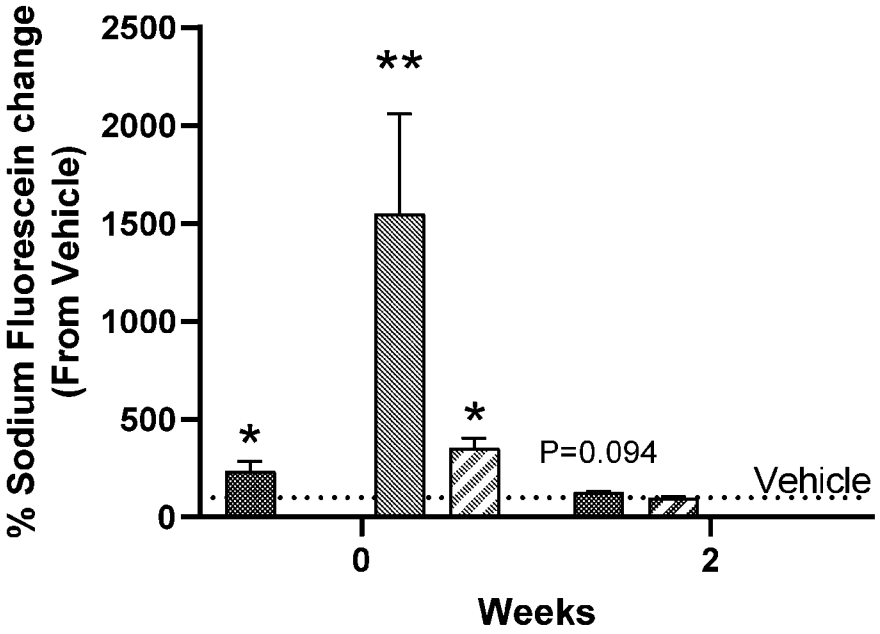
% Sodium Fluorescein change
(From Vehicle)
2500
2000
1500
1000
500
0
**
*
*
P=0.094
Vehicle
0
2
Weeks

FIG. 2C

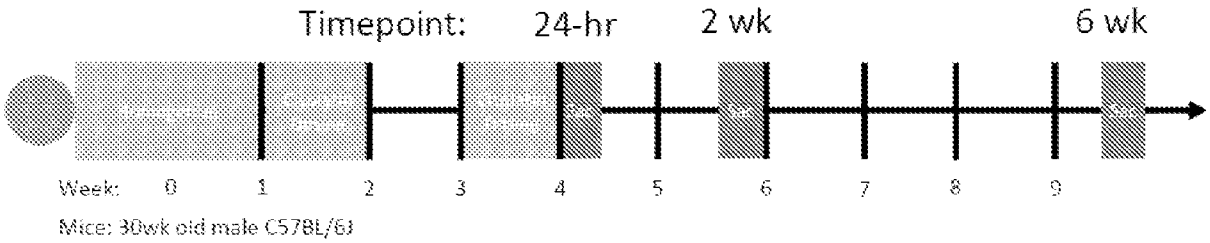
Timepoint:
24-hr
2 wk
6 wk
Week:
0
1
2
3
4
5
6
7
8
9
Mice: 30wk old male C57BL/6J

FIG. 3A

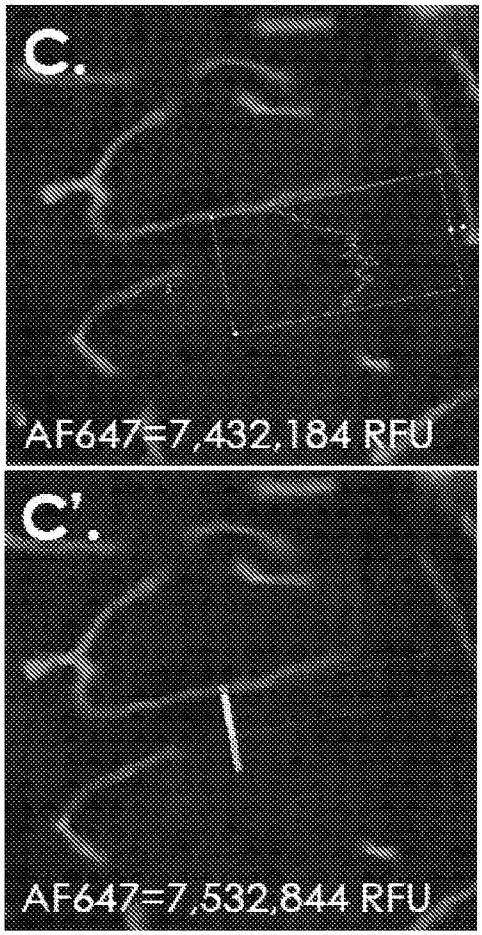

FIG. 3B

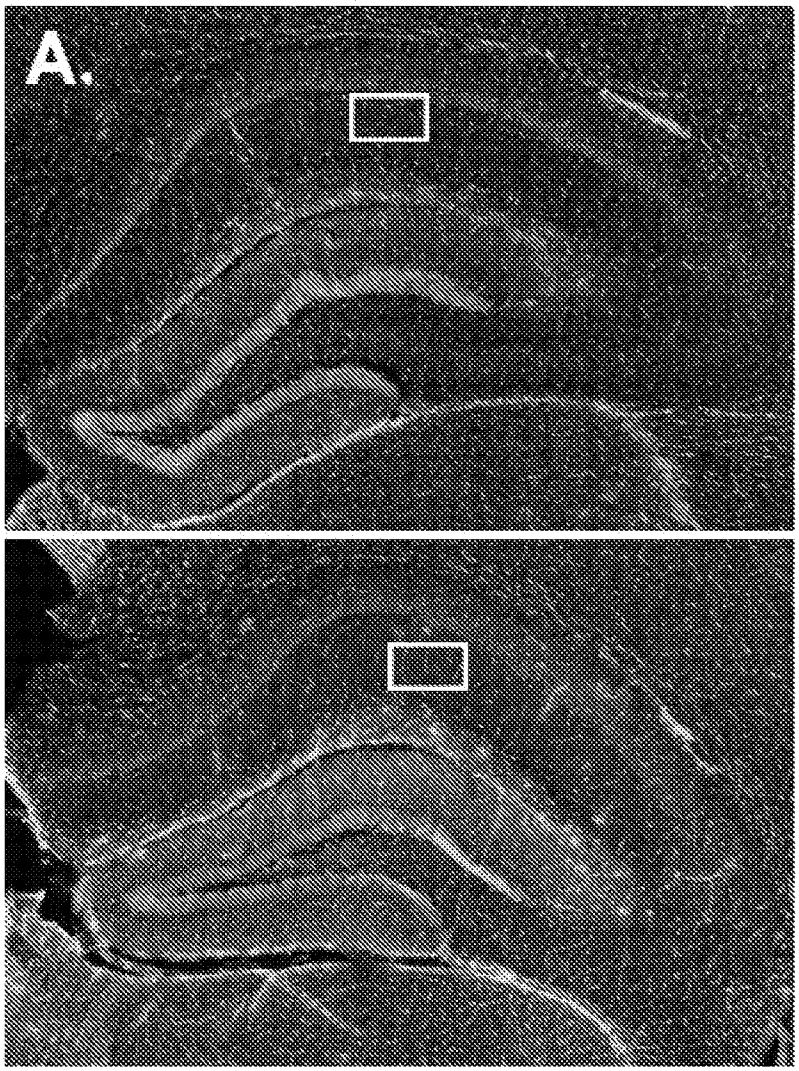

FIG. 4A

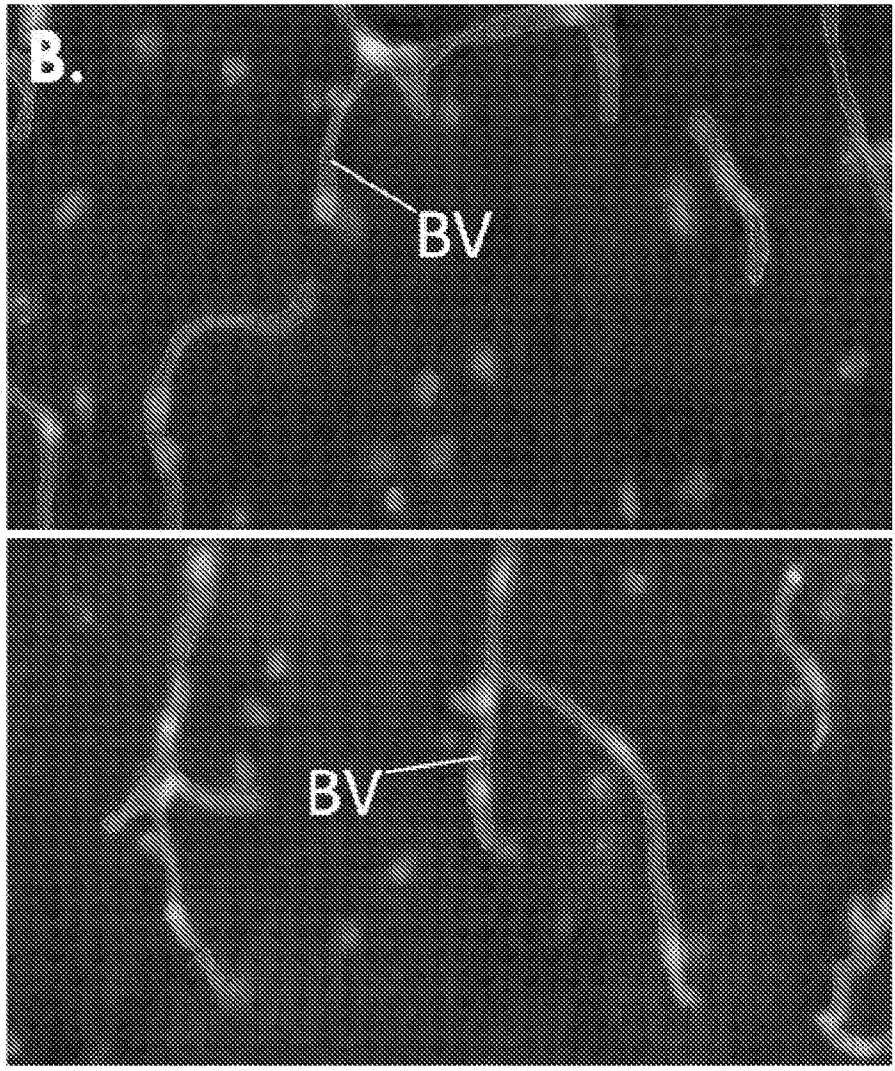

FIG. 4B

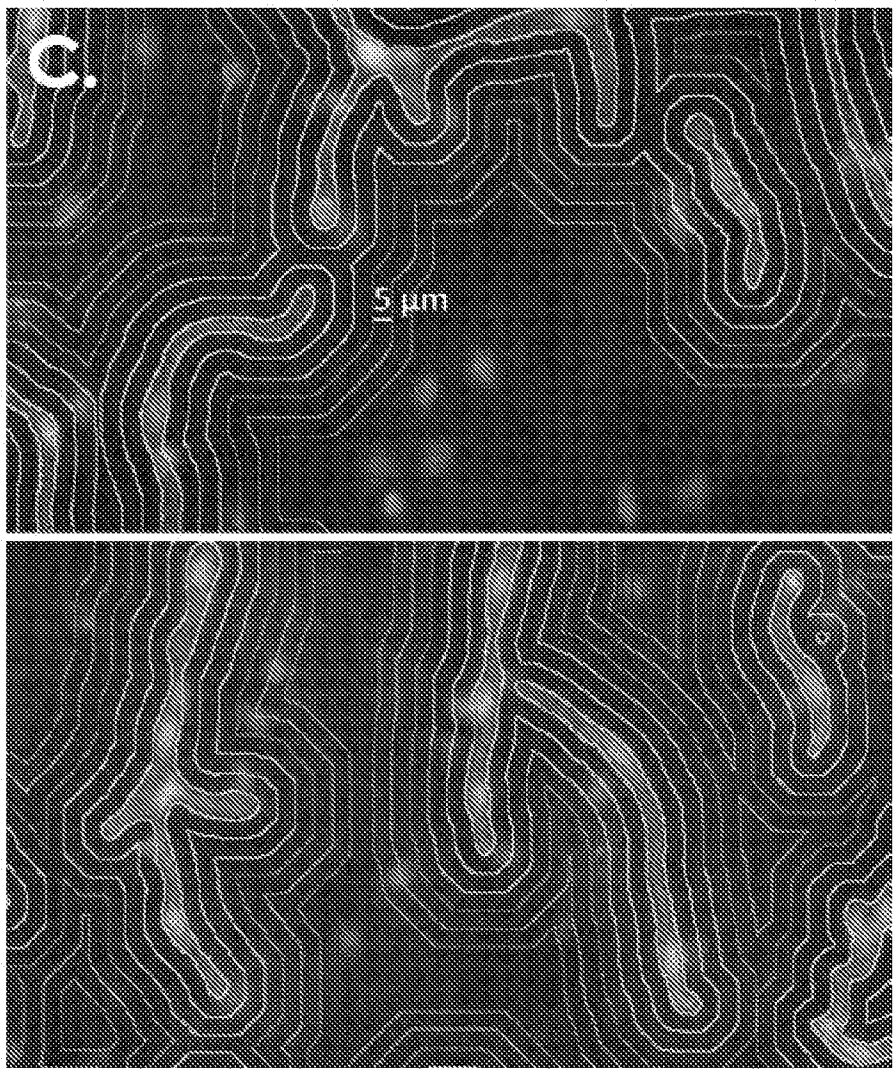

FIG. 4C

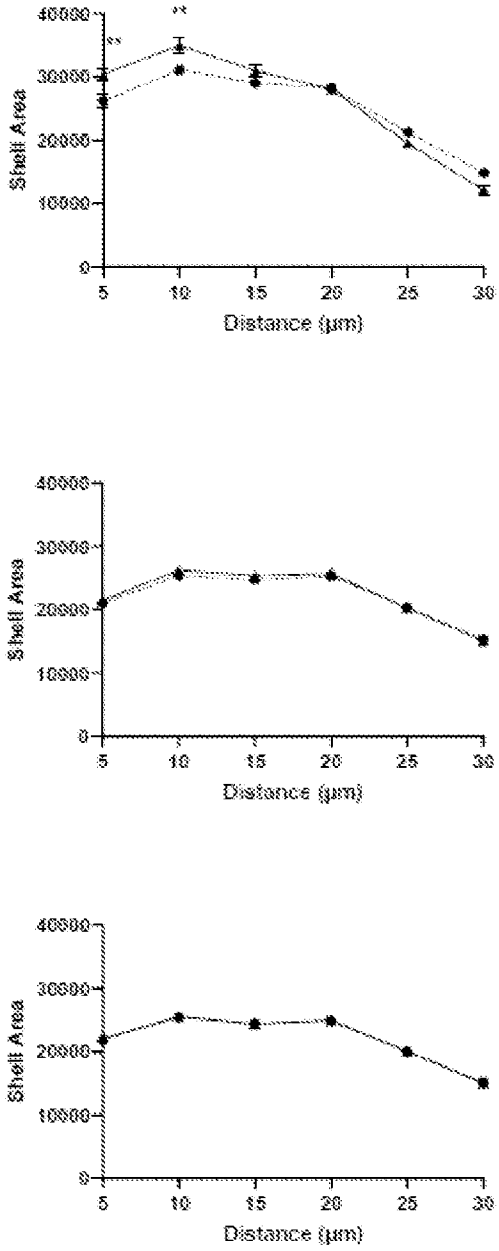
Shell Area
Distance (µm)
Shell Area
Distance (µm)
Shell Area
Distance (µm)

FIG. 5A

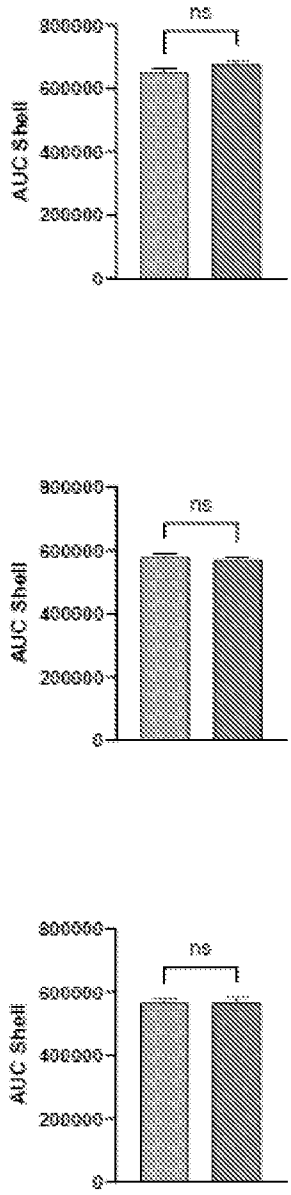
ns
AUC Shell
800000
600000
400000
200000
0
ns
AUC Shell
800000
600000
400000
200000
0
ns
AUC Shell
800000
600000
400000
200000
0

FIG. 5B

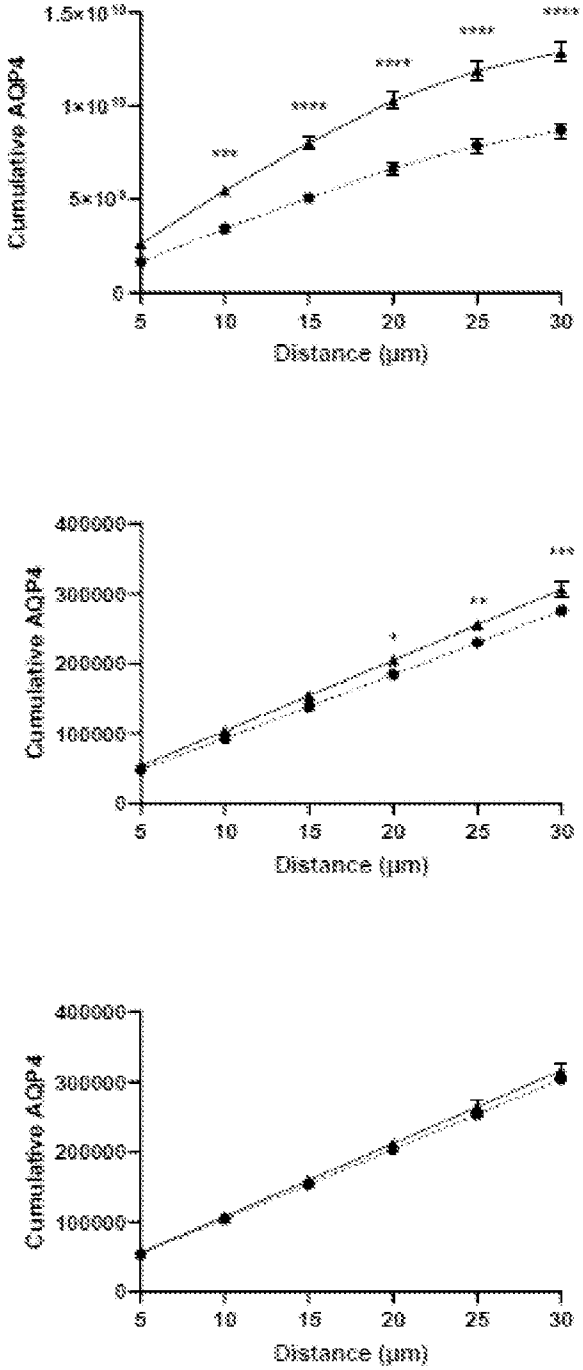
Cumulative AQP4
Distance (µm)
5
10
15
20
25
30
0
400000
300000
200000
100000
****
***
**
*

FIG. 5C

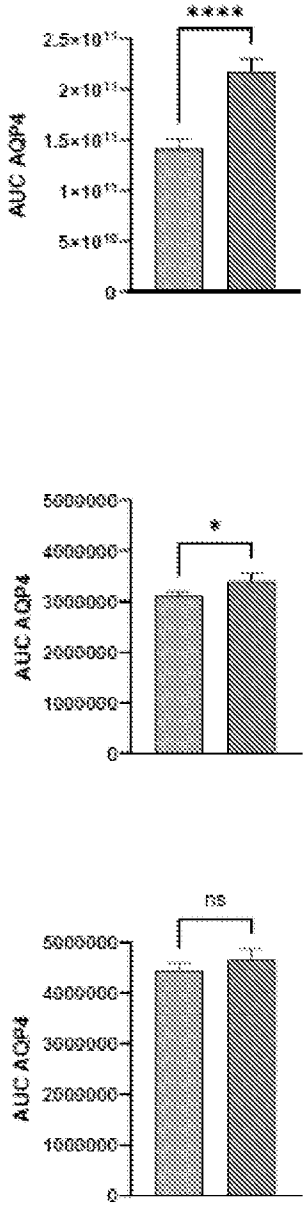
****
AUC AQP4
2.5×10
2×10
1.5×10
1×10
5×10
0
*
AUC AQP4
5000000
4000000
3000000
2000000
1000000
0
ns
AUC AQP4
5000000
4000000
3000000
2000000
1000000
0

FIG. 5D

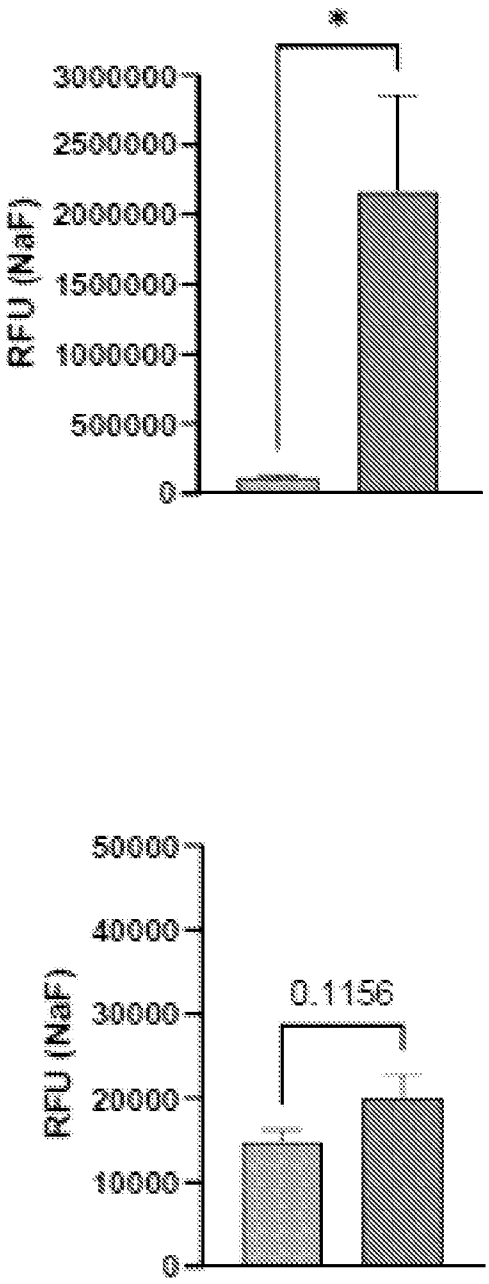
*
3000000
2500000
2000000
1500000
1000000
500000
0
RFU (NaF)
50000
40000
30000
20000
10000
0
RFU (NaF)
0.1156

FIG. 6A

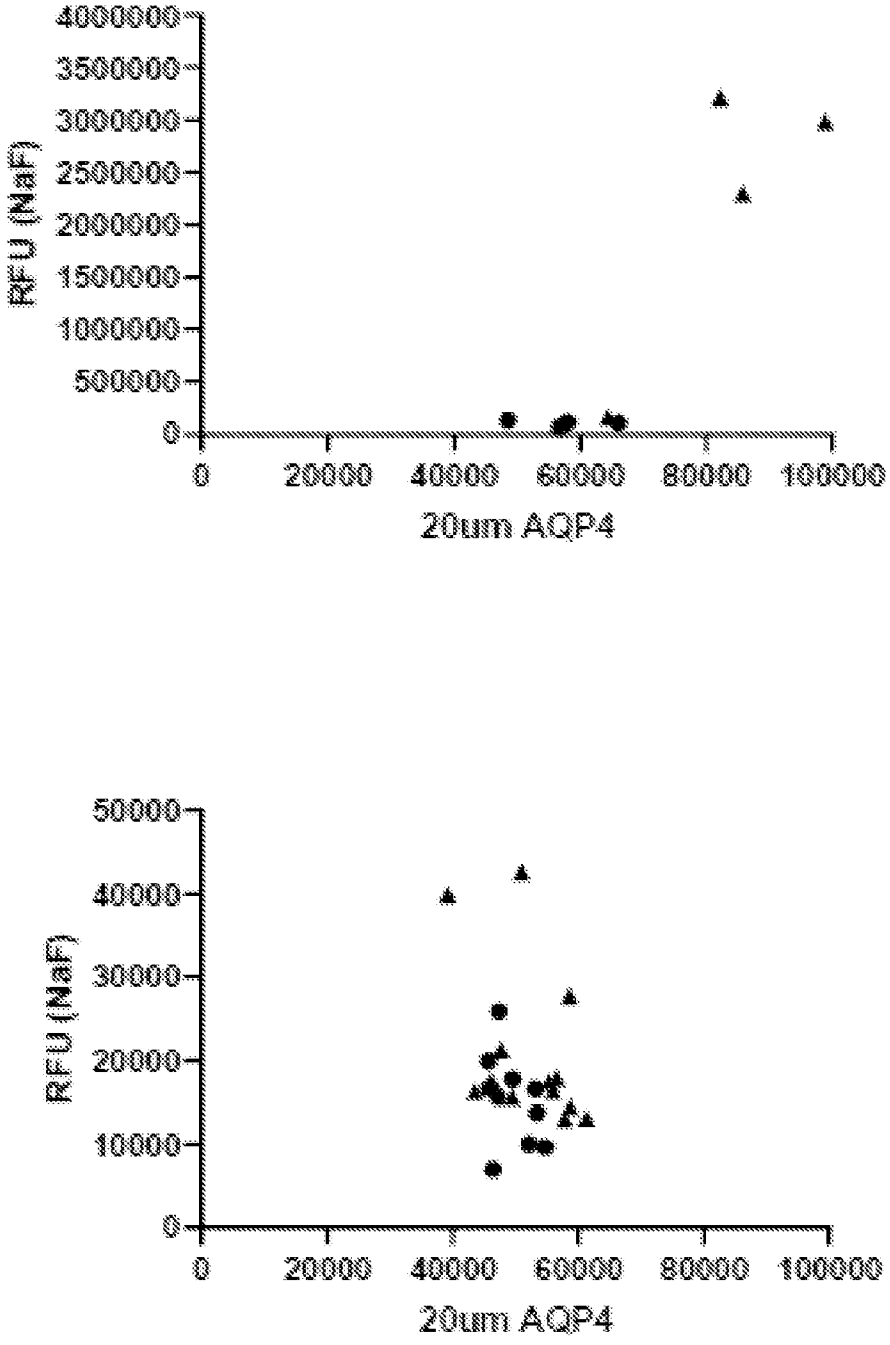
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
RFU (NaF)
0
20000
40000
60000
80000
100000
20um AQP4
50000
40000
30000
20000
10000
0
RFU (NaF)
0
20000
40000
60000
80000
100000
20um AQP4

FIG. 6B

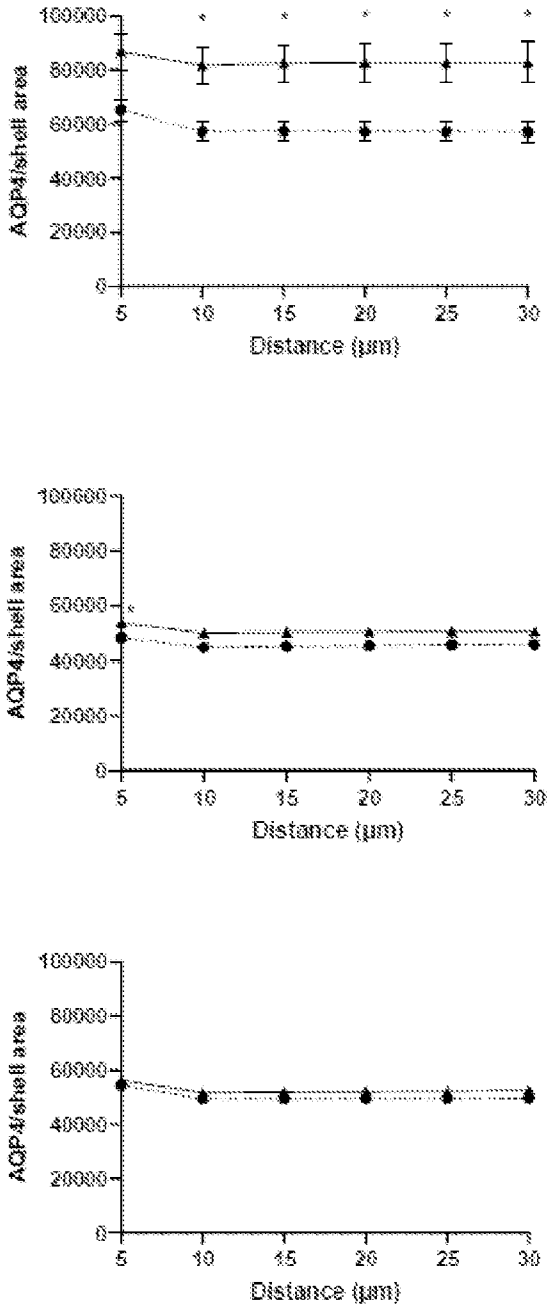
AQP4/shell area
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
Distance (µm)
AQP4/shell area
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
Distance (µm)
AQP4/shell area
100000
80000
60000
40000
20000
0
5
10
15
20
25
30
Distance (µm)

FIG. 7A

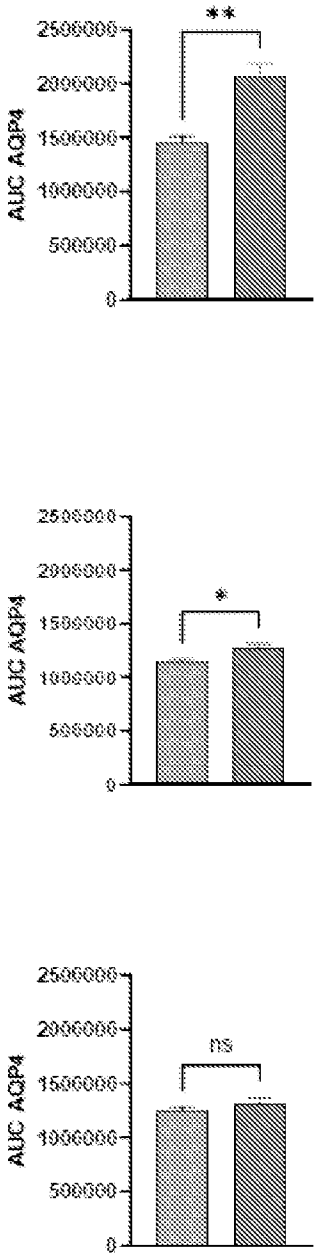
**
2500000
2000000
1500000
1000000
500000
0
AUC AQP4
*
2500000
2000000
1500000
1000000
500000
0
AUC AQP4
ns
2500000
2000000
1500000
1000000
500000
0
AUC AQP4

FIG. 7B

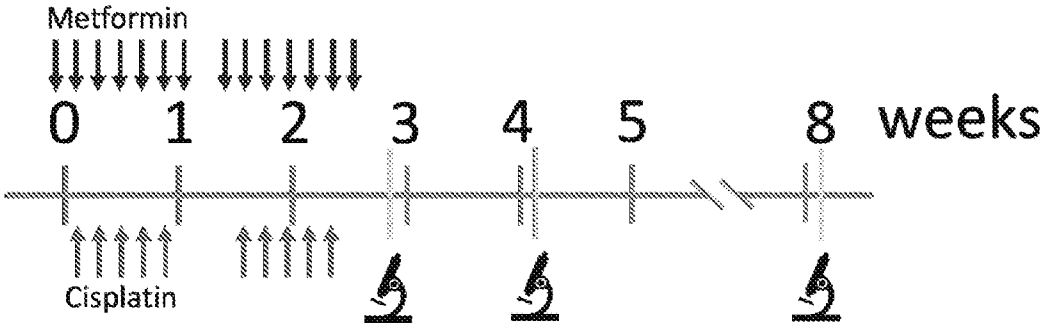

FIG. 8A

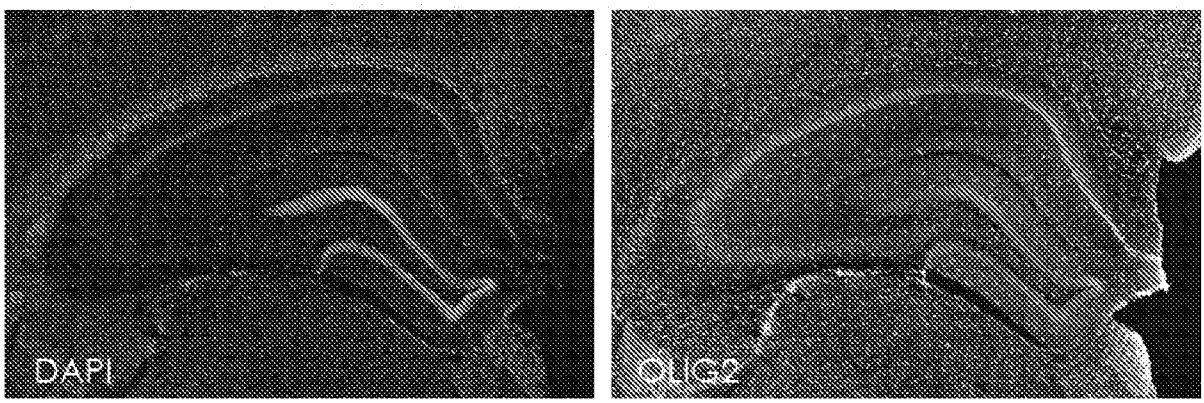

FIG. 8B

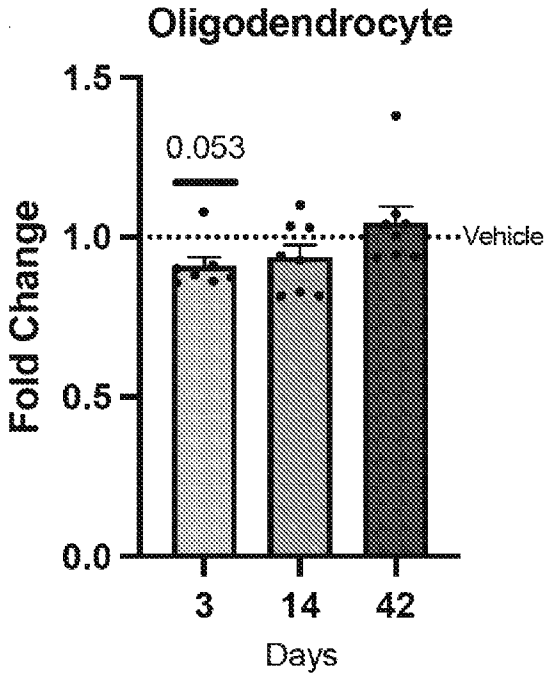

FIG. 8C

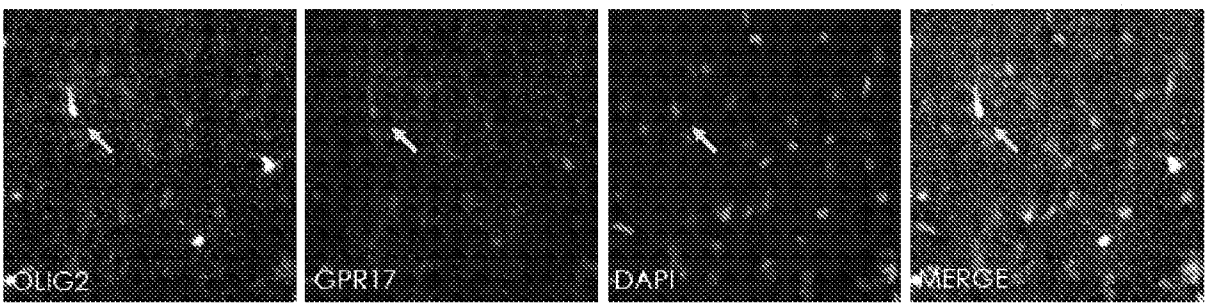

FIG. 9A

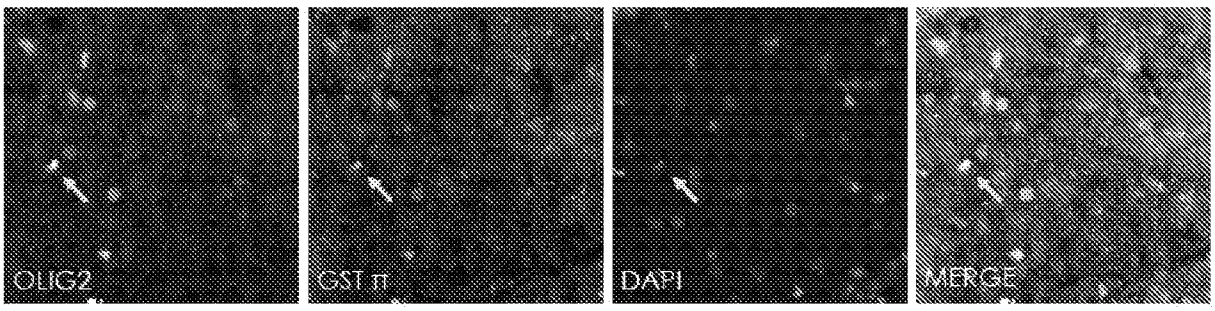

FIG. 9B

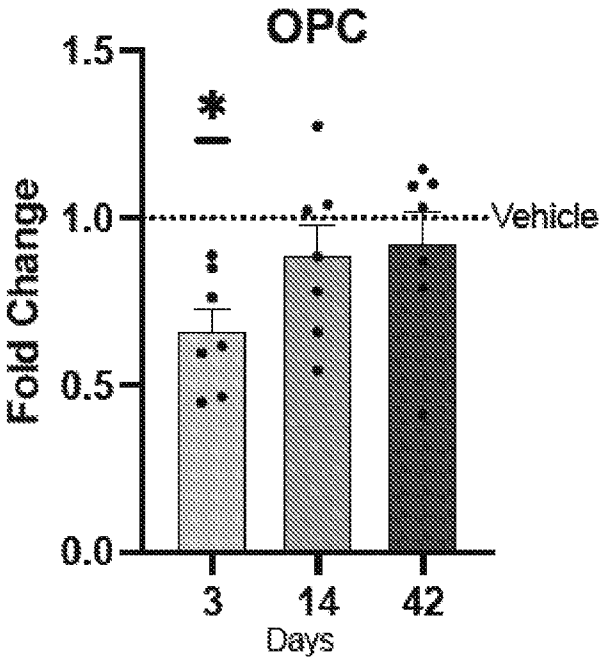

FIG. 9C

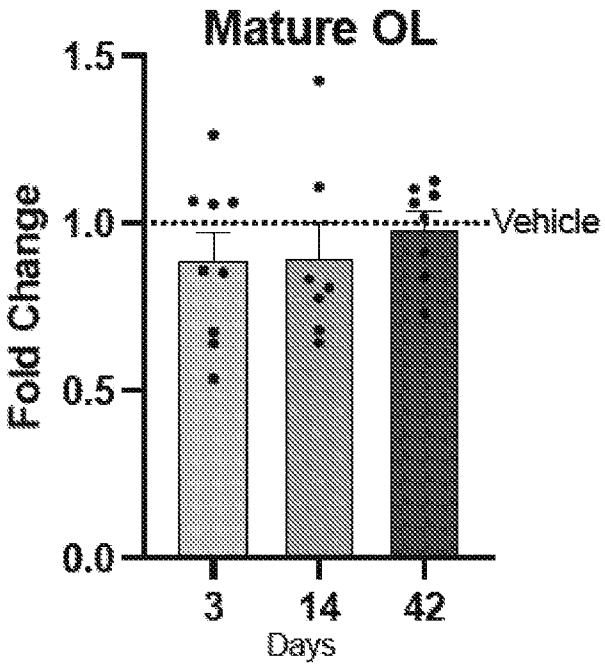

FIG. 9D

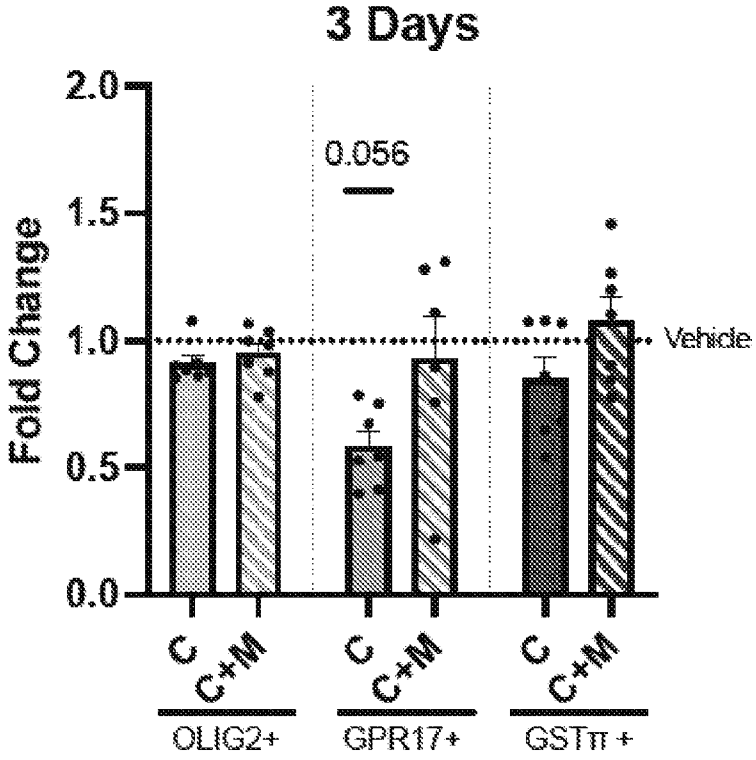

FIG. 10A

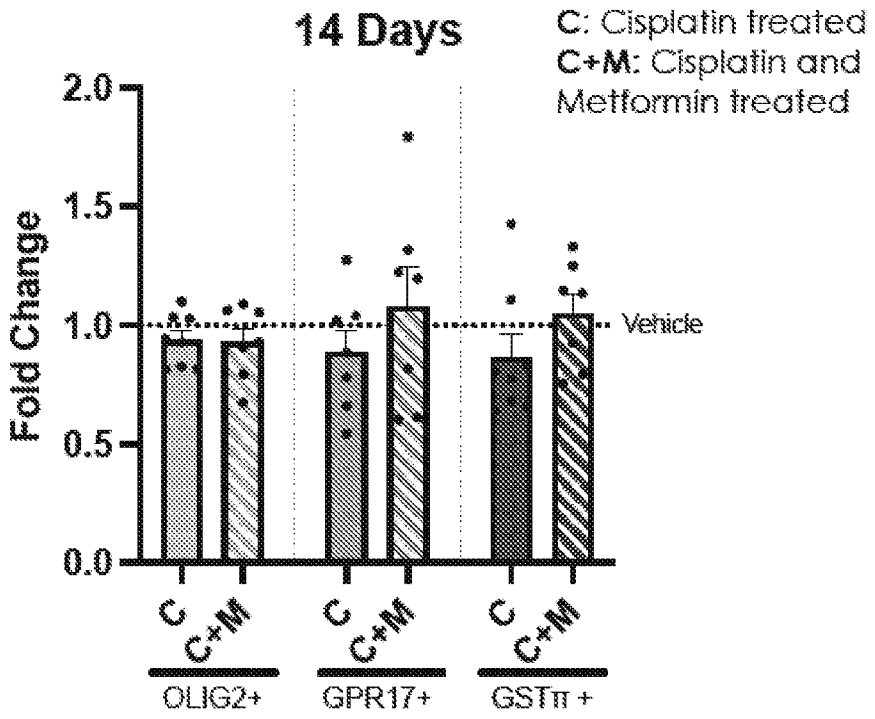

FIG. 10B

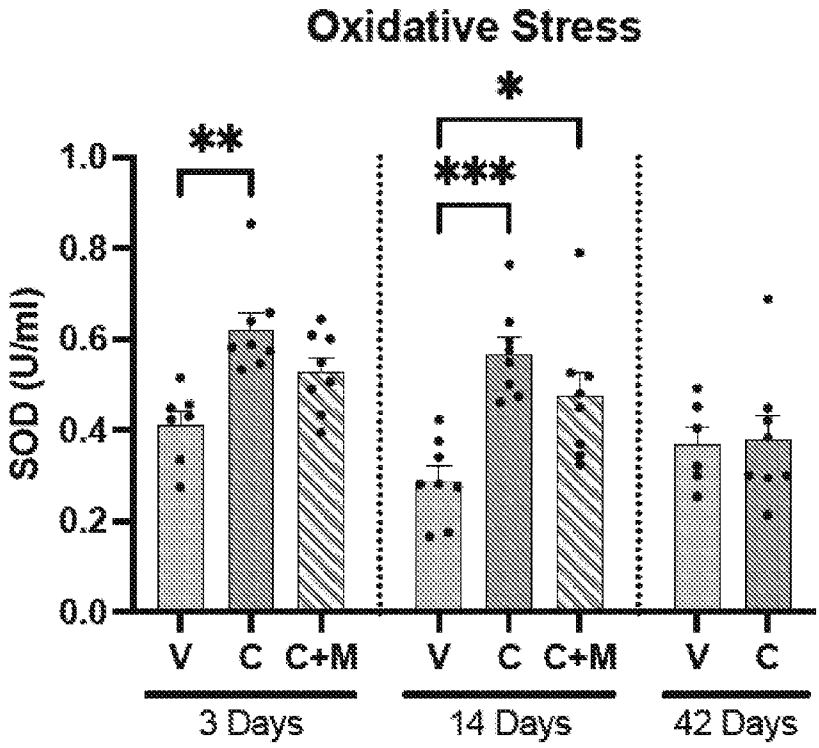

FIG. 11

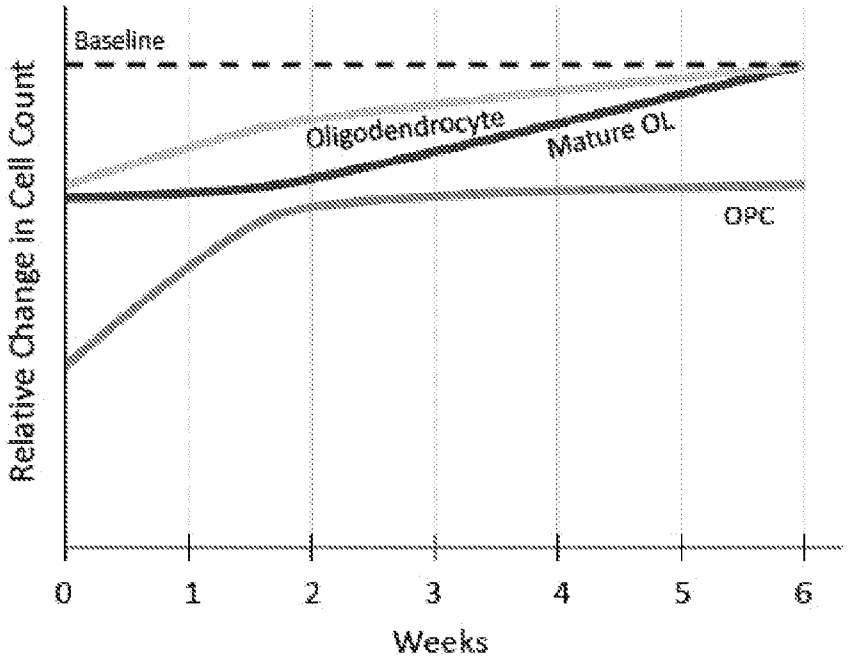

FIG. 12A

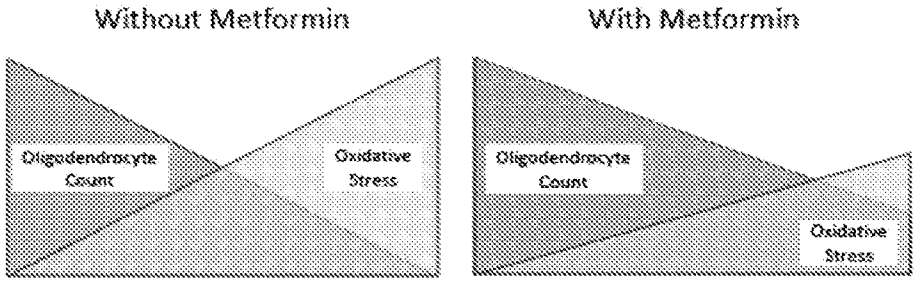

FIG. 12B

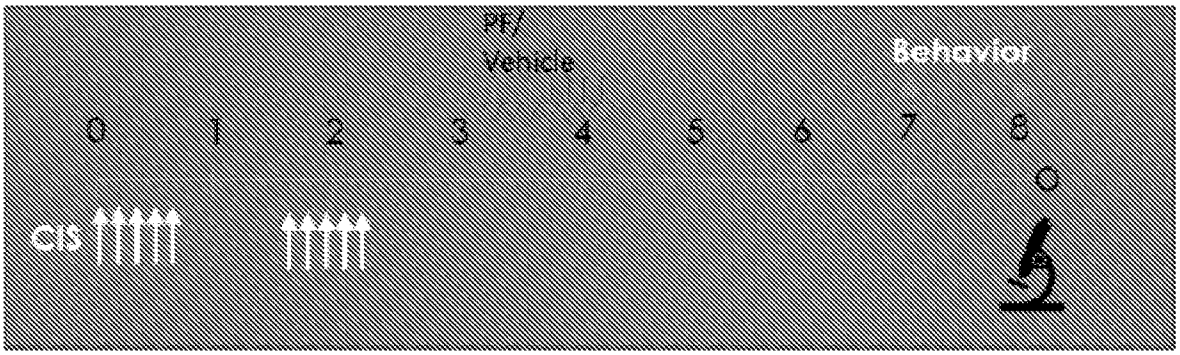

FIG. 13A

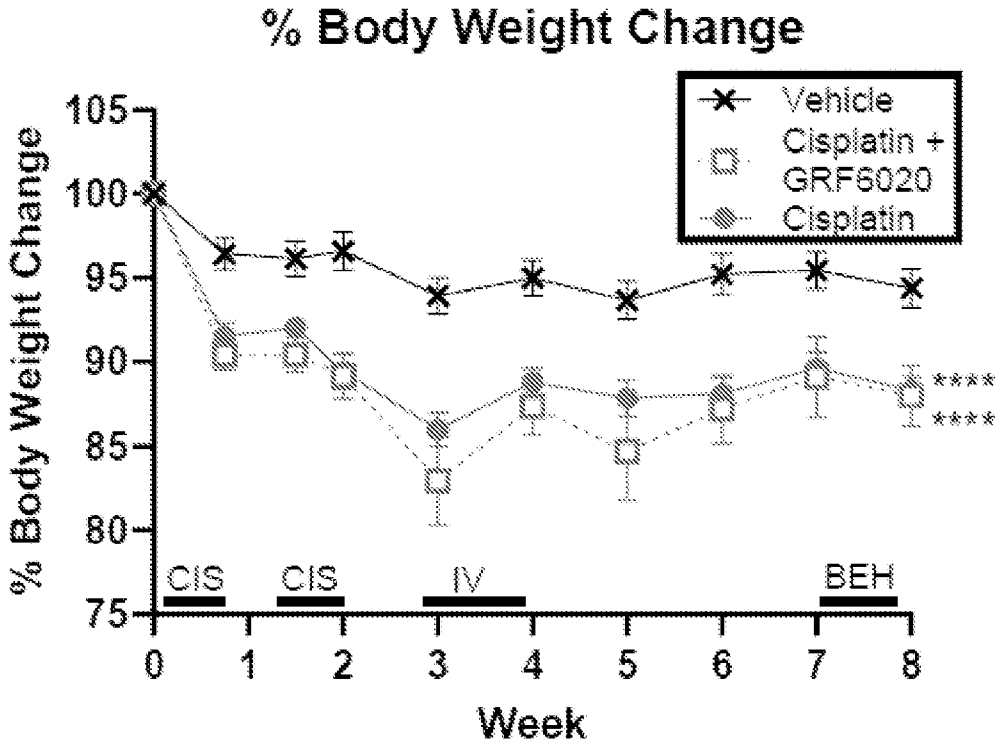

FIG. 13B

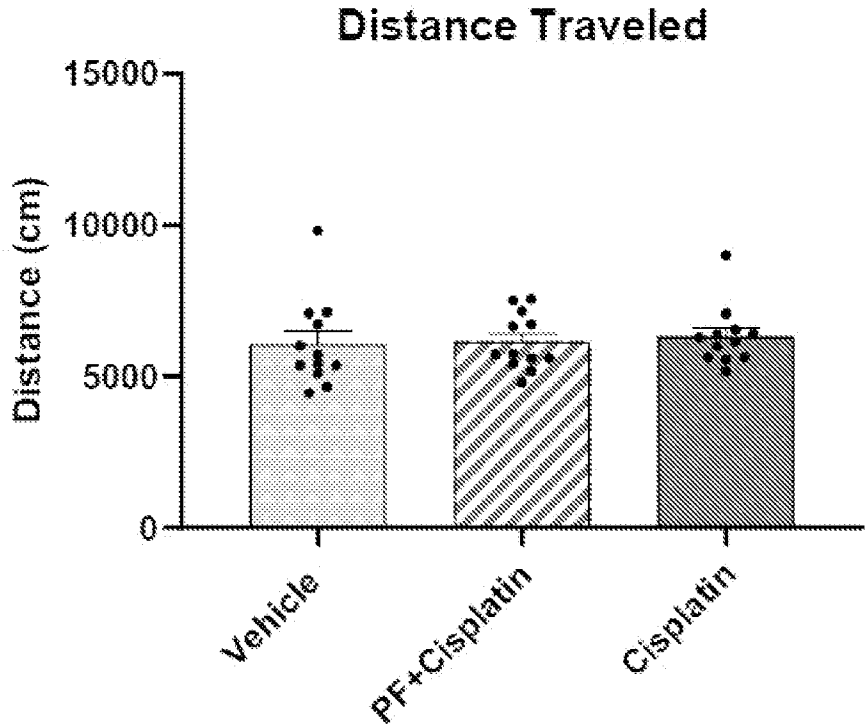

FIG. 13C

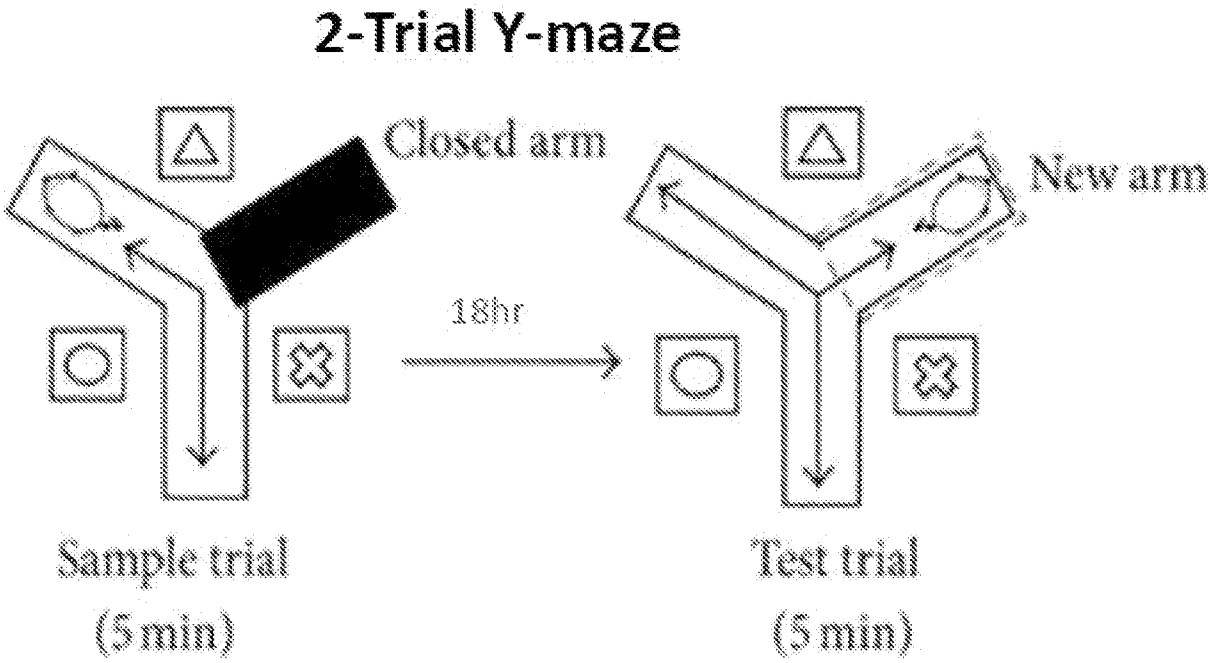

FIG. 13D

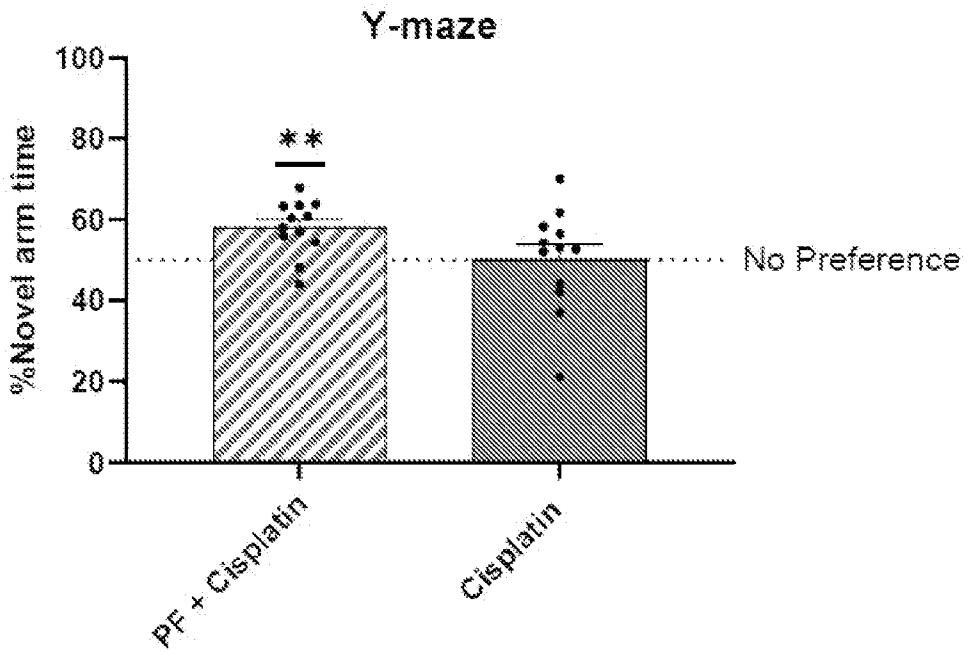

FIG. 13E

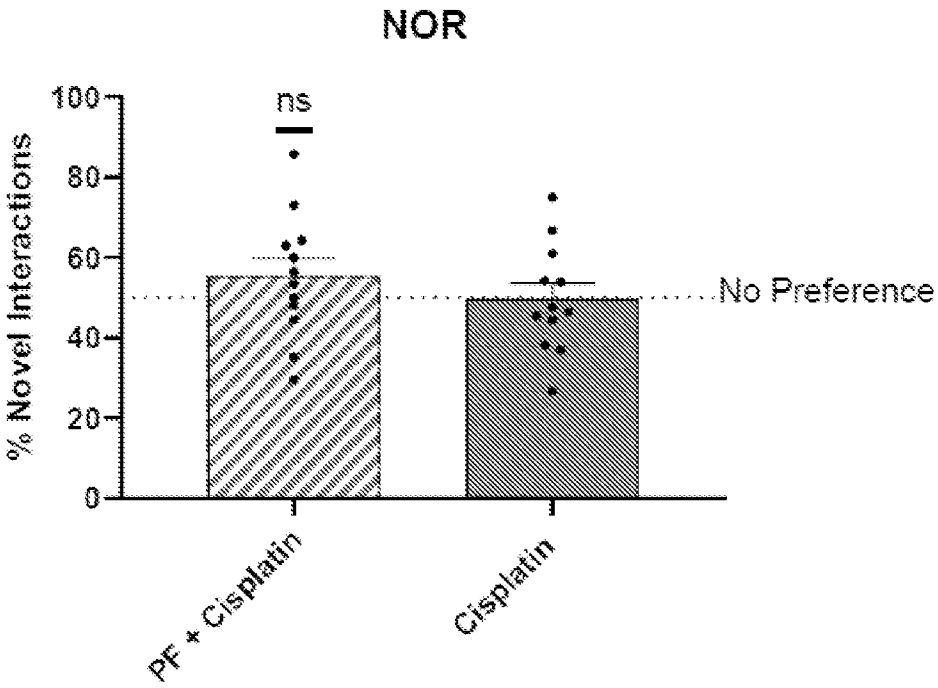

FIG. 13F

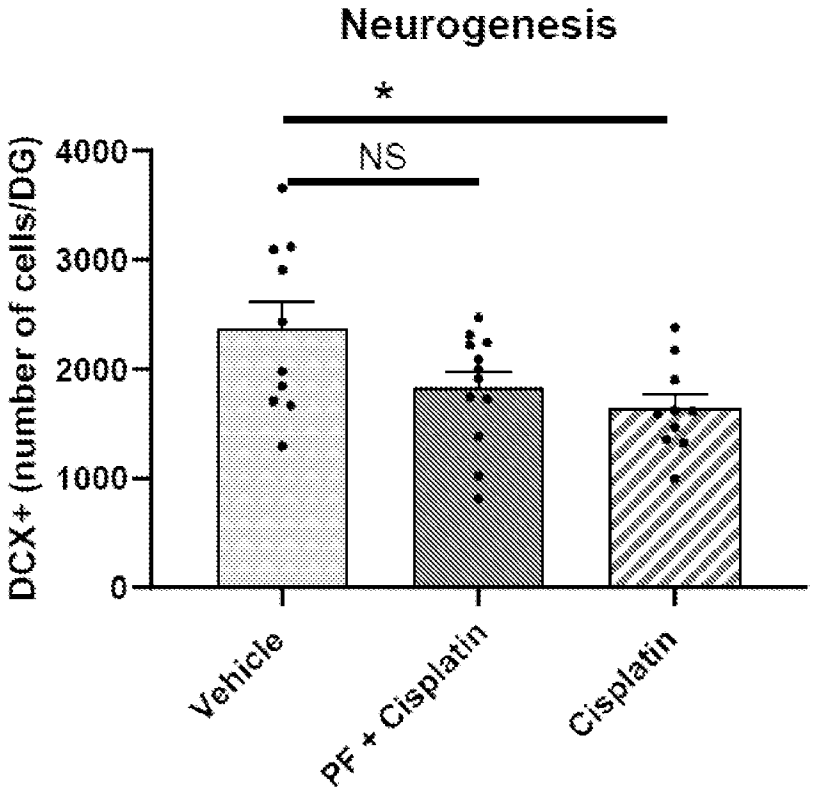

FIG. 14A

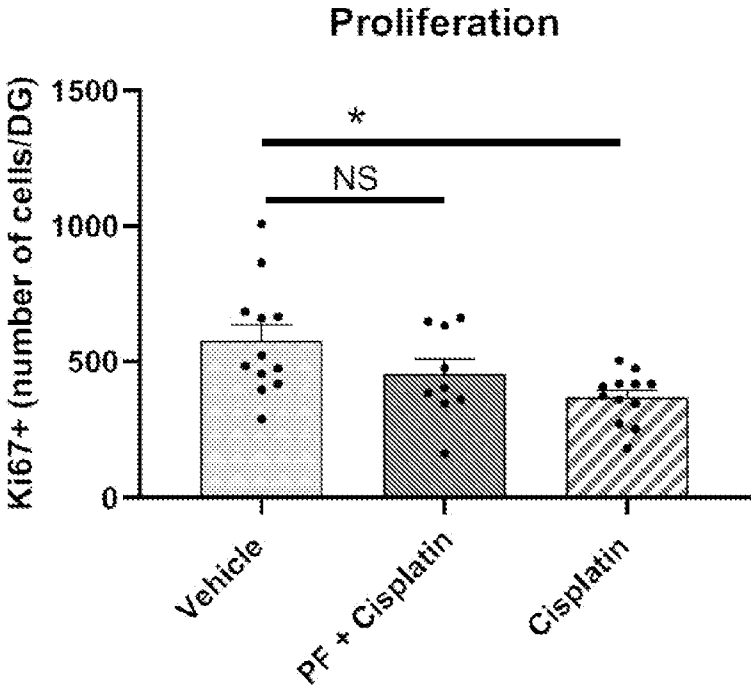

FIG. 14B

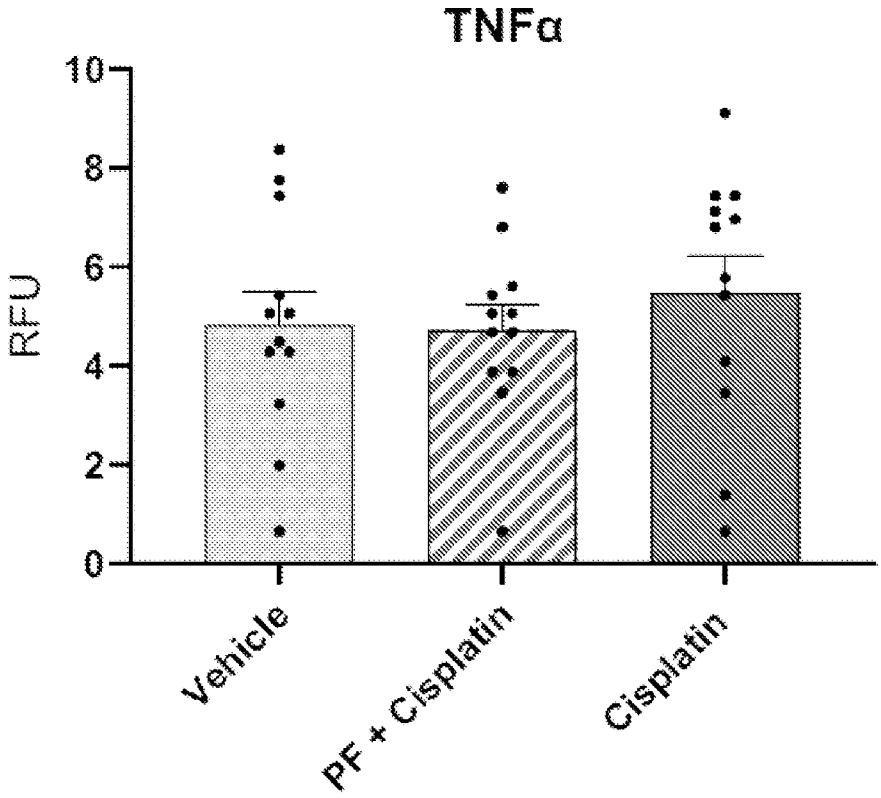

FIG. 14C

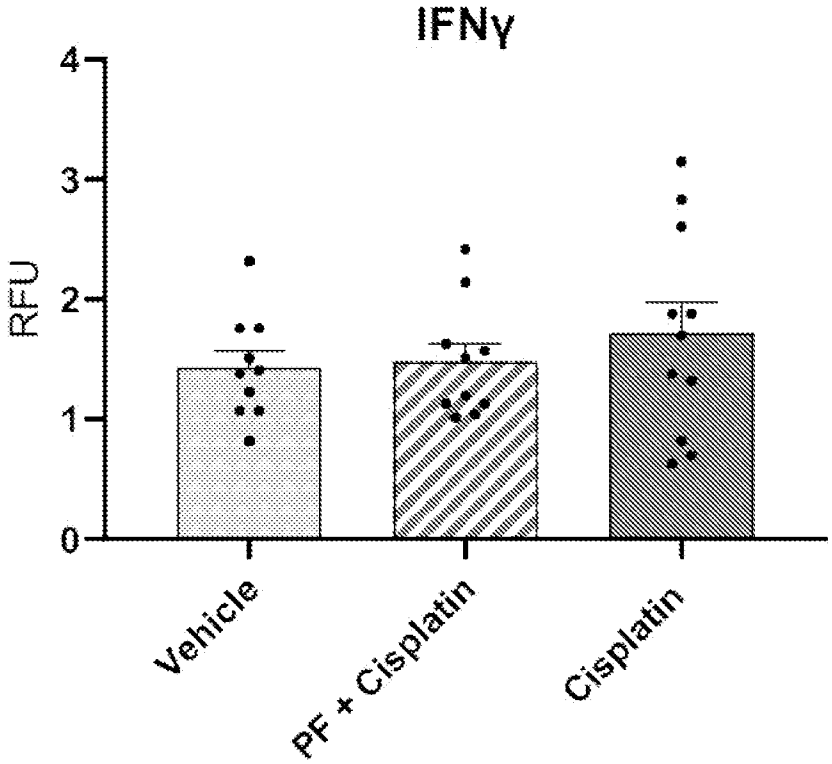

FIG. 14D

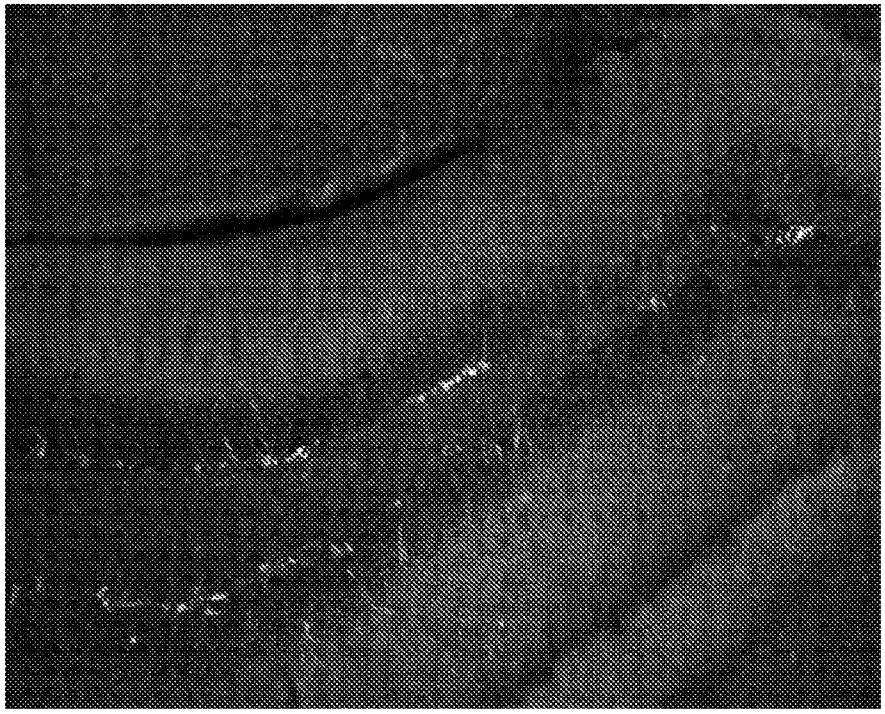

FIG. 14E

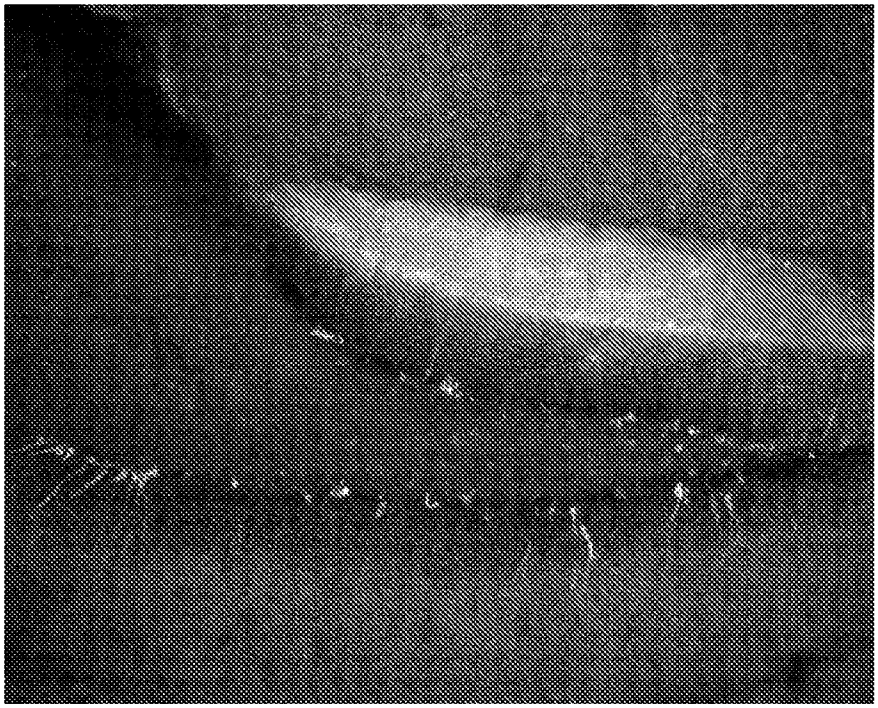

FIG. 14F

FIG. 14G

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/031597

A. CLASSIFICATION OF SUBJECT MATTER

A61K 35/16(2006.01)i; **A61K 38/38**(2006.01)i; **A61K 33/243**(2019.01)i; **A61K 45/06**(2006.01)i; **A61P 25/28**(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 35/16(2006.01); A61K 31/192(2006.01); A61K 31/337(2006.01); A61K 38/38(2006.01); A61P 25/02(2006.01); A61P 25/28(2006.01); A61P 29/00(2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: a chemotherapy induced cognitive disorder, plasma fraction, albumin, cisplatin, cancer, treatment

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2021-0106616 A1 (ALKAHEST, INC.) 15 April 2021 (2021-04-15) claims 6, 8, 10-12; paragraphs [0062], [0076], [0078], [0086], [0096], [0106], [0120], [0122]-[0124]	1-10,19
Y	LIN, Y. et al., "Relationship between peripheral neuropathy and cognitive performance in the elderly population", Medicine, 2021, Vol. 100, No. 20, pages 1-6 abstract; pages 4, 5	1-10,19
A	WO 2016-176437 A1 (NEWSOUTH INNOVATIONS PTY LIMITED et al.) 03 November 2016 (2016-11-03) claims 1, 2	1-10,19
A	US 2019-0321449 A1 (ALKAHEST, INC.) 24 October 2019 (2019-10-24) claims 1-11, 15	1-10,19
A	US 2023-0048576 A1 (CITY OF HOPE) 16 February 2023 (2023-02-16) abstract; claim 1	1-10,19

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2024	**13 September 2024**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office **189 Cheongsa-ro, Seo-gu, Daejeon** **35208, Republic of Korea**	**HEO, Joo Hyung**
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/031597

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☑ Claims Nos.: **12,14,17**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 12,14,17 are unclear since they are referring to the multiple dependent claims which do not comply with PCT Rule 6.4(a).

3. ☑ Claims Nos.: **11,13,15,16,18**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2024/031597

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Information on patent family members

International application No.
PCT/US2024/031597

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Information on patent family members

International application No.
PCT/US2024/031597

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