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(54) **STABLE FORMULATIONS FOR CNS DELIVERY OF ARYLSULFATASE A**

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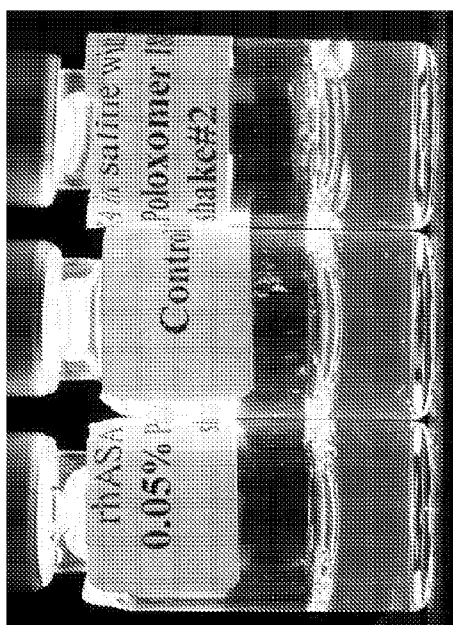
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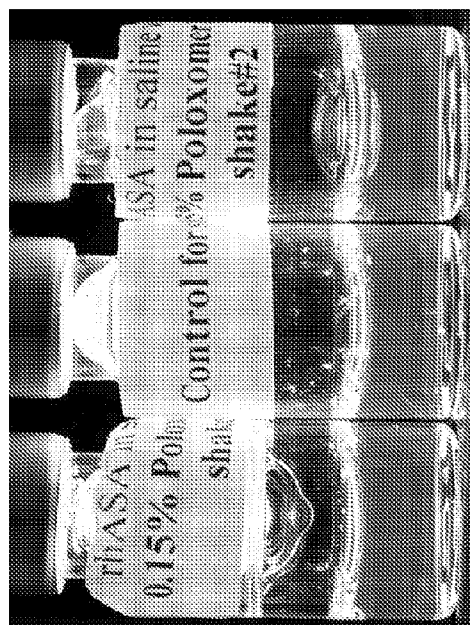
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(57) **ABSTRACT**

The present invention provides, among other things, compositions and methods for CNS delivery of lysosomal enzymes (e.g., recombinant human arylsulfatase A (rhASA)) for effective treatment of lysosomal storage diseases (e.g. Metachromatic Leukodystrophy Disease). In some embodiments, the present invention includes a stable formulation for intrathecal administration comprising an ASA protein and a poloxamer, wherein less than 3% of the ASA protein exists in aggregated form.



0.05% P188



0.15% P188

Fig. 1

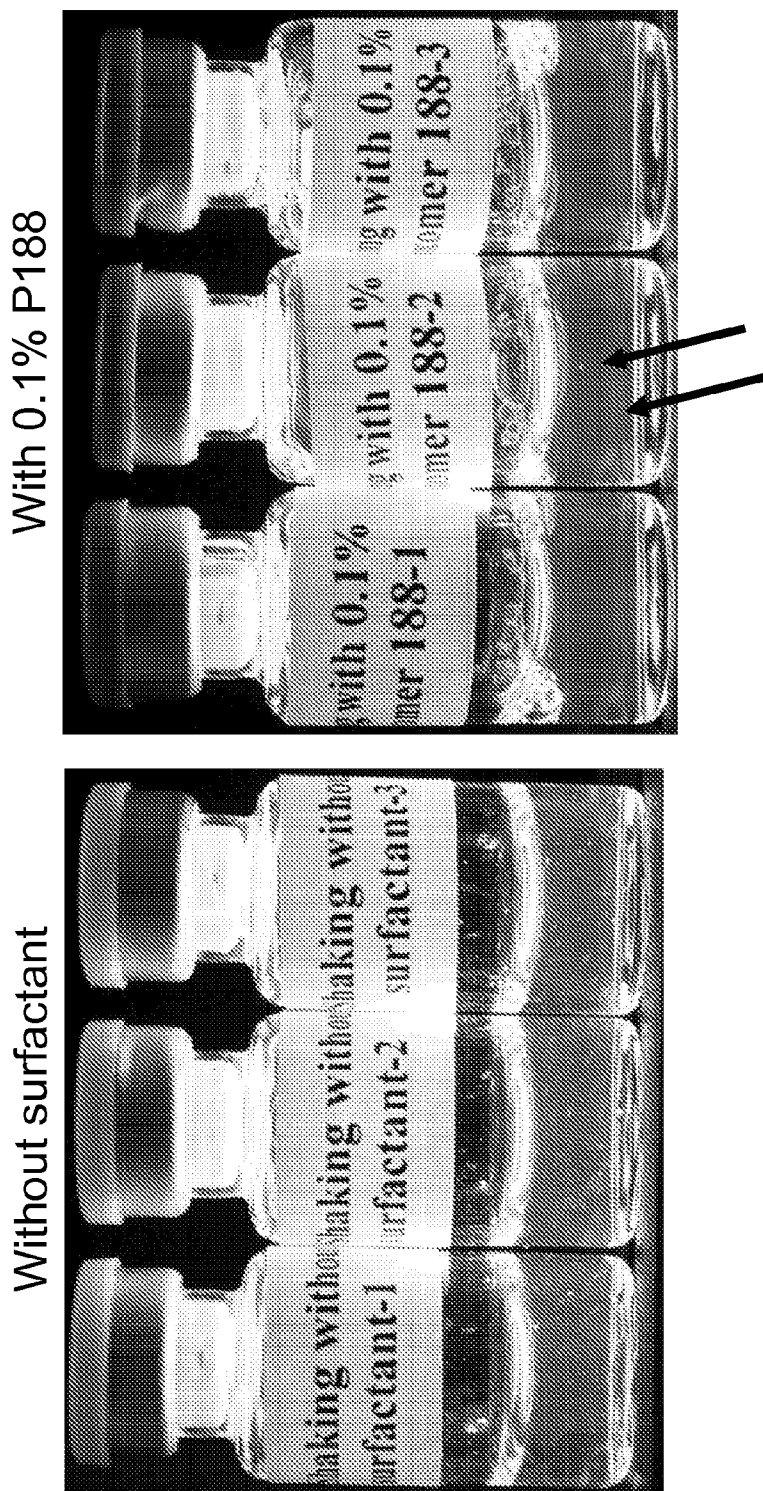


Fig. 2

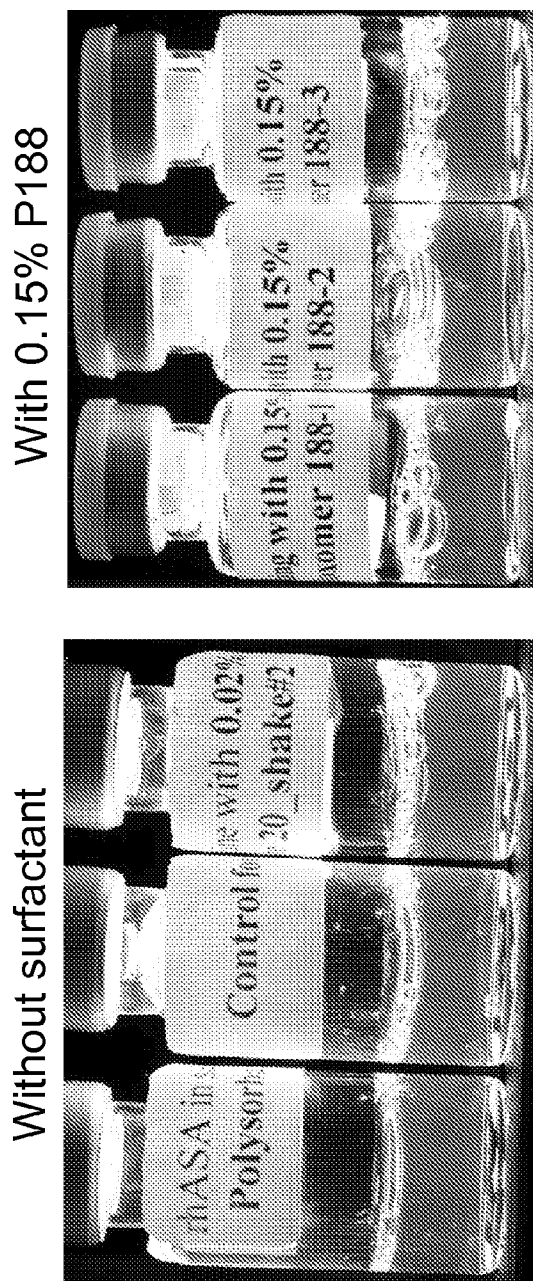


Fig. 3

STABLE FORMULATIONS FOR CNS DELIVERY OF ARYLSULFATASE A

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/580,064, filed Dec. 23, 2011, the entire contents of all of which are incorporated herein by reference.

BACKGROUND

[0002] Enzyme replacement therapy (ERT) involves the systemic administration of natural or recombinantly-derived proteins and/or enzymes to a subject. Approved therapies are typically administered to subjects intravenously and are generally effective in treating the somatic symptoms of the underlying enzyme deficiency. As a result of the limited distribution of the intravenously administered protein and or enzyme into the cells and tissues of the central nervous system (CNS), the treatment of diseases having a CNS etiology has been especially challenging because the intravenously administered proteins and or enzymes do not adequately cross the blood-brain barrier (BBB).

[0003] The blood-brain barrier (BBB) is a structural system comprised of endothelial cells that functions to protect the central nervous system (CNS) from deleterious substances in the blood, stream, such as bacteria, macromolecules (e.g., proteins) and other hydrophilic, molecules, by limiting the diffusion of such substances across the BBB and into the underlying cerebrospinal fluid (CSF) and CNS.

[0004] There are several ways of circumventing the BBB to enhance brain delivery of a therapeutic agent including direct intra-cranial injection, transient permeabilization of the BBB, and modification of the active agent to alter tissue distribution. Direct injection of a therapeutic agent into brain tissue bypasses the vasculature completely, but suffers primarily from the risk of complications (infection, tissue damage, immune responsive) incurred by intra-cranial injections and poor diffusion of the active agent from the site of administration. To date, direct administration of proteins into the brain substance has not achieved significant therapeutic effect due to diffusion barriers and the limited volume of therapeutic that can be administered. Convection-assisted diffusion has been studied via catheters placed in the brain parenchyma using slow, long-term infusions (Bobo, et al., Proc. Natl. Acad. Set. USA 91, 2076-2080 (1994); Nguyen, et al, J. Neurosurg. 98, 584-590 (2003)), but no approved therapies currently use this approach for long-term therapy in addition, the placement of intracerebral catheters is very invasive and less desirable as a clinical alternative.

[0005] Intrathecal (IT) injection, or the administration of proteins to the cerebrospinal fluid (CSF), has also been attempted but has not yet yielded therapeutic success. A major challenge in this treatment has been the tendency of the active agent to bind the ependymal lining of the ventricle very tightly which prevented subsequent diffusion. Currently, there are no approved products for the treatment of brain genetic disease by administration directly to the CSF.

[0006] In fact, many believed that the barrier to diffusion at the brains surface, as well as the lack of effective and convenient delivery methods, were too great an obstacle to achieve adequate therapeutic effect in the brain for any disease.

[0007] Many lysosomal storage disorders affect the nervous system and thus demonstrate unique challenges in treating these diseases with traditional therapies. There is often a

large build-up of glycosaminoglycans (GAGS) in neurons and meninges of affected individuals, leading to various forms of CNS symptoms. To date, no CNS symptoms resulting from a lysosomal disorder has successfully been treated by any means available.

[0008] Furthermore, because proteins utilized in enzyme replacement therapy are larger and more complex than traditional organic and inorganic drugs (i.e., possessing multiple functional groups in addition to complex three-dimensional structures), the formulation, packaging and preservation of such proteins poses special problems.

[0009] Thus, there remains a great need for formulations that can be used to safely and effectively deliver therapeutic agents, such as lysosomal enzymes, to the brain.

SUMMARY OF THE INVENTION

[0010] The present invention provides improved formulations for intrathecal delivery of lysosomal enzymes (e.g., arylsulfatase A (ASA)) for the treatment of lysosomal storage diseases (e.g., MLD). As described in the Example section, the present invention is, in part, based on the surprising discovery that poloxamer is particularly effective in suppressing enzyme precipitation/aggregation, under thermal and mechanical stress, including particulate formation such as flakes and/or fibers in saline or buffer based formulations. It is contemplated that enzyme precipitation is in part caused by protein aggregation that forms high molecular weight species (HMS), which are typically insoluble and denatured. Protein aggregation is a common issue encountered during manufacture and other bioprocessing. For protein therapeutics, the presence of aggregates of an type is typically considered to be undesirable because of the concern that the aggregates may lead to an immunogenic reaction (small aggregates) or may cause adverse events on administration (particulates). The present invention encompasses the recognition that formulations containing a poloxamer stabilize lysosomal enzymes (e.g., ASA) and reduce protein aggregation during lyophilization, reconstitution, storage and patient infusion. Thus, stable formulations according to the present invention can be used to effectively deliver lysosomal enzymes such as an ASA protein, intrathecally for more effective treatment of lysosomal storage diseases that have CNS components without causing substantial immunogenic reaction and adverse effects. The invention further encompasses the recognition that use of a poloxamer allows for stable, ready-to-use liquid formulations, which can be stored at various temperatures.

[0011] As described in detail below, the present inventors have successfully developed stable formulations for effective intrathecal (IT) administration of an arylsulfatase A (ASA) protein. It is contemplated, however, that various stable formulations described herein are generally suitable for CNS delivery of therapeutic agents, including various other lysosomal enzymes. Indeed, stable formulations according to the present invention can be used for CNS delivery via various techniques and routes including, but not limited to, intraparenchymal, intracerebral, intraventricular cerebral (ICV), intrathecal (e.g., IT-Lumbar, IT-cisterna magna) administrations and any other techniques and routes for injection directly or indirectly to the CNS and/or CSF.

[0012] It is also contemplated that various stable formulations described herein are generally suitable for CNS delivery of other therapeutic agents, such as therapeutic proteins including various replacement enzymes for lysosomal stor-

age diseases. In some embodiments, a replacement enzyme can be a synthetic, recombinant, gene-activated or natural enzyme.

[0013] In various aspects, the present invention includes a stable formulation air intrathecal administration comprising an arylsulfatase A (ASA) protein and a poloxamer, wherein less than about 5% (e.g., less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%) of the arylsulfatase A (ASA) protein exists in aggregated form. In some embodiments, the poloxamer is selected, from the group consisting of Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, 407, and combination thereof. In certain embodiments, the poloxamer is poloxamer 188.

[0014] In some embodiments, the poloxamer is present at a concentration of approximately 0.15%. In some embodiments, the poloxamer is present at a concentration of approximately 0.1%. In certain embodiments, the poloxamer is present at a concentration of approximately 0.05-0.5%, 0.05-0.4%, 0.05-0.3%, 0.05%-0.2%, 0.05%-0.15%, 0.1-0.5%, 0.1-0.4%, 0.1-0.3%, 0.1%-0.2%, or 0.1%-0.15%.

[0015] In certain embodiments, less than about 4% of the arylsulfatase A (ASA) protein exists in aggregated form. In certain embodiments, less than about 3% of the arylsulfatase A (ASA) protein exists in aggregated form. In certain embodiments, less than about 2% of the arylsulfatase A (ASA) protein exists in aggregated form. In certain embodiments, less than about 1% of the arylsulfatase A (ASA) protein exists in aggregated form. In certain embodiments, less than about 0.5% of the arylsulfatase A (ASA) protein exists in aggregated form.

[0016] In some embodiments, the ASA protein is present at a concentration ranging from approximately 0.1-100 mg/ml (e.g., approximately 1-100 mg/ml, 5-100 mg/ml, 5-90 mg/ml, 5-80 mg/ml, 5-70 mg/ml, 5-60 mg/ml, 5-50 mg/ml, 5-40 mg/ml, 5-30 mg/ml, 5-20 mg/ml). In some embodiments, the ASA protein is present at or greater than a concentration selected from about 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, 45 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, or 100 mg/ml. In certain embodiments, the ASA protein is present at a concentration selected from about 1 mg/ml, 10 mg/ml, 20 mg/ml, 30 mg/ml, 50 mg/ml, or 100 mg/ml.

[0017] In some embodiments, the ASA protein comprises an amino acid sequence having at least about 60% (e.g., at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) identity to SEQ ID NO: 1. In some embodiments, the ASA protein comprises an amino acid sequence of SEQ ID NO: 1.

[0018] In various embodiments, the ASA protein is produced from a human cell line. In certain embodiments, the ASA protein is produced from CHO cells.

[0019] In various embodiments the formulation further comprises salt. In some embodiments, the salt is NaCl. In certain embodiments, the NaCl is present as a concentration ranging from approximately 0-300 mM (e.g., 0-50 mM, 0-100 mM, 0-120 mM, 0-140 mM, 0-150 mM, 0-160 mM, 0-170 mM, 0-180 mM, 0-200 mM, 0-250 mM). In certain embodiments, the NaCl is present at a concentration ranging from approximately 50-300 mM, approximately 50-200 mM, approximately 80-160 mM, approximately 100-160 mM,

approximately 110-180 mM, approximately 130-170 mM, or approximately 1.30-160 mM. In certain embodiments, the NaCl is present at a concentration of approximately 0 mM, 60 mM, 70 mM, 80 mM, 88 mM, 90 mM, 100 mM, 110 mM, 120 mM, 130 mM, 132 mM, 137 mM, 138 mM, 140 mM, 142 mM, 144 mM, 146 mM, 148 mM, 150 mM, 152 mM, 154 mM, 156 mM, 158 mM, or 160 mM.

[0020] In various embodiments, the stable formulation further comprises a buffering agent. In certain embodiments, the buffering agent is selected from the group consisting of phosphate, acetate, histidine, succinate, citrate, Tris, and combinations thereof. In some embodiments, the buffering agent is phosphate. In some embodiments, the formulation has a pH of approximately 3-8.0 (e.g., approximately 4-7.5, 5-8, 5-7.5, 5-6.5, 5-7.0, 5.5-8.0, 5.5-7.7, 5.5-6.5, 6-7.5, or 6-7.0). In some embodiments, the formulation has a pH of approximately 6.0-6.5 (e.g., 6.0, 6.1, 6.2, 6.3, 6.4, or 6.5). In certain embodiments, the formulation has a pH of approximately 6.0. In some embodiments, a stable formulation according to the invention does not comprise a buffering agent. As used herein, a formulation without a buffering agent is also known as a saline based formulation.

[0021] In various embodiments, the formulation is a liquid formulation. In various embodiments, the formulation is formulated as lyophilized dry powder.

[0022] In various embodiments, the formulation further comprises a stabilizing agent. In certain embodiments, the stabilizing agent is selected from the group consisting of sucrose, glucose, mannitol, sorbitol, polyethylene glycol PEG2000, PEG3250, PEG4000, PEG5000, etc.), histidine, arginine, lysine, phospholipids, trehalose and combination thereof.

[0023] In some embodiments, the present invention provides a stable formulation for intrathecal administration comprising an arylsulfatase A (ASA) protein and a poloxamer, wherein the ASA protein is present at a concentration of at least approximately 1 mg/ml (e.g., at least approximately 5 mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/ml, 30 mg/ml, 40 mg/ml, 45 mg/ml, 50 mg/ml). In some embodiments, the poloxamer is at a concentration of approximately 0.05%, 0.10%, 0.15%, 0.20%, 0.25%, or 0.30%. In certain embodiments, the poloxamer is present at a concentration of 0.05%-0.5%, 0.05%-0.2%, 0.05%-0.15%, 0.1%-0.2%, or 0.1%-0.15%. In some embodiments, the poloxamer is selected from the group consisting of Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, 407, and combination thereof. In certain embodiments, the poloxamer is poloxamer 188.

[0024] In some embodiments, less than about 5% (e.g., less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, or less than about 0.1%) of the arylsulfatase A (ASA) protein exists in aggregated form. In some embodiments, less than 3% of the arylsulfatase A (ASA) protein exists in aggregated form. In some embodiments, less than about 1% of the arylsulfatase A (ASA) protein exists in aggregated form.

[0025] In various embodiments, the ASA protein is at a concentration ranging from approximately 0.1-100 mg/ml. In some embodiments, the ASA protein is present at or greater than a concentration selected from about 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, 45 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, or 100 mg/ml.

[0026] In various aspects, the present invention includes a container comprising a single dosage form of a stable formulation according to embodiments described herein. In some embodiments, the container is selected from an ampule, a vial, a cartridge, a reservoir, a lyo-ject, or a pre-filled syringe. In certain embodiments, the container is a pre-filled syringe. In particular embodiments, the pre-filled syringe is selected from borosilicate glass syringes with baked silicone coating, borosilicate glass syringes with sprayed silicone, or plastic resin syringes without silicone.

[0027] In some embodiments, the stable formulation is present in a volume of less than about 50.0 mL (e.g., less than about 45 ml, 40 ml, 35 ml, 30 ml, 25 ml, 20 ml, 15 ml, 10 ml, 5 ml, 4 ml, 3 ml, 2.5 ml, 2.0 ml, 1.5 ml, 1.0 ml, or 0.5 ml). In certain embodiments, the stable formulation is present in a volume of less than about 5.0 mL (e.g., less than about 5 ml, 4 ml, 2.5 ml, 2.0 ml, 1.5 ml, 1.0 ml, or 0.5 ml).

[0028] In various aspects, the present invention includes a method of treating metachromatic leukodystrophy (MLD) disease comprising a step of administering intrathecally to a subject in need of treatment a formulation according to any of the embodiments described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The drawings are for illustration purposes only and not for limitation.

[0030] FIG. 1 illustrates exemplary arylsulfatase A (rhASA) formulation agitation studies.

[0031] FIGS. 2 and 3 illustrate exemplary rhASA pumping and shaking studies.

DEFINITIONS

[0032] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0033] Approximately or about: As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0034] Amelioration: As used herein, the term “amelioration” is meant the prevention, reduction or palliation of a state, or improvement of the state of a subject. Amelioration includes, but does not require complete recovery or complete prevention of a disease condition. In some embodiments, amelioration includes increasing levels of relevant protein or its activity that is deficient in relevant disease tissues.

[0035] Biologically active: As used herein, the phrase “biologically active” refers to a characteristic of any agent that has activity in a biological system, and particularly in an organism. For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, where a protein or polypeptide is biologically active, a portion of that protein or polypeptide that shares at least one biologi-

cal activity of the protein or polypeptide is typically referred to as a “biologically active” portion.

[0036] Bulking agent: As used herein, the term “bulking agent” refers to a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake (e.g., facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Exemplary bulking agents include mannitol, glycine, sodium chloride, hydroxyethyl starch, lactose, sucrose, trehalose, polyethylene glycol and dextran.

[0037] Cation-independent mannose-6-phosphate receptor (CI-MPR): As used herein, the term “cation-independent mannose-6-phosphate receptor (CI-MPR)” refers to a cellular receptor that binds mannose-6-phosphate (M6P) tags on acid hydrolase precursors in the Golgi apparatus that are destined for transport to the lysosome. In addition to mannose-6-phosphates, the CI-MPR also binds other proteins including IGF-II. The CI-MPR is also known as “M6P/IGF-II receptor,” “CI-MPR/IGF-II receptor,” “IGF-II receptor” or “IGF2 Receptor.” These terms and abbreviations thereof are used interchangeably herein.

[0038] Concurrent immunosuppressant therapy: As used herein, the term “concurrent immunosuppressant therapy” includes any immunosuppressant therapy used as pre-treatment, preconditioning or in parallel to a treatment method.

[0039] Diluent: As used herein, the term “diluent” refers to a pharmaceutically acceptable (e.g., safe and non-toxic for administration to a human) diluting substance useful for the preparation of a reconstituted formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer’s solution or dextrose solution.

[0040] Dosage form: As used herein, the terms “dosage form” and “unit dosage form” refer to a physically discrete unit of a therapeutic protein for the patient to be treated. Each unit contains a predetermined quantity of active material calculated to produce the desired therapeutic effect. It will be understood, however, that the total dosage of the composition will be decided by the attending physician within the scope of sound medical judgment.

[0041] Enzyme replacement therapy (ERT): As used herein, the term “enzyme replacement therapy (ERT)” refers to any therapeutic strategy that corrects an enzyme deficiency by providing the missing enzyme. In some embodiments, the missing enzyme is provided, by intrathecal administration. In some embodiments, the missing enzyme is provided by infusing into bloodstream. Once administered, enzyme is taken up by cells and transported to the lysosome, where the enzyme acts to eliminate material that has accumulated in the lysosomes due to the enzyme deficiency. Typically, for lysosomal enzyme replacement therapy to be effective, the therapeutic enzyme is delivered to lysosomes in the appropriate cells in target tissues where the storage defect is manifest.

[0042] Improve, increase, or reduce: As used herein, the terms “improve,” “increase” or “reduce,” or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A “control individual” is an individual afflicted with the same form of lysosomal storage disease as the individual being treated, who is about the same age as the individual being

treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

[0043] Individual, subject, patient: As used herein, the terms “subject,” “individual” or “patient” refer to a human or a non-human mammalian subject. The individual (also referred to as “patient” or “subject”) being treated is an individual (fetus, infant, child, adolescent, or adult human) suffering from a disease.

[0044] Intrathecal administration: As used herein, the term “intrathecal administration” or “intrathecal injection” refers to an injection into the spinal canal (intrathecal space surrounding the spinal cord). Various techniques may be used including, without limitation, lateral cerebroventricular injection through a burrhole or cisternal or lumbar puncture or the like. In some embodiments, “intrathecal administration” or “intrathecal delivery” according to the present invention refers to IT administration or delivery via the lumbar area or region, i.e., lumbar IT administration or delivery. As used herein, the term “lumbar region” or “lumbar area” refers to the area between the third and fourth lumbar (lower back) vertebrae and, more inclusively the L2-S1 region of the spine.

[0045] Linker: As used herein, the term “linker” refers to, in a fusion protein, an amino acid sequence other than that appearing at a particular position in the natural protein and is generally designed to be flexible or to interpose a structure, such as an α -helix, between two protein moieties. A linker is also referred to as a spacer.

[0046] Lyoprotectant: As used herein, the term “lyoprotectant” refers to a molecule that prevents or reduces chemical and/or physical instability of a protein or other substance upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars such as sucrose or trehalose; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, e.g. glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronic; and combinations thereof. In some embodiments, a lyoprotectant is a non-reducing sugar, such as trehalose or sucrose.

[0047] Lysosomal enzyme: As used herein, the term “lysosomal enzyme” refers to any enzyme that is capable of reducing accumulated materials in mammalian lysosomes or that can rescue or ameliorate one or more lysosomal storage disease symptoms. Lysosomal enzymes suitable for the invention include both wild-type or modified lysosomal enzymes and can be produced using recombinant and synthetic methods or purified from nature sources. Exemplary lysosomal enzymes are listed in Table 1.

[0048] Lysosomal enzyme deficiency: As used herein, “lysosomal enzyme deficiency” refers to a group of genetic disorders that result from deficiency in at least one of the enzymes that are required to break macromolecules (e.g., enzyme substrates) down to peptides, amino acids, monosaccharides, nucleic acids and fatty acids in lysosomes. As a result, individuals suffering from lysosomal enzyme deficiencies have accumulated materials in various tissues (e.g., CNS, liver, spleen, gut, blood vessel walls and other organs).

[0049] Lysosomal Storage Disease: As used herein, the term “lysosomal storage disease” refers to any disease resulting from the deficiency of one or more lysosomal enzymes necessary for metabolizing natural macromolecules. These diseases typically result in the accumulation of un-degraded molecules in the lysosomes, resulting in increased numbers of

storage granules (also termed storage vesicles). These diseases and various examples are described in more detail below.

[0050] Polypeptide: As used herein, a “polypeptide”, generally speaking, is a string of at least two amino acids attached to one another by a peptide bond. In some embodiments, a polypeptide may include at least 3-5 amino acids, each of which is attached to others by way of at least one peptide bond. Those of ordinary skill in the art will appreciate that polypeptides sometimes include “non-natural” amino acids or other entities that nonetheless are capable of integrating into as polypeptide chain, optionally.

[0051] Replacement enzyme: As used herein, the term “replacement enzyme” refers to any enzyme that can act to replace at least in part the deficient or missing enzyme in a disease to be treated. In some embodiments, the term “replacement enzyme” refers to any enzyme that can act to replace at least in part the deficient or missing lysosomal enzyme in a lysosomal storage disease to be treated. In some embodiments, a replacement enzyme is capable of reducing accumulated materials in mammalian lysosomes or that can rescue or ameliorate one or more lysosomal storage disease symptoms. Replacement enzymes suitable for the invention include both wild-type or modified lysosomal enzymes and can be produced using recombinant and synthetic methods or purified from nature sources. A replacement enzyme can be a recombinant, synthetic, gene-activated or natural enzyme.

[0052] Soluble: As used herein, the term “soluble” refers to the ability of a therapeutic agent to form a homogenous solution. In some embodiments, the solubility of the therapeutic agent in the solution into which it is administered and by which it is transported to the target site of action (e.g., the cells and tissues of the brain) is sufficient to permit the delivery of a therapeutically effective amount of the therapeutic agent to the targeted, site of action. Several factors can impact the solubility of the therapeutic agents. For example, relevant factors which may impact protein solubility include ionic strength, amino acid sequence and the presence of other cosolubilizing agents or salts (e.g., calcium salts). In some embodiments, the pharmaceutical compositions are formulated such that calcium salts are excluded from such compositions. In some embodiments, therapeutic agents in accordance with the present invention are soluble in its corresponding pharmaceutical composition. It will be appreciated that, while isotonic solutions are generally preferred for parenterally administered drugs, the use of isotonic solutions may limit adequate solubility for some therapeutic agents and, in particular some proteins and/or enzymes. Slightly hypertonic solutions (e.g., up to 175 mM sodium chloride in 5 mM sodium phosphate at pH 7.0) and sugar-containing solutions (e.g., up to 2% sucrose in 5 mM sodium phosphate at pH 7.0) have been demonstrated to be well tolerated in monkeys. For example, the most common approved CNS bolus formulation composition is saline (150 mM NaCl in water).

[0053] Stability: As used herein, the term “stable” refers to the ability of the therapeutic agent (e.g., a recombinant enzyme) to maintain its therapeutic efficacy (e.g., all or the majority of its intended biological activity and/or physiochemical integrity) over extended periods of time. The stability of a therapeutic agent, and the capability of the pharmaceutical composition to maintain stability of such therapeutic agent, may be assessed over extended periods of time (e.g., for at least 1, 3, 6, 12, 18, 24, 30, 36 months or more). In

general, pharmaceutical compositions described herein have been formulated such that they are capable of stabilizing, or alternatively slowing or preventing the degradation, of one or more therapeutic agents formulated therewith (e.g., recombinant proteins). In the context of a formulation a stable formulation is one in which the therapeutic agent therein essentially retains its physical and/or chemical integrity and biological activity upon storage and during processes (such as freeze/thaw, mechanical mixing and lyophilization). For protein stability, it can be measured by formation of high molecular weight (HMW) aggregates, loss of enzyme activity, generation of peptide fragments and shift of charge profiles.

[0054] Subject: As used herein, the term “subject” means any mammal, including humans. In certain embodiments of the present invention the subject is an adult, an adolescent or an infant. Also contemplated by the present invention are the administration of the pharmaceutical compositions and/or performance of the methods of treatment in-utero.

[0055] Substantial homology: The phrase “substantial homology” is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be “substantially homologous” if they contain homologous residues in corresponding positions. Homologous residues may be identical residues. Alternatively, homologous residues may be non-identical residues will appropriately similar structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as “hydrophobic” or “hydrophilic” amino acids, and/or as having “polar” or “non-polar” side chains. Substitution of one amino acid for another of the same type may often be considered a “homologous” substitution.

[0056] As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J. Mol. Biol.* 215(3): 403-410, 1990; Altschul, et al., *Methods in Enzymology*; Altschul, et al., “Gapped BLAST and PSI-BLAST: a new generation of protein database search programs”, *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis, et al., *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying homologous sequences, the programs mentioned above typically provide an indication of the degree of homology. In some embodiments, two sequences are considered to be substantially homologous if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are homologous over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues.

[0057] Substantial identity: The phrase “substantial identity” is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally

considered to be “substantially identical” if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, *J. Mol. Biol.*, 215(3): 403-410, 1990; Altschul, et al., *Methods Enzymology*; Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1997; Baxevanis et al., *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins*, Wiley, 1998; and Misener, et al., (eds.), *Bioinformatics Methods and Protocols* (Methods in Molecular Biology, Vol. 132), Humana Press, 1999, in addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues.

[0058] Synthetic CSF: As used herein, the term “synthetic CSF” refers to a solution that has pH, electrolyte composition, glucose content and osmolarity consistent with the cerebrospinal fluid. Synthetic CSF is also referred to as artificial CSF. In some embodiments, synthetic CSF is an Elliott’s B solution.

[0059] Suitable for CNS delivery: As used herein, the phrase “suitable for CNS delivery” or “suitable for intrathecal delivery” as it relates to the pharmaceutical compositions of the present invention generally refers to the stability, tolerability, and solubility properties of such compositions, as well as the ability of such compositions to deliver an effective amount of the therapeutic agent contained therein to the targeted site of delivery (e.g., the CSF or the brain).

[0060] Target tissues: As used herein, the term “target tissues” refers to any tissue that is affected by the lysosomal storage disease to be treated or any tissue in which the deficient lysosomal enzyme is normally expressed. In some embodiments, target tissues include those tissues in which there is a detectable or abnormally high amount of enzyme substrate, for example stored in the cellular lysosomes of the tissue, in patients suffering from or susceptible to the lysosomal storage disease. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature. In some embodiments, target tissues include those tissues in which the deficient lysosomal enzyme is normally expressed at an elevated level. As used herein, a target tissue may be a brain target tissue, a spinal cord target tissue and/or a peripheral target tissue. Exemplary target tissues are described in detail below.

[0061] Therapeutic moiety: As used herein, the term “therapeutic moiety” refers to a portion of a molecule that renders the therapeutic effect of the molecule. In some embodiments, a therapeutic moiety is a polypeptide having therapeutic activity.

[0062] Therapeutically effective amount: As used herein, the term “therapeutically effective amount” refers to an

amount of a therapeutic protein (e.g., replacement enzyme) which confers a therapeutic effect on the treated subject, at a reasonable benefit/risk ratio applicable to any medical treatment. The therapeutic effect may be objective measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). In particular, the “therapeutically effective amount” refers to an amount of a therapeutic protein or composition effective to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease. A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular therapeutic protein, as therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. Also, the specific therapeutically effective amount (and/or unit dose) for any particular patient may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and/or rate of excretion or metabolism of the specific fusion protein employed; the duration of the treatment; and like factors as is well known in the medical arts.

[0063] Tolerable: As used herein, the terms “tolerable” and “tolerability” refer to the ability of the pharmaceutical compositions of the present invention to not elicit an adverse reaction in the subject to whom such composition is administered, or alternatively not to elicit a serious adverse reaction in the subject to whom such composition is administered. In some embodiments, the pharmaceutical compositions of the present invention are well tolerated by the subject to whom such compositions is administered.

[0064] Treatment: As used herein, the term “treatment” (also “treat” or “treating”) refers to any administration of a therapeutic protein lysosomal enzyme) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of a particular disease, disorder, and/or condition (e.g., Hunter syndrome, Sanfilippo B syndrome). Such treatment may be of a subject who does not exhibit signs oldie relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition.

DETAILED DESCRIPTION OF THE INVENTION

[0065] The present invention provides, among other things, improved methods and compositions for effective direct delivery of a therapeutic agent to the central nervous system (CNS). The present invention is, in pan, based on the surprising discovery that the including surfactant, in particular, a poloxamer such as poloxamer 188, in a formulation significantly reduced or eliminated enzyme precipitation. As discussed above, enzyme precipitation is in part caused by protein aggregation that forms high molecular weight species (HMS), which are typically insoluble and denatured. Protein

aggregation is a common issue encountered during manufacture and other bioprocessing (e.g., lyophilization, reconstitution, storage and patient infusion). For protein therapeutics, the presence of aggregates of any type is typically considered to be undesirable because of the concern that the aggregates may lead to an immunogenic reaction (small aggregates) or may cause adverse events on administration (particulates). Thus, stable formulations according to the present invention can be used to effectively deliver lysosomal enzymes, such as an ASA protein, intrathecally for more effective treatment of lysosomal storage diseases that have CNS components without causing substantial immunogenic reaction and adverse effects.

[0066] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention in this application, the use of “or” means “and/or” unless stated otherwise.

Therapeutic Proteins

[0067] In some embodiments, inventive methods and compositions provided by the present invention are used to deliver an arylsulfatase A (ASA) protein to the CNS for treatment of Metachromatic Leukodystrophy Disease. A suitable ASA protein can be any molecule or as portion of a molecule that can substitute for naturally-occurring arylsulfatase A (ASA) protein activity or rescue one or more phenotypes or symptoms associated with ASA-deficiency. In some embodiments, as replacement enzyme suitable for the invention is a polypeptide having an N-terminus and a C-terminus and an amino acid sequence substantially similar or identical to mature human ASA protein.

[0068] Typically, human ASA is produced as a precursor molecule that is processed to a mature form. This process generally occurs by removing the 18 amino acid signal peptide. Typically, the precursor form is also referred to as full-length precursor or full-length ASA protein, which contains 507 amino acids. The N-terminal 18 amino acids are cleaved, resulting in a mature form that is 489 amino acids in length. Thus, it is contemplated that the N-terminal 18 amino acids is generally not required for the ASA protein activity. The amino acid sequences of the mature form (SEQ ID NO:1) and full-length precursor (SEQ ID NO:2) of a typical wild-type or naturally-occurring human ASA protein are shown in Table 1.

TABLE 1

| Human Arylsulfatase A | |
|-----------------------|---|
| Mature Form | RPPNIVLIFADDLGYGDLGCYGHPSSTTPNLDQLA AGGLRFDFYVVPVSLCTPSRAALLTGRLPVRMGMY PGVLVPSRRGGLPLEEVVAEVLAAARGYLTMAGK WHLGVPPEGAFLLPHQGFHRLGIPYSHDQGPQCM LTCFPPATPCDGGCDQGLVPIPLLANLSVEAQPPW LPGLEARYMAFAHDLMDAQRDRPFLLYASHHT HYPOFSGQSFAERSGRGPFGLMELDAAVGLTMT AIGDLGLEETLVIFTADNGPETMRMSRGGCSGLL RCGKTTYEGGVRPALAFWPGHIPAGVTHELASS LDLLPTLAALAGAPLPNVTLTLDGFDLSPLLLGTGKS PRQSLFFYPSPYDVEVRGVFAVRTGKYKAHFPTQGS AHSDDTADPACHASSSLTAHEPPLLYDLKDPGEN YNLLGGVAGATPEVLQALKQLQLLKAQLDAAVTFG PSQVARGEDPALQICCHPGCTPRPACCHCPDPHA (SEQ ID NO: 1) |

TABLE 1-continued

| Human Arylsulfatase A | |
|-----------------------|---|
| Full-Length | MGAPRSLLLALAAGLAVARPPNIVLIFADDLGYGD |
| Precursor | LGCYGHPSSTTPNLDQLAAGGLRFTDFYVPVSLCT PSRAALLTGRLPVRMGMYPGVLVPSRGGGLPLEEV TVAEVLAAARGYLTMAGKWHLGVGPEGAFPPHQQ FHRFLGIPYSHDQGPCQNLTCFPPATPCDGGCDQG LVPIPLLANLSVEAQPWLPGLREARYMAFAHDLMA DAQQRDRPFLLYYASHHHTYPOFSGQSFASRGRG PFGDSELMELDAVGTLMTAIGDLGLEETLVIPTA DNGPETMRMSRGGCSGLLRGCGKTTYEGGVREPAL APWPGHIAPGVTHELASSLDLLPTLAALAGAPLPN VTLDGFDLSPDLLGTGKSPRQSLFFYPSYPDEVRG VFAVRTGKYKAHFFFTQGSASDITADPACHASSSL TAHEPPLLVDLSKDPGENYNLLGGVAGATPEVLQA LRQLQLLKAQLDAAVTFGPSQVARGEDPALQICCH PGCTPRPACCHCPDPA (SEQ ID NO: 2) |

[0069] Thus, in some embodiments, a therapeutic moiety suitable for the present invention is mature human ASA protein (SEQ ID NO:1). In some embodiments, a suitable therapeutic moiety may be a homologue or an analogue of mature human ASA protein. For example, as homologue or an analogue of mature human ASA protein may be as modified mature human ASA protein containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring ASA protein (e.g., SEQ ID NO:1), while retaining substantial ASA protein activity. Thus, in some embodiments, a therapeutic moiety suitable for the present invention is substantially homologous to mature human ASA protein (SEQ ID NO:1). In some embodiments, a therapeutic moiety suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:1. In some embodiments, a therapeutic moiety suitable for the present invention is substantially identical to mature human ASA protein (SEQ ID NO:1). In some embodiments, a therapeutic moiety suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:1. In some embodiments, a therapeutic moiety suitable for the present invention contains a fragment or a portion of mature human ASA protein.

[0070] Alternatively, a replacement enzyme suitable for the present invention is full-length ASA protein. In some embodiments, a suitable replacement enzyme may be a homologue or an analogue of full-length human ASA protein. For example, a homologue or an analogue of full-length human ASA protein may be a modified full-length human ASA protein containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring full-length ASA protein (e.g., SEQ ID NO:2), while retaining substantial ASA protein activity. Thus, in some embodiments, a replacement enzyme suitable for the present invention is substantially homologous to full-length human ASA protein (SEQ ID NO:2). In some embodiments, at replacement enzyme suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 97%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:2. In

some embodiments, a replacement enzyme suitable for the present invention is substantially identical to SEQ ID NO:2. In some embodiments, a replacement enzyme suitable for the present invention has an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2. In some embodiments, a replacement enzyme suitable for the present invention contains a fragment or a portion of full-length human ASA protein. As used herein, a full-length ASA protein typically contains signal peptide sequence.

[0071] In some embodiments, a therapeutic protein includes a targeting moiety (e.g., a lysosome targeting sequence) and/or a membrane-penetrating peptide. In some embodiments, a targeting sequence and/or a membrane-penetrating peptide is an intrinsic part of the therapeutic moiety (e.g., via a chemical linkage, via a fusion protein). In some embodiments, a targeting sequence contains a mannose-6-phosphate moiety. In some embodiments, a targeting sequence contains an IGF-I moiety. In some embodiments, a targeting sequence contains an moiety.

Other Lysosomal Storage Diseases and Replacement Enzymes

[0072] It is contemplated that inventive methods and compositions according to the present invention can be used to treat other lysosomal storage diseases, in particular those lysosomal storage diseases having CNS etiology and/or symptoms, including, but are not limited to, aspartylglucosaminuria, cholesterol ester storage disease, Wolman disease, cystinosis, Danon disease, Fabry disease, Farber lipogranulomatosis, Farber disease, fucosidosis, galactosialidosis types I/II, Gaucher disease types I/II/III, globoid cell leukodystrophy, Krabbe disease, glycogen storage disease II, Pompe disease, GM1-gangliosidosis types I/II/III, GM2-gangliosidosis type I, Tay Sachs disease, GM2-gangliosidosis type II, Sandhoff disease, GM2-gangliosidosis, α -mannosidosis types I/II, β -mannosidosis, metachromatic leukodystrophy, mucopolipidosis type I, sialidosis types I/II, mucopolipidosis types II/III, I-cell disease, mucopolipidosis type IIIC pseudo-Hurler polydystrophy, mucopolysaccharidosis type I, mucopolysaccharidosis type II, mucopolysaccharidosis type IIIA, Sanfilippo syndrome, mucopolysaccharidosis type IIIB, mucopolysaccharidosis type IIIC, mucopolysaccharidosis type IIID, mucopolysaccharidosis type IVA, Morquio syndrome, mucopolysaccharidosis type IVB, mucopolysaccharidosis type VI, mucopolysaccharidosis type VII, Sly syndrome, mucopolysaccharidosis type IX, multiple sulfatase deficiency, neuronal ceroid lipofuscinosis, CLN1 Batten disease, CLN2 Batten disease, Niemann-Pick disease types A/B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, pycnodysostosis, Schindler disease types I/II, Gaucher disease and sialic acid storage disease.

[0073] A detailed review of the genetic etiology, clinical manifestations, and molecular biology of the lysosomal storage diseases are detailed in Scriver et al., eds., *The Metabolic and Molecular Basis of Inherited Disease*, 7th ed., Vol. II, McGraw Hill, (1995). Thus, the enzymes deficient in the above diseases are known to those of skill in the art, some of these are exemplified in Table 2 below:

TABLE 2

| Disease Name | Enzyme Deficiency | Substance Stored |
|---|--|--|
| Pompe Disease | Acid- α 1,4-Glucosidase | Glycogen α 1-4 linked Oligosaccharides |
| GM1 Gangliosidosis | β -Galactosidase | GM ₁ Gangliosides |
| Tay-Sachs Disease | β -Hexosaminidase A | GM ₂ Ganglioside |
| GM2 Gangliosidosis: | GM ₂ Activator | GM ₂ Ganglioside |
| AB Variant | Protein | |
| Sandhoff Disease | β -Hexosaminidase A&B | GM ₂ Ganglioside |
| Fabry Disease | α -Galactosidase A | Globosides |
| Gaucher Disease | Glucocerebrosidase | Glucosylceramide |
| Metachromatic Leukodystrophy | Arylsulfatase A | Sulphatides |
| Krabbe Disease | Galactosylceramidase | Galactocerebroside |
| Niemann Pick, Types A & B | Acid Sphingomyelinase | Sphingomyelin |
| Niemann-Pick, Type C | Cholesterol Esterification Defect | Sphingomyelin |
| Niemann-Pick, Type D | Unknown | Sphingomyelin |
| Farber Disease | Acid Ceramidase | Ceramide |
| Wolman Disease | Acid Lipase | Cholesteryl Esters |
| Hurler Syndrome (MPS IH) | α -L-Iduronidase | Heparan & Dermatan Sulfates |
| Scheie Syndrome (MPS IS) | α -L-Iduronidase | Heparan & Dermatan, Sulfates |
| Hurler-Scheie (MPS IH/S) | α -L-Iduronidase | Heparan & Dermatan Sulfates |
| Hunter Syndrome (MPS II) | Iduronate Sulfatase | Heparan & Dermatan Sulfates |
| Sanfilippo A (MPS IIIA) | Heparan N-Sulfatase | Heparan Sulfate |
| Sanfilippo B (MPS IIIB) | α -N-Acetylglucosaminidase | Heparan Sulfate |
| Sanfilippo C (MPS IIIC) | Acetyl-CoA-Glucosaminidase | Heparan Sulfate |
| Sanfilippo D (MPS IIID) | Acetyltransferase | |
| Morquio B (MPS IVB) | N-Acetylglucosamine-6-Sulfatase | Heparan Sulfate |
| Maroteaux-Lamy (MPS VI) | β -Galactosidase | Keratan Sulfate |
| Sly Syndrome (MPS VII) | Arylsulfatase B | Dermatan Sulfate |
| α -Mannosidosis | β -Glucuronidase | |
| β -Mannosidosis | α -Mannosidase | Mannose/ Oligosaccharides |
| Fucosidosis | β -Mannosidase | Mannose/ Oligosaccharides |
| Aspartylglucosaminuria | α -L-Fucosidase | Fucosyl Oligosaccharides |
| Sialidosis (Mucopolipidosis I) | N-Aspartyl- β -Glucosaminidase | Aspartylglucosamine Asparagines |
| Galactosialidosis (Goldberg Syndrome) | α -Neuraminidase | Sialyloligosaccharides |
| Schindler Disease | Lysosomal Protective Protein Deficiency | Sialyloligosaccharides |
| Mucopolipidosis II (I-Cell Disease) | α -N-Acetyl-Galactosaminidase | |
| Mucopolipidosis III (Pseudo-Hurler Polydystrophy) | N-Acetylglucosamine-1-Phosphotransferase | Heparan Sulfate |
| Cystinosis | Same as ML II | |
| Salla Disease | Cystine Transport Protein | Free Cystine |
| | Sialic Acid Transport Protein | Free Sialic Acid and Glucuronic Acid |

TABLE 2-continued

| Disease Name | Enzyme Deficiency | Substance Stored |
|--|--------------------------------|--------------------------------------|
| Infantile Sialic Acid Storage Disease | Sialic Acid Transport Protein | Free Sialic Acid and Glucuronic Acid |
| Infantile Neuronal Ceroid Lipofuscinosis | Palmitoyl-Protein Thioesterase | Lipofuscins |
| Mucopolipidosis IV | Unknown | Gangliosides & Hyaluronic Acid |
| Prosaposin | Saposins A, B, C or D | |

[0074] Inventive methods according to the present invention may be used to deliver various other replacement enzymes. As used herein, replacement enzymes suitable for the present invention may include any enzyme that can act to replace at least partial activity of the deficient or missing lysosomal enzyme in a lysosomal storage disease to be treated. In some embodiments, a replacement enzyme is capable of reducing accumulated substance in lysosomes or that can rescue or ameliorate one or more lysosomal storage disease symptom.

[0075] In some embodiments, a suitable replacement enzyme may be any lysosomal enzyme known to be associated with the lysosomal storage disease to be treated. In some embodiments, a suitable replacement enzyme is an enzyme selected from the enzyme listed in Table 2 above.

[0076] In some embodiments, a replacement enzyme suitable for the invention may have a wild-type or naturally occurring sequence. In some embodiments, a replacement enzyme suitable for the invention may have a modified sequence having substantial homology or identify to the wild-type or naturally-occurring sequence (e.g., having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% sequence identity to the wild-type or naturally-occurring sequence).

[0077] A replacement enzyme suitable for the present invention may be produced by any available means. For example, replacement enzymes may be recombinantly produced by utilizing a host cell system engineered to express a replacement enzyme-encoding nucleic acid. Alternatively or additionally, replacement enzymes may be produced by activating endogenous genes. Alternatively or additionally, replacement enzymes may be partially or fully prepared by chemical synthesis. Alternatively or additionally, replacement enzymes may also be purified from natural sources.

[0078] Where enzymes are recombinantly produced, any expression system can be used. To give but a few examples, known expression systems include, for example, egg, baculovirus, plant, yeast, or mammalian cells.

[0079] In some embodiments, enzymes suitable for the present invention are produced in mammalian cells. Non-limiting examples of mammalian cells that may be used in accordance with the present invention include BALB/c mouse myeloma line (NSO/1, ECACC No: 85110503); human retinoblasts (PER.C6, CruCell, Leiden, The Netherlands); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36:59, 1977); human fibrosarcoma cell line (e.g., HT1080); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells +/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 774216, 1980); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251, 1980); monkey kidney cells (CV1 ATCC CCL

70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); human cervical carcinoma cells (HeLa, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.*, 383:44-68, 1982); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0080] In some embodiments, inventive methods according to the present invention are used to deliver replacement enzymes produced from human cells. In some embodiments, inventive methods according to the present invention are used to deliver replacement enzymes produced from CHO cells.

[0081] In some embodiments, replacement enzymes delivered using a method of the invention contain a moiety that binds to a receptor on the surface of brain cells to facilitate cellular uptake and/or lysosomal targeting. For example, such a receptor may be the cation, independent mannose-6-phosphate receptor (CI-MPR) which binds the mannose-6-phosphate (M6P) residues. In addition, the CI-MPR also binds other proteins including IGF-II. In some embodiments, a replacement enzyme suitable for the present invention contains M6P residues on the surface of the protein. In some embodiments, a replacement enzyme suitable for the present invention may contain bis-phosphorylated oligosaccharides which have higher binding affinity to the CI-MPR. In some embodiments, a suitable enzyme contains up to about an average of about at least 20% bis-phosphorylated oligosaccharides per enzyme. In other embodiments, a suitable enzyme may contain about 10%, 15%, 18%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% bis-phosphorylated oli-

gosaccharides per enzyme. While such bis-phosphorylated oligosaccharides may be naturally present on the enzyme, it should be noted that the enzymes may be modified to possess such oligosaccharides. For example, suitable replacement enzymes may be modified by certain enzymes which are capable of catalyzing the transfer of N-acetylglucosamine-L-phosphate from UDP-GlcNAc to the 6' position of α -1,2-linked mannoses on lysosomal enzymes. Methods and compositions for producing and using such enzymes are described by, for example, Canfield et al. in U.S. Pat. No. 6,537,785, and U.S. Pat. No. 6,534,300, each incorporated herein by reference.

[0082] In some embodiments, replacement enzymes for use in the present invention may be conjugated or fused to a lysosomal targeting moiety that is capable of binding to a receptor on the surface of brain cells. A suitable lysosomal targeting moiety can be IGF-II, RAP, p97, and variants, homologues or fragments thereof (e.g., including those peptide having a sequence at least 70%, 75%, 80%, 85%, 90%, or 95% identical to a wild-type mature human IGF-I, IGF-II, RAP, p97 peptide sequence).

[0083] In some embodiments replacement enzymes suitable for the present invention have not been modified to enhance delivery or transport of such agents across the BBB and into the CNS.

[0084] In some embodiments, a therapeutic protein includes a targeting moiety (e.g., lysosome targeting sequence) and/or a membrane-penetrating peptide. In some embodiments, a targeting sequence and/or a membrane-penetrating peptide is an intrinsic part of the therapeutic moiety (e.g., via a chemical linkage, via a fusion protein). In some embodiments, a targeting sequence contains a mannose-6-phosphate moiety. In some embodiments, a targeting sequence contains an IGF-I moiety. In some embodiments, a targeting sequence contains an IGF-II moiety.

Formulations

[0085] Aqueous pharmaceutical solutions and compositions (i.e., formulations) that are traditionally used to deliver therapeutic agents to the CNS of a subject include unbuffered isotonic saline and Elliott's B solution, which is artificial CSF. A comparison depicting the compositions of CSF relative to Elliott's B solution is included in Table 3 below. As shown in Table 3, the concentration of Elliott's B Solution closely parallels that of the CSF. Elliott's B Solution, however contains a very low buffer concentration and accordingly may not provide the adequate buffering capacity needed to stabilize therapeutic agents (e.g., proteins), especially over extended periods of time (e.g., during storage conditions). Furthermore Elliott's B Solution contains certain salts which may be incompatible with the formulations intended to deliver some therapeutic agents, and in particular proteins or enzymes. For example, the calcium salts present in Elliott's B Solution are capable of mediating protein precipitation and thereby reducing the stability of the formulation.

TABLE 3

| Solution | Na ⁺ mEq/L | K ⁺ mEq/L | Ca ⁺⁺ mEq/L | Mg ⁺⁺ mEq/L | HCO ₃ ⁻ mEq/L | Cl ⁻ mEq/L | pH | Phosphorous mg/L | Glucose mg/L |
|----------------------|--------------------------|-------------------------|---------------------------|---------------------------|--|--------------------------|---------|---------------------|-----------------|
| CSF | 117-137 | 2.3 | 2.2 | 2.2 | 22.9 | 113-127 | 7.31 | 1.2-2.1 | 45-80 |
| Elliott's B Sol'n | 149 | 2.6 | 2.7 | 2.4 | 22.6 | 132 | 6.0-7.5 | 2.3 | 80 |

[0086] The present invention provides formulations, in either aqueous, pre-lyophilized, lyophilized or reconstituted form for therapeutic agents that have been formulated such that they are capable of stabilizing, or alternatively slowing or preventing the degradation, of one or more therapeutic agents form dated therewith (e.g., recombinant proteins). In some embodiments, the present formulations provide lyophilization formulation for therapeutic agents. In some embodiments, the present formulations provide aqueous formulations for therapeutic agents. In some embodiments the formulations are stable formulations.

Stable Formulations

[0087] As used herein, the term "stable" refers to the ability of the therapeutic agent (e.g., a recombinant enzyme) to maintain its therapeutic efficacy (e.g., all or the majority of its intended biological activity and/or physicochemical integrity) over extended periods of time. The stability of a therapeutic agent, and the capability of the pharmaceutical composition to maintain stability of such therapeutic agent, may be assessed over extended periods of time (e.g., preferably for at

least 1, 3, 6, 9, 12, 18, 24, 30, 36 months or more). In the context of a formulation a stable formulation is one in which the therapeutic agent therein essentially retains its physical and/or chemical integrity and biological activity upon storage and during processes (such as freeze/thaw, mechanical mixing and lyophilization). For protein stability, it can be measured by formation of high molecular weight (HMW) aggregates, loss of enzyme activity, generation of peptide fragments and shift of charge profiles.

[0088] Stability of the therapeutic agent is of particular importance with respect to the maintenance of the specified range of the therapeutic agent concentration required to enable the agent to serve its intended therapeutic function. Stability of the therapeutic agent may be further assessed relative to the biological activity or physiochemical integrity of the therapeutic agent over extended periods of time. For example, stability at a time point may be compared against stability at an earlier time point (e.g., upon formulation day 0) or against unformulated therapeutic agent and the results of this comparison expressed as a percentage. Preferably, the pharmaceutical compositions of the present invention maintain at least 100%, at least 99%, at least 98%, at least 97% at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55% or at least 50% of the therapeutic agent's biological activity or physiochemical integrity over an extended period of time (e.g., as measured over at least about 1-36 (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 24, 30, or 36) months, at room temperature, 2-8° C. or under accelerated storage conditions).

[0089] The therapeutic agents are preferably soluble in the pharmaceutical compositions of the present invention. The term "soluble" as it relates to the therapeutic agents of the present invention refer to the ability of such therapeutic agents to form a homogenous solution. Preferably the solubility of the therapeutic agent in the solution into which it is administered and by which it is transported to the target site of action (e.g., the cells and tissues of the brain) is sufficient to permit the delivery of a therapeutically effective amount of the therapeutic agent to the targeted site of action. Several factors can impact the solubility of the therapeutic agents. For example, relevant factors which may impact protein solubility include ionic strength, amino acid sequence and the presence of other co-solubilizing agents or salts (e.g., calcium salts.) In some embodiments, the pharmaceutical compositions are formulated such that calcium salts are excluded from such compositions.

[0090] Suitable formulations, in either aqueous, pre-lyophilized, lyophilized or reconstituted form, may contain a therapeutic agent of interest at various concentrations. In some embodiments, formulations may contain a protein or therapeutic agent of interest at a concentration in the range of about 0.1 mg/ml to 100 mg/ml (e.g., about 0.1 mg/ml to 80 mg/ml, about 0.1 mg/ml to 60 mg/ml, about 0.1 mg/ml to 50 mg/ml, about 0.1 mg/ml to 40 mg/ml, about 0.1 mg/ml to 30 mg/ml, about 0.1 mg/ml to 25 mg/ml, about 0.1 mg/ml to 20 mg/ml, about 0.1 mg/ml to 60 about 0.1 mg/ml to 50 mg/ml, about 0.1 mg/ml to 40 mg/ml, about 0.1 mg/ml to 30 mg/ml, about 0.1 mg/ml to 25 mg/ml, about 0.1 mg/ml to 20 about 0.1 mg/ml to 15 mg/ml, about 0.1 mg/ml to 10 mg/ml, about 0.1 mg/ml to 5 mg/ml, about 1 mg/ml to 10 mg/ml, about 1 mg/ml to 20 mg/ml, about 1 mg/ml to 40 mg/ml, about 5 mg/ml to 100 mg/ml, about 5 mg/ml to 50 mg/ml, or about 5 mg/ml to 25 mg/ml). In some embodiments, formulations according to the invention may contain a therapeutic agent at or above a

concentration of approximately 1 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, 45 mg/ml, 50 mg/ml, 55 mg/ml, 60 mg/ml, 65 mg/ml, 70 mg/ml, 75 mg/ml, 80 mg/ml, 85 mg/ml, 90 mg/ml, 95 mg/ml, or 100 mg/ml.

[0091] The formulations of the present invention are characterized by their tolerability either as aqueous solutions or as reconstituted lyophilized solutions. As used herein, the terms "tolerable" and "tolerability" refer to the ability of the pharmaceutical compositions of the present invention to not elicit an adverse reaction in the subject to whom such composition is administered, or alternatively not to elicit a serious adverse reaction in the subject to whom such composition is administered. In some embodiments, the pharmaceutical compositions of the present invention are well tolerated by the subject to whom such compositions is administered.

[0092] Many therapeutic agents, and in particular the proteins and enzymes of the present invention, require controlled pH and specific excipients to maintain their solubility and stability in the pharmaceutical compositions of the present invention. Table 4 below identifies typical aspects of protein formulations considered to maintain the solubility and stability of the protein therapeutic agents of the present invention.

TABLE 4

| Parameter | Typical Range/Type | Rationale |
|----------------------|---|--|
| pH | 5 to 7.5 | For stability Sometimes also for solubility |
| Buffer type | acetate, succinate, citrate, histidine, phosphate or Tris | To maintain optimal pH May also affect stability |
| Buffer concentration | 5-50 mM | To maintain pH May also stabilize or add ionic strength |
| Tonicifier | NaCl, sugars, mannitol | To render iso-osmotic or isotonic solutions |
| Surfactant | Polysorbate 20, polysorbate 80 | To stabilize against interfaces and shear |
| Other | Amino acids (e.g. arginine) at tens to hundreds of mM | For enhanced solubility or stability |

Buffers

[0093] The pH of the formulation, is an additional factor which is capable of altering the solubility of a therapeutic agent e.g., an enzyme or protein) in an aqueous formulation or for a pre-lyophilization formulation. Accordingly, in some embodiments, formulations of the present invention may comprise one or more buffers. In some embodiments the aqueous formulations comprise an amount of buffer sufficient to maintain the optimal pH of said composition between about 4.0-8.0 (e.g., about 4.0, 4.5, 5.0, 5.5, 6.0, 6.2, 6.4, 6.5, 6.6, 6.8, 7.0, 7.5, or 8.0). In some embodiments, the pH of the formulation is between about 5.5-7.0, between about 6.0-7.0, between about 5.5-6.0, between about 5.5-6.5, between about 5.0-6.0, between about 5.0-6.5 and between about 6.0-7.5, Suitable buffers include, for example acetate, citrate, histidine, phosphate, succinate, tris(hydroxymethyl) aminomethane ("Tris") and other organic acids. The buffer concentration and pH range of the pharmaceutical compositions of the present invention are factors in controlling or adjusting the tolerability of the formulation. In some embodiments, a buffering agent is present at a concentration ranging between about 1 mM to about 150 mM, or between about 10

mM to about 50 mM, or between about 15 mM to about 50 mM, or between about 20 mM to about 50 mM, or between about 25 mM to about 50 mM. In some embodiments, a suitable buffering agent is present at a concentration of approximately 1 mM, 5 mM, 10 mM, 0.15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 75 mM, 100 mM, 125 mM or 150 mM.

[0094] In other embodiments, formulations according to the present invention contain no buffers. As used herein, a formulation without buffer is referred to as saline-based formulations.

Tonicity

[0095] In some embodiments, formulations, in either aqueous, pre-lyophilized, lyophilized or reconstituted form, contain an isotonicity agent to keep the formulations isotonic. Typically, by "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 240 mOsm/kg to about 350 mOsm/kg. Isotonicity can be measured using, for example, a vapor pressure or freezing point type osmometers. Exemplary isotonicity agents include, but are not limited to, glycine, sorbitol, mannitol, sodium chloride and asinine. In some embodiments, suitable isotonic agents may be present in aqueous and/or pre-lyophilized formulations at a concentration from about 0.01-5% (e.g., 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0 or 5.0%) by weight. In some embodiments, formulations for lyophilization contain an isotonicity agent to keep the pre-lyophilization formulations or the reconstituted formulations isotonic.

[0096] While generally isotonic solutions are preferred for parenterally administered drugs, the use of isotonic solutions may change solubility for some therapeutic agents and in particular some proteins and/or enzymes. Slightly hypertonic solutions (e.g., up to 175 mM sodium chloride in 5 mM sodium phosphate at pH 7.0) and sugar-containing solutions (e.g., up to 2% sucrose in 5 mM sodium phosphate at pH 7.0) have been demonstrated to be well tolerated. The most common approved CNS bolus formulation composition is saline (about 150 mM NaCl in water).

Stabilizing Agents

[0097] In some embodiments, formulations may contain a stabilizing agent, or lyoprotectant, to protect the protein. Typically, a suitable stabilizing agent is a sugar, a non-reducing sugar and/or an amino acid. Exemplary sugars include, but are not limited to, dextran, lactose, mannitol, mannose, sorbitol, raffinose, sucrose and trehalose. Exemplary amino acids include, but are not limited to, arginine, glycine and methionine. Additional stabilizing agents may include sodium chloride, hydroxyethyl starch and polyvinylpyrrolidone. The amount of stabilizing agent in the lyophilized formulation is generally such that the formulation will be isotonic. However, hypertonic reconstituted formulations may also be suitable. In addition, the amount of stabilizing agent must not be too low such that an unacceptable amount of degradation/aggregation of the therapeutic agent occurs. Exemplary stabilizing agent concentrations in the formulation may range from about mM to about 400 mM (e.g., from about 30 mM to about 300 mM, and from about 50 mM to about 100 mM), or alternatively, from 0.1% to 15% (e.g., from 1% to 10%, from 5% to 15%, from 5% to 10%) by

weight. In some embodiments, the ratio of the mass amount of the stabilizing agent and the therapeutic agent is about 1:1. In other embodiments, the ratio of the mass amount of the stabilizing agent and the therapeutic agent can be about 0.1:1, 0.2:1, 0.25:1, 0.4:1, 0.5:1, 1:1, 2:1, 2.6:1, 3:1, 4:1, 5:1, 10:1, or 20:1. In some embodiments, suitable for lyophilization, the stabilizing agent is also a lyoprotectant.

[0098] In some embodiments, liquid formulations suitable for the present invention contain amorphous materials. In some embodiments, liquid formulations suitable for the present invention contain a substantial amount of amorphous materials (e.g., sucrose-based formulations). In some embodiments, liquid formulations suitable for the present invention contain partly crystalline/partly amorphous materials.

Bulking Agents

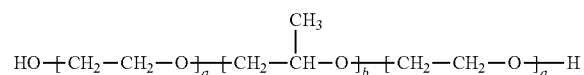
[0099] In some embodiments, suitable formulations for lyophilization may further include one or more bulking agents. A "bulking agent" is a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake. For example, a bulking agent may improve the appearance of lyophilized cake (e.g., essentially uniform lyophilized cake). Suitable bulking agents include, but are not limited to, sodium chloride, lactose, mannitol, glycine, sucrose, trehalose, hydroxyethyl starch. Exemplary concentrations of bulking agents are from about 1% to about 10% (e.g., 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 5.5%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5%, 9.0%, 9.5%, and 10.0%).

Surfactants

[0100] In some embodiments, it is desirable to add a surfactant to formulations. Exemplary surfactants include non-ionic surfactants such as Polysorbates (e.g., Polysorbates 20 or 80); poloxamers (e.g., poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-aurate; and the MONAQUAT™ series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g., Pluronic, PF68, etc).

[0101] Poloxamer

[0102] In some embodiments, formulations provided by the present invention contain a poloxamer as a surfactant. Typically, poloxamers are non-ionic, triblock copolymers. Poloxamers typically have an amphiphilic structure comprised of as central hydrophobic block, between two hydrophilic blocks. Generally, the central hydrophobic block is polyoxypropylene (poly(propylene oxide)) ("PPO") and the two hydrophilic blocks are polyoxyethylene (poly(ethylene oxide)) ("PEO"). A general structure for an exemplary poloxamer is shown as follows:



[0103] Poloxamers are also known by the trade name Pluronic® (manufactured by BASF). Poloxamers/Pluronic® are available in various grades differing in molecular weights, ratios of hydrophilic to hydrophobic blocks and physical forms, (i.e., liquid, flakes/solids or paste). Exemplary poloxamers/Pluronic® products suitable for the present invention are shown in Table 5 below.

TABLE 5

| Poloxamer/Pluronic ® grades and their chemical composition | | | | | |
|--|-----------|-----|----|----------------------------------|------------------|
| Pluronic ® | Poloxamer | a | b | Content of Oxyethylene (Percent) | Molecular weight |
| L 44 NF | 124 | 12 | 20 | 44.8-48.6 | 2090-2360 |
| F 68 NF | 188 | 79 | 28 | 79.9-83.7 | 7680-9510 |
| F 87 NF | 237 | 64 | 37 | 70.5-74.3 | 6840-8830 |
| F 108 NF | 338 | 141 | 44 | 81.4-84.9 | 12700-17400 |
| F 127 NF | 407 | 101 | 56 | 71.5-74.9 | 9840-14600 |

[0104] In some embodiments, a poloxamer suitable for the present invention is Poloxamer 188 (e.g., Pluronic® F-68). Other suitable poloxamers include, but are not limited to, Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 215, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, 407, and combination thereof.

[0105] A surfactant can be included in a formulation of the invention at various concentrations. In particular, a surfactant may be present in a formulation at a concentration of approximately 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.11%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.18%, 0.19%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4%, 0.45%, 0.5%, 0.55%, 0.6%, 0.65%, 0.7%, 0.75%, 0.8%, 0.85%, 0.9%, 0.95%, or 1.0%, etc. In some embodiments, a surfactant is present at a concentration ranging approximately between 0.001% and (e.g., between 0.001% and 0.8%, between 0.001% and 0.6%, between 0.001% and 0.5%, between 0.001% and 0.4%, between 0.001% and 0.3%, between 0.001% and 0.2%, between 0.005% and 0.05%, between 0.005% and 0.02%, between 0.05% and 1.0%, between 0.05% and 0.75%, between 0.05% and 0.5%, between 0.05% and 0.4%, between 0.05% and 0.3%, between 0.05 and 0.2%, or between 0.05% and 0.15%) by weight.

[0106] Alternatively, or in addition, the surfactant may be added to the lyophilized formulation, pre-lyophilized formulation and/or the reconstituted formulation.

[0107] Typically, the amount of surfactant in a formulation is such that it reduces aggregation of the protein and minimizes the formation of particulates or effervescences. For example, a surfactant may be present in an amount such that aggregation, particulate formation and/or effervescences is decreased by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more as compared to a control formulation lacking the surfactant. A formulation in accordance with the present invention may be one wherein less than about 10% (e.g., less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%) and preferably less than about (e.g., less than 4%, 3%, 2%, 1%, 0.5%) of the

protein is present as an aggregate in the formulation also referred to as high molecular weight species (“HMW”).

[0108] In some embodiments, the present invention provides “stable” formulations for lysosomal enzymes described herein. As used herein, the term “stable” refers to the ability of the therapeutic agent (e.g., a recombinant enzyme) to maintain its therapeutic efficacy (e.g., all or the majority of its intended biological activity and/or physicochemical integrity) over extended periods of time. The stability of a therapeutic agent, and the capability of the pharmaceutical composition to maintain stability of such therapeutic agent, may be assessed over extended periods of time (e.g., for at least 1, 3, 6, 12, 18, 24, 30, 34 months or more). In general, pharmaceutical compositions described herein have been formulated such that they are capable of stabilizing, or alternatively slowing or preventing the degradation, of one or more therapeutic agents formulated therewith (e.g., recombinant proteins), in the context of a formulation, a stable formulation is one in which the therapeutic agent therein essentially retains its physical and/or chemical integrity and biological activity upon storage and during processes (such as freeze/thaw, mechanical mixing and lyophilization). For protein stability, it can be measured by formulation of high molecular weight (HMW) aggregates, loss of enzyme activity, generation of peptide fragments and shift of charge profiles. Among other things, formulations provided by the present invention reduce or eliminate formation of high molecule weight aggregates, protein degradation during freeze-drying, storage, shipping and infusion. In particular embodiments, a stable formulation provided by the present invention reduces or eliminates enzyme precipitation during freeze-drying, storage, Shipping and infusion.

[0109] Stability can be measured after storage at a selected temperature (e.g., 0° C., 5° C., 25° C. (room temperature), 30° C., 40° C.) for a selected time period (e.g., 2 weeks, 1 month, 1.5 months, 2 months, 3 months, 4 months, 5 months, 6 months, 12 months, 18 months, 24 months, etc.). For rapid screening, the formulation may be kept at 40° C. for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8° C., generally the formulation should be stable at 25° C. (i.e., room temperature) or 40° C. for at least 1 month and/or stable at 2-8° C. for at least 3 months, 6 months, 1 year or 2 years. Where the formulation is to be stored at 30° C., generally the formulation should be stable for at least 3 months, 6 months, 1 year or 2 years at 30° C. and/or stable at 40° C. for at least 2 weeks, 1 month, 3 months or 6 months. In some embodiments, the extent of aggregation following lyophilization and storage can be used as an indicator of protein stability. As used herein, the term “high molecular weight (“HMW”) aggregates” refers to an association of at least two protein monomers. For the purposes of this invention, a monomer refers to the single unit of any biologically active form of the protein of interest. The association may be covalent, non-covalent, disulfide, non-reducible crosslinking, or by other mechanism.

[0110] In some embodiments, aggregation and/or particulate formation can be measured after shaking the formulation (e.g., for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 18 hours, 24 hours, 36 hours, 48 hours or more). In some embodiments, aggregation and/or particulate formation can be measured after pumping the formulation at high speed (e.g., through an IVEC pump). In some embodiments, aggregation and/or particulate formation can be verified visually

after pumping the formulation at high speed and shaking the formulation. In some embodiments, aggregation and/or particulate formation can be measured by an increase in aggregate formation following lyophilization and storage of the lyophilized formulation.

[0111] Other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the formulation (and/or the lyophilized formulation and/or the reconstituted formulation) provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include, but are not limited to, additional buffering agents; preservatives; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g., Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counter-ions such as sodium.

[0112] Formulations, in either aqueous, pre-lyophilized, lyophilized or reconstituted form, in accordance with the present invention can be assessed based on product quality analysis, reconstitution time (if lyophilized), quality of reconstitution if lyophilized, high molecular weight, moisture, and glass transition temperature. Typically, protein quality and product analysis, including product degradation rate analysis and protein aggregation analysis, may use methods including, but not limited to size exclusion HPLC (SE-HPLC), cation exchange-HPLC (CEX-HPLC), X-ray diffraction (XRD), modulated differential scanning calorimetry (mDSC), reversed phase HPLC (RP-HPLC), multi-angle light scattering (MALS), fluorescence, ultraviolet absorption, nephelometry, capillary electrophoresis (CE), SDS-PAGE, and combinations thereof. In some embodiments, evaluation of product in accordance with the present invention may include a step of evaluating appearance (either liquid or cake appearance). In some embodiments, aggregation is determined by visual examination.

[0113] Generally, formulations (lyophilized or aqueous) can be stored for extended periods of time at room temperature. Storage temperature may typically range from 0° C. to 45° C. (e.g., 4° C., 20° C., 25° C., 45° C. etc.). Formulations may be stored for a period of months to a period of years. Storage time generally will be 2.4 months, 12 months, 6 months, 4.5 months, 3 months, 2 months or 1 month. Formulations can be stored directly in the container used for administration, eliminating transfer steps.

[0114] In some embodiments, a "stable" lyophilized formulation may be one wherein the increase in aggregate in the lyophilized formulation is less than about 5% (e.g., less than 4%, 3%, 2%, 0.5%) and preferably less than about 3% (e.g., 2%, 1%, 0.5%, 0.2%, 0.1%) when the lyophilized formulation is stored at 25° C. (i.e., room temperature) or 40° C. for at least 2 weeks, 1 month, 3 months or 6 months, or at 2-8° C. for at least 3 months, 6 months, 1 year or 2 years.

[0115] Formulations can be stored directly in the lyophilization container (if lyophilized), which may also function as the reconstitution vessel, eliminating transfer steps. Alternatively, lyophilized product formulations may be measured into smaller increments for storage. Storage should generally avoid circumstances that lead to degradation of the proteins, including but not limited to exposure to sunlight,

UV radiation, other forms of electromagnetic radiation, excessive heat or cold, rapid thermal shock, and mechanical shock.

Lyophilization

[0116] Inventive methods in accordance with the present invention can be utilized to lyophilize any materials, in particular, therapeutic agents. Typically, a pre-lyophilization formulation further contains an appropriate choice of excipients or other components such as stabilizers, buffering agents, bulking agents, and surfactants to prevent compound of interest from degradation (e.g., protein aggregation, deamidation, and/or oxidation) during freeze-drying and storage. The formulation for lyophilization can include one or more additional ingredients including lyoprotectants or stabilizing agents, buffers, bulking agents, isotonicity agents and surfactants.

[0117] After the substance of interest and any additional components are mixed together, the formulation is lyophilized. Lyophilization generally includes three main stages: freezing, primary drying and secondary drying. Freezing is necessary to convert water to ice or some amorphous formulation components to the crystalline form. Primary drying is the process step when ice is removed from the frozen product by direct sublimation at low pressure and temperature. Secondary drying is the process step when bounded water is removed from the product matrix utilizing the diffusion of residual water to the evaporation surface. Product temperature during secondary drying is normally higher than during primary drying. See, Tang X. et al. (2004) "Design of freeze-drying processes for pharmaceuticals: Practical advice," *Pharm. Res.*, 21:191-200; Nail S. L. et al. (2002) "Fundamentals of freeze-drying," in Development and manufacture of protein pharmaceuticals. Nail S. L. editor New York: Kluwer Academic/Plenum Publishers, pp 281-353; Wang et al. (2000) "Lyophilization and development of solid protein Pharmaceuticals," *Int. J. Pharm.*, 203; 1-60; Williams N. A. et al. (1984) "The lyophilization of pharmaceuticals; A literature review," *J. Parenteral Sci. Technol.*, 38:48-59. Generally, any lyophilization process can be used in connection with the present invention.

[0118] In some embodiments, an annealing step may be introduced during the initial freezing of the product. The annealing step may reduce the overall cycle time. Without wishing to be bound by any theories, it is contemplated that the annealing step can help promote excipient crystallization and formation of larger ice crystals due to re-crystallization of small crystals formed during supercooling, which, in turn, improves reconstitution. Typically, an annealing step includes an interval or oscillation in the temperature during freezing. For example, the freeze temperature may be -40° C., and the annealing step will increase the temperature to, for example, -10° C. and maintain this temperature for a set period of time. The annealing step time may range from 0.5 hours to 8 hours (e.g., 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 6, and 8 hours). The annealing temperature may be between the freezing temperature and 0° C.

[0119] Lyophilization may be performed in a container, such as a tube, a bag, a bottle, a tray, a vial (e.g., a glass vial), syringe or any other suitable containers. The containers may be disposable. Lyophilization may also be performed in a large scale or small scale. In some instances, it may be desirable to lyophilize the protein formulation in the container in which reconstitution of the protein is to be carried out in order

to avoid as transfer step. The container in this instance may, for example, be a 3, 4, 5, 10, 20, 50 or 100 cc vial.

[0120] Many different freeze-dryers are available for this purpose such as Hull pilot scale dryer (SP Industries, USA), Genesis (SP industries) laboratory freeze-dryers, or any freeze-dryers capable of controlling the given lyophilization process parameters. Freeze-drying is accomplished by freezing the formulation and subsequently subliming ice from the frozen content at a temperature suitable for primary drying. Initial freezing brings the formulation to a temperature below about -20°C . (e.g., -50°C ., -45°C ., -40°C ., -35°C ., -30°C ., -25°C ., etc.) in typically not more than about 4 hours (e.g., not more than about 3 hours, not more than about 2.5 hours, not more than about 2 hours). Under this condition, the product temperature is typically below the eutectic point or the collapse temperature of the formulation. Typically, the shelf temperature for the primary drying will range from about -30 to 25°C . (provided the product remains below the melting point during primary drying) at a suitable pressure, ranging typically from about 20 to 250 nTorr. The formulation, size and type of the container holding the sample (e.g., glass vial) and the volume of liquid will mainly dictate the time required for drying, which can range from a few hours to several days. A secondary drying stage is carried out at about 0 - 60°C ., depending primarily on the type and size of container and the type of therapeutic protein employed. Again, volume of liquid will mainly dictate the time required for drying, which can range from a few hours to several days.

[0121] As a general proposition, lyophilization will result in a lyophilized formulation in which the moisture content thereof is less than about 5%, less than about 4% less than about 3%, less than about 2%, less than about 1%, and less than about 0.5%,

Reconstitution

[0122] While the pharmaceutical compositions of the present invention are generally in an aqueous form upon administration to a subject, in some embodiments the pharmaceutical compositions of the present invention are lyophilized. Such compositions must be reconstituted by adding one or more diluents thereto prior to administration to a subject. At the desired stage, typically at an appropriate time prior to administration to the patient, the lyophilized formulation may be reconstituted with a diluent such that the protein concentration in the reconstituted formulation is desirable.

[0123] Various diluents may be used in accordance with the present invention, in some embodiments, a suitable diluent for reconstitution is water. The water used as the diluent can be treated in a variety of ways including reverse osmosis, distillation, deionization, filtrations (e.g., activated carbon, microfiltration, nanofiltration) and combinations of these treatment methods. In general, the water should be suitable for injection including, but not limited to, sterile water or bacteriostatic water for injection.

[0124] Additional exemplary diluents include a pH buffered solution (e.g., phosphate buffered saline), sterile saline solution, Elliot's solution, Ringers solution or dextrose solution. Suitable diluents may optionally contain a preservative. Exemplary preservatives include aromatic alcohols such as benzyl or phenol alcohol. The amount of preservative employed is determined by assessing different preservative concentrations for compatibility with the protein and preservative efficacy testing. For example, if the preservative is an

aromatic alcohol (such as benzyl alcohol), it can be present in an amount from about 0.1-2.0%, from about 0.5-1.5%, or about 1.0-1.2%.

[0125] Diluents suitable for the invention may include a variety of additives, including, but not limited to, pH buffering agents, (e.g. Tris, histidine,) salts (e.g., sodium chloride) and other additives (e.g., sucrose) including those described above (e.g. stabilizing agents, isotonicity agents).

[0126] According to the present invention, a lyophilized substance (e.g., protein) can be reconstituted to a concentration of at least 25 mg/ml (e.g. at least 50 mg/ml, at least 75 mg/ml, at least 100 mg/ml) and in any ranges therebetween. In some embodiments, a lyophilized substance (e.g., protein) may be reconstituted to a concentration ranging from about 1 mg/ml to 100 mg/ml (e.g., from about 1 mg/ml to 50 mg/ml, from 1 mg/ml to 100 mg/ml, from about 1 mg/ml to about 5 mg/ml, from about 1 mg/ml to about 10 mg/ml, from about 1 to about 25 mg/ml, from about 1 mg/ml to about 75 mg/ml, from about 10 mg/ml to about 30 mg/ml, from about 10 mg/ml to about 50 mg/ml, from about 10 mg/ml to about 75 mg/ml, from about 10 mg/ml to about 100 mg/ml, from about 25 mg/ml to about 50 mg/ml, from about 25 mg/ml to about 7.5 mg/ml, from about 25 mg/ml to about 100 mg/ml, from about 50 mg/ml to about 75 mg/ml, from about 50 mg/ml to about 100 mg/ml). In some embodiments, the concentration of protein in the reconstituted formulation may be higher than the concentration in the pre-lyophilization formulation. High protein concentrations in the reconstituted formulation are considered to be particularly useful where subcutaneous or intramuscular delivery of the reconstituted formulation is intended. In some embodiments, the protein concentration in the reconstituted formulation may be about 2-30 times (e.g., about 2-20, about 2-10 times, or about 2-5 times) of the pre-lyophilized formulation. In some embodiments, the protein concentration in the reconstituted formulation may be at least about 2 times (e.g., at least about 3, 4, 5, 10, 20, 40 times) of the pre-lyophilized formulation.

[0127] Reconstitution according to the present invention may be performed in any container. Exemplary containers suitable for the invention include, but are not limited to, such as tubes, vials, syringes (e.g., single-chamber or dual-chamber), bags, bottles, and trays. Suitable containers may be made of any materials such as glass, plastics, metal. The containers may be disposable or reusable. Reconstitution may also be performed in a large scale or small scale.

[0128] In some instances, it may be desirable to lyophilize the protein formulation in the container in which reconstitution of the protein is to be carried out in order to avoid a transfer step. The container in this instance may, for example, be a 3, 4, 5, 10, 20, 50 or 100 cc vial. In some embodiments, a suitable container for lyophilization and reconstitution is a dual chamber syringe (e.g., Lyo-Ject,[®] (Vetter) syringes). For example, a dual chamber syringe may contain both the lyophilized substance and the diluent, each in a separate chamber, separated by a stopper (see Example 5). To reconstitute, a plunger can be attached to the stopper at the diluent side and pressed to move diluent into the product chamber so that the diluent can contact the lyophilized substance and reconstitution may take place as described herein (see Example 5).

[0129] The pharmaceutical compositions, formulations and related methods of the invention are useful for delivering a variety of therapeutic agents to the CNS of a subject (e.g., intrathecally, intraventricularly or intracisternally) and for the treatment of the associated diseases. The pharmaceutical

compositions of the present invention are particularly useful for delivering proteins and enzymes (e.g., enzyme replacement therapy) to subjects suffering from lysosomal storage disorders. The lysosomal storage diseases represent a group of relatively rare inherited metabolic disorders that result from defects in lysosomal function. The lysosomal diseases are characterized by the accumulation of undigested macromolecules within the lysosomes, which results in an increase in the size and number of such lysosomes and ultimately in cellular dysfunction and clinical abnormalities.

CNS Delivery

[0130] It is contemplated that various stable formulations described herein are generally suitable for CNS delivery of therapeutic agents. Stable formulations according to the present invention can be used for CNS delivery via various techniques and routes including, but not limited to, intraparenchymal, intracerebral, intraventricular cerebral (ICV), intrathecal (e.g., IT-Lumbar, IT-cisterna magna) administrations and any other techniques and routes for injection directly or indirectly to the CNS and/or CSF. Exemplary CNS delivery of stable formulations are described in PCT/US2011/041926, filed on Jun. 25, 2011 entitled "Methods and Compositions for CNS Delivery of Arylsulfatase A," the entire contents of which are incorporated herein by reference.

Intrathecal Delivery

[0131] In some embodiments, a replacement enzyme is delivered to the CNS in a formulation described herein. In some embodiments, a replacement enzyme is delivered to the CNS by administering into the cerebrospinal fluid (CSF) of a subject in need of treatment. In some embodiments, intrathecal administration is used to deliver a desired replacement enzyme (e.g., an ASA protein) into the CSF. As used herein, intrathecal administration (also referred to as intrathecal injection) refers to an injection into the spinal canal (intrathecal space surrounding the spinal cord). Various techniques may be used including, without limitation, lateral cerebroventricular injection through a burrhole or cisternal or lumbar puncture or the like. Exemplary methods are described in Lazorthes et al. *Advances in Drug Delivery Systems and Applications in Neurosurgery*, 143-192 and Omayya et al., *Cancer Drug Delivery*, 1: 169-179, the contents of which are incorporated herein by reference.

[0132] According to the present invention, an enzyme may be injected at any region surrounding the spinal canal. In some embodiments, an enzyme is injected into the lumbar area or the cisterna magna or intraventricularly into a cerebral ventricle space. As used herein, the term "lumbar region" or "lumbar area" refers to the area between the third and fourth lumbar (lower back) vertebrae and, more inclusively, the L2-S1 region of the spine. Typically, intrathecal injection via the lumbar region or lumbar area is also referred to as "lumbar IT delivery" or "lumbar IT administration." the term "cisterna magna" refers to the space around and below the cerebellum via the opening between the skull and the top of the spine. Typically, intrathecal injection via cisterna magna is also referred to as "cisterna magna delivery." The term "cerebral ventricle" refers to the cavities in the brain that are continuous with the central canal of the spinal cord. Typically, injections via the cerebral ventricle cavities are referred to as intraventricular Cerebral (ICV) delivery.

[0133] In some embodiments, "intrathecal administration" or "intrathecal delivery" according to the present invention refers to lumbar IT administration or delivery, for example, delivered between the third and fourth lumbar (lower back) vertebrae and, more inclusively, the L2-S1 region of the spine. It is contemplated that lumbar IT administration or delivery distinguishes over cisterna magna delivery in that lumbar IT administration or delivery according to our invention provides better and more effective delivery to the distal spinal canal, while cisterna magna delivery, among other things, typically does not deliver well to the distal spinal canal.

[0134] Device for Intrathecal Delivery

[0135] Various devices may be used for intrathecal delivery according to the present invention. In some embodiments, a device for intrathecal administration contains a fluid access port (e.g., injectable port); a hollow body (e.g., catheter) having a first flow orifice in fluid communication with the fluid access port and a second flow orifice configured for insertion into spinal cord; and a securing mechanism for securing the insertion of the hollow body in the spinal cord. As a non-limiting example shown in FIG. 62, a suitable securing mechanism contains one or more knobs mounted on the surface of the hollow body and a sutured ring adjustable over the one or more knobs to prevent the hollow body (e.g., catheter) from slipping out of the spinal cord. In various embodiments, the fluid access port comprises a reservoir. In some embodiments, the fluid access port comprises a mechanical pump (e.g., an infusion pump). In some embodiments, an implanted catheter is connected to either a reservoir (e.g., for bolus delivery), or an infusion pump. The fluid access port may be implanted or external.

[0136] In some embodiments, intrathecal administration may be performed by either lumbar puncture (i.e., slow bolus) or via a port-catheter delivery system (i.e., infusion or bolus). In some embodiments, the catheter is inserted between the laminae of the lumbar vertebrae and the tip is threaded up the thecal space to the desired level (generally L3-L4) (FIG. 63).

[0137] Relative to intravenous administration, a single dose volume suitable for intrathecal administration is typically small. Typically, intrathecal delivery according to the present invention maintains the balance of the composition of the CST as well as the intracranial pressure of the subject. In some embodiments, intrathecal delivery is performed absent the corresponding removal of CSF from a subject. In some embodiments, a suitable single dose volume may be e.g., less than about 10 ml, 8 ml, 6 ml, 5 ml, 4 ml, 3 ml, 2 ml, 1.5 ml, 1 ml, or 0.5 ml. In some embodiments, a suitable single dose volume may be about 0.5-5 ml, 0.5-4 ml, 0.5-3 ml, 0.5-2 ml, 0.5-1 ml, 1-3 ml, 1-5 ml, 1.5-3 ml, 1-4 ml, or 0.5-1.5 ml. In some embodiments, intrathecal delivery according to the present invention involves a step of removing a desired amount of CSF first. In some embodiments, less than about 10 ml (e.g., less than about 9 ml, 8 ml, 7 ml, 6 ml, 5 ml, 4 ml, 3 ml, 2 ml, 1 ml) of CSF is first removed before IT administration. In those cases, a suitable single dose volume may be e.g., more than about 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml, 15 ml, or 20 ml.

[0138] Various other devices may be used to effect intrathecal administration of a therapeutic composition. For example, formulations containing desired enzymes may be given using an Ommaya reservoir which is in common use for intrathecally administering drugs for meningeal carcinomatosis (*Lancet* 2: 983-84, 1963). More specifically, in this

method, a ventricular tube is inserted through a hole formed in the anterior horn and is connected to an Ommaya reservoir installed under the scalp, and the reservoir is subcutaneously punctured to intrathecally deliver the particular enzyme being replaced, which is injected into the reservoir. Other devices for intrathecal administration of therapeutic compositions or formulations to an individual are described in U.S. Pat. No. 6,217,552, incorporated herein by reference. Alternatively, the drug may be intrathecally given, for example, by a single injection, or continuous infusion. It should be understood that the dosage treatment may be in the form of a single dose administration or multiple doses.

[0139] For injection, formulations of the invention can be formulated in liquid solutions. In addition, the enzyme may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. The injection can be, for example, in the form of a bolus injection or continuous infusion (e.g., using infusion pumps) of the enzyme.

[0140] In one embodiment of the invention, the enzyme is administered by lateral cerebro ventricular injection into the brain of a subject. The injection can be made, for example, through a burr hole made in the subject's skull. In another embodiment, the enzyme and/or other pharmaceutical formulation is administered through a surgically inserted shunt into the cerebral ventricle of a subject. For example, the injection can be made into the lateral ventricles, which are larger. In some embodiments, injection into the third and fourth smaller ventricles can also be made.

[0141] In yet another embodiment, the pharmaceutical compositions used in the present invention are administered by injection into the cisterna magna, or lumbar area of a subject.

[0142] In another embodiment of the method of the invention, the pharmaceutically acceptable formulation provides sustained delivery, e.g., "slow release" of the enzyme or other pharmaceutical composition used in the present invention, to a subject for at least one, two, three, four weeks or longer periods of time after the pharmaceutically acceptable formulation is administered to the subject.

[0143] As used herein, the term "sustained delivery" refers to continual delivery of a pharmaceutical formulation of the invention in vivo over a period of time following administration, preferably at least several days, a week or several weeks. Sustained delivery of the composition can be demonstrated by, for example, the continued therapeutic effect of the enzyme over time (e.g., sustained delivery of the enzyme can be demonstrated by continued reduced amount of storage granules in the subject). Alternatively, sustained delivery of the enzyme may be demonstrated by detecting the presence of the enzyme in vivo over time.

Delivery to Target Tissues

[0144] As discussed above, one of the surprising and important features of the present invention is that therapeutic agents, in particular, replacement enzymes administered using inventive methods and compositions of the present invention are able to effectively and extensively diffuse across the brain surface and penetrate various layers or regions of the brain, including deep brain regions. In addition, inventive methods and compositions of the present invention effectively deliver therapeutic agents (e.g., an ASA enzyme) to various tissues, neurons or cells of spinal cord, including the lumbar region, which is hard to target by existing CNS deliv-

ery methods such as ICV injection. Furthermore, inventive methods and compositions of the present invention deliver sufficient amount of therapeutic agents (e.g., an ASA enzyme) to blood stream and various peripheral organs and tissues.

[0145] Thus, in some embodiments, a therapeutic protein (e.g. an ASA enzyme) is delivered to the central nervous system of a subject. In some embodiments, a therapeutic protein (e.g., an ASA enzyme) is delivered to one or more of target tissues of brain, spinal cord, and/or peripheral organs. As used herein, the term "target tissues" refers to any tissue that is affected by the lysosomal storage disease to be treated or any tissue in which the deficient lysosomal enzyme is normally expressed. In some embodiments, target tissues include those tissues in which there is a detectable or abnormally high amount of enzyme substrate, for example stored in the cellular lysosomes of the tissue, in patients suffering, from or susceptible to the lysosomal storage disease. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature. In some embodiments, target tissues include those tissues in which the deficient lysosomal enzyme is normally expressed at an elevated level. As used herein, a target tissue may be a brain target tissue, a spinal cord target tissue and/or a peripheral target tissue. Exemplary target tissues are described in detail below.

[0146] Brain Target Tissues

[0147] In general, the brain can be divided into different regions, layers and tissues. For example, meningeal tissue is a system of membranes which envelops the central nervous system, including the brain. The meninges contain three layers, including dura matter, arachnoid matter, and pia matter. In general, the primary function of the meninges and of the cerebrospinal fluid is to protect the central nervous system. In some embodiments, a therapeutic protein in accordance with the present invention is delivered to one or more layers of the meninges.

[0148] The brain has three primary subdivisions, including the cerebrum, cerebellum, and brain stem. The cerebral hemispheres, which are situated above most other brain structures and are covered with a conical layer. Underneath the cerebrum lies the brainstem, which resembles a stalk on which the cerebrum is attached. At the rear of the brain, beneath the cerebrum and behind the brainstem, is the cerebellum.

[0149] The diencephalon, which is located near the midline of the brain and above the mesencephalon, contains the thalamus, metathalamus, hypothalamus, epithalamus, prethalamus, and pretectum. The mesencephalon, also called the mid-brain, contains the tectum, tegumentum, ventricular mesocoelia, and cerebral peduncles, the red nucleus, and the cranial nerve III nucleus. The mesencephalon is associated with vision, hearing, motor control, sleep/wake, alertness, and temperature regulation.

[0150] Regions of tissues of the central nervous system, including the brain, can be characterized based on the depth of the tissues. For example CNS (e.g., brain) tissues can be characterized as surface or shallow tissues, mid-depth tissues, and/or deep tissues.

[0151] According to the present invention, a therapeutic protein (e.g., a replacement enzyme) may be delivered to any appropriate brain target tissue(s) associated with a particular disease to be treated in a subject. In some embodiments, a therapeutic protein (e.g., a replacement enzyme) in accordance with the present invention is delivered to surface or

shallow brain target tissue. In some embodiments, a therapeutic protein in accordance with the present invention is delivered, to mid-depth brain target tissue. In some embodiments, a therapeutic protein in accordance with the present invention is delivered to deep brain target tissue. In some embodiments, a therapeutic protein in accordance with the present invention is delivered to a combination of surface or shallow brain target tissue, mid-depth brain target tissue, and/or deep brain target tissue. In some embodiments, a therapeutic protein in accordance with the present invention is delivered to a deep brain tissue at least 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm or more below (or internal to) the external surface of the brain.

[0152] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to one or more surface or shallow tissues of cerebrum. In some embodiments, the targeted surface or shallow tissues of the cerebrum are located within 4 mm from the surface of the cerebrum. In some embodiments, the targeted surface or shallow tissues of the cerebrum are selected from pia mater tissues, cerebral cortical ribbon tissues, hippocampus, Virchow Robin space, blood vessels within the VR space, the hippocampus, portions of the hypothalamus on the inferior surface of the brain, the optic nerves and tracts, the olfactory bulb and projections, and combinations thereof.

[0153] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to one or more deep tissues of the cerebrum. In some embodiments, the targeted surface or shallow tissues of the cerebrum are located 4 mm (e.g., 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, or 10 mm) below (or internal to) the surface of the cerebrum. In some embodiments, targeted deep tissues of the cerebrum include the cerebral conical ribbon. In some embodiments, targeted deep tissues of the cerebrum include one or more of the diencephalon (e.g., the hypothalamus, thalamus, prethalamus, subthalamus, etc.), metencephalon lentiform nuclei, the basal ganglia, caudate, putamen, amygdala, globus pallidus, and combinations thereof.

[0154] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to one or more tissues of the cerebellum. In certain embodiments, the targeted one or more tissues of the cerebellum are selected from the group consisting of tissues of the molecular layer, tissues of the Purkinje cell layer, tissues of the Granular cell layer, cerebellar peduncles, and combination thereof. In some embodiments, therapeutic agents (e.g., enzymes) are delivered to one or more deep tissues of the cerebellum including, but not limited to, tissues of the Purkinje cell layer, tissues of the Granular cell layer, deep cerebellar white matter tissue (e.g., deep relative to the Granular cell layer), and deep cerebellar nuclei tissue.

[0155] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to one or more tissues of the brainstem. In some embodiments, the targeted one or more tissues of the brainstem include brain stem white matter tissue and/or brain stem nuclei tissue.

[0156] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to various brain tissues including, but not limited to matter, white matter, periventricular areas, pia-arachnoid, meninges, neocortex, cerebellum, deep tissues in cerebral cortex, molecular layer, caudate/putamen region, midbrain, deep regions of the pons or medulla, and combinations thereof.

[0157] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to various cells in the brain including, but not limited to, neurons, glial cells, perivascular cells and/

or meningeal cells. In some embodiments, a therapeutic protein is delivered to oligodendrocytes of deep white matter.

[0158] Spinal Cord

[0159] In general, regions or tissues of the spinal cord can be characterized based on the depth of the tissues. For example, spinal cord tissues can be characterized as surface or shallow tissues, mid-depth tissues, and/or deep tissues.

[0160] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to one or more surface or shallow tissues of the spinal cord. In some embodiments, a targeted surface or shallow tissue of the spinal cord is located within 4 mm from the surface of the spinal cord. In some embodiments, a targeted surface or shallow tissue of the spinal cord contains pia matter and/or the tracts of white matter.

[0161] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to one or more deep tissues of the spinal cord. In some embodiments, a targeted deep tissue of the spinal cord is located internal to 4 mm from the surface of the spinal cord, in some embodiments, a targeted deep tissue of the spinal cord contains spinal cord grey matter and/or ependymal cells.

[0162] In some embodiments, therapeutic agents (e.g., enzymes) are delivered to neurons of the spinal cord.

[0163] Peripheral Target Tissues

[0164] As used herein, peripheral organs or tissues refer to any organs or tissues that are not part of the central nervous system (CNS). Peripheral target tissues may include, but are not limited to, blood system, liver, kidney, heart, endothelium, bone marrow and bone marrow derived cells, spleen, lung, lymph node, bone, cartilage, ovary and testis. In some embodiments, a therapeutic protein (e.g., a replacement enzyme) in accordance with the present invention is delivered to one or more of the peripheral target tissues.

Biodistribution and Bioavailability

[0165] In various embodiments, once delivered to the target tissue, a therapeutic agent (e.g., an ASA enzyme) is localized intracellularly. For example, a therapeutic agent (e.g., enzyme) may be localized to exons, axons, lysosomes, mitochondria or vacuoles of a target cell (e.g., neurons such as Purkinje cells). For example, in some embodiments intrathecally-administered enzymes demonstrate translocation dynamics such that the enzyme moves within the perivascular space (e.g., by pulsation-assisted convective mechanisms). In addition, active axonal transport mechanisms relating to the association of the administered protein or enzyme with neurofilaments may also contribute to or otherwise facilitate the distribution of intrathecally-administered proteins or enzymes into the deeper tissues of the central nervous system.

[0166] In some embodiments, a therapeutic agent (e.g., an ASA enzyme) delivered according to the present invention may achieve therapeutically or clinically effective levels or activities in various target tissues described herein. As used herein, a therapeutically or clinically effective level or activity is a level or activity sufficient to confer a therapeutic effect in a target tissue. The therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). For example, a therapeutically or clinically effective level or activity may be an enzymatic level or activity that is sufficient to ameliorate symptoms associated with the disease in the target tissue (e.g., GAG storage).

[0167] In some embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present

invention may achieve an enzymatic level or activity that is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% of the normal level or activity of the corresponding lysosomal enzyme in the target tissue. In some embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present invention may achieve an enzymatic level or activity that is increased by at least 1-fold, 2-fold, 3-fold, 4-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold as compared to a control (e.g., endogenous levels or activities without the treatment). In some embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present invention may achieve an increased enzymatic level or activity at least approximately 10 nmol/hr/mg, 20 nmol/hr/mg, 40 nmol/hr/mg, 50 nmol/hr/mg, 60 nmol/hr/mg, 70 nmol/hr/mg, 80 nmol/hr/mg, 90 nmol/hr/mg, 100 nmol/hr/mg, 150 nmol/hr/mg, 200 nmol/hr/mg, 250 nmol/hr/mg, 300 nmol/hr/mg, 350 nmol/hr/mg, 4110 nmol/hr/mg, 450 nmol/hr/mg, 500 nmol/hr/mg, 550 nmol/hr/mg or 600 nmol/hr/mg in a target tissue.

[0168] In some embodiments, inventive methods according to the present invention are particularly useful for targeting the lumbar region. In some embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present invention may achieve an increased enzymatic level or activity in the lumbar region of at least approximately 500 nmol/hr/mg, 600 nmol/hr/mg, 700 nmol/hr/mg, 800 nmol/hr/mg, 900 nmol/hr/mg, 1000 nmol/hr/mg, 1500 nmol/hr/mg, 2000 nmol/hr/mg, 3000 nmol/hr/mg, 4000 nmol/hr/mg, 5000 nmol/hr/mg, 6000 nmol/hr/mg, 7000 nmol/hr/mg, 8000 nmol/hr/mg, 9000 nmol/hr/mg, or 10,000 nmol/hr/mg.

[0169] In general, therapeutic agents (e.g., replacement enzymes) delivered according to the present invention have sufficiently long half time in CSF and target tissues of the brain, spinal cord, and peripheral organs. In some embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present invention may have a half-life of at least approximately 30 minutes, 45 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 12 hours, 16 hours, 18 hours, 20 hours, 25 hours, 30 hours, 35 hours, 40 hours, up to 3 days, up to 7 days, up to 14 days, up to 21 days or up to a month. In some embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present invention may retain detectable level or activity in CSF or bloodstream after 12 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 66 hours, 72 hours, 78 hours, 84 hours, 90 hours, 96 hours, 0.102 hours, or a week following administration. Detectable level or activity may be determined using, various methods known in the art.

[0170] In certain embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present invention achieves a concentration of at least 30 µg/ml in the CNS tissues and cells of the subject following administration (e.g., one week, 3 days, 48 hours, 36 hours, 24 hours, 18 hours, 2 hours, 8 hours, 6 hours, 4 hours, 3 hours, 2 hours, 1 hour, 30 minutes, or less, following intrathecal administration of the pharmaceutical composition to the subject). In certain embodiments, a therapeutic agent (e.g., a replacement enzyme) delivered according to the present invention achieves a concentration of at least 20 µg/ml, at least 15 µg/ml, at least 10 µg/ml, at least 7.5 µg/ml, at least 5 µg/ml, at least 2.5 µg/ml, at least 1.0 µg/ml or at least 0.5 µg/ml in the targeted tissues or cells of the subject (e.g., brain tissues or neurons) following administration to such subject (e.g., One

week, 3 days, 4 hours, 36 hours, 24 hours, 18 hours, 12 hours, 8 hours, 6 hours, 4 hours, 3 hours, 2 hours, 1 hour, 30 minutes, or less following intrathecal administration of such pharmaceutical compositions to the subject).

[0171] Treatment of Metachromatic Leukodystrophy Disease (MLD)

[0172] Metachromatic Leukodystrophy Disease (MLD), is an autosomal recessive disorder resulting from a deficiency of the enzyme Arylsulfatase A (ASA). ASA, which is encoded by the ARSA gene in humans, is an enzyme that breaks down cerebroside 3-sulfate or sphingolipid 3-O-sulfogalactosylceramide (sulfatide) into cerebroside and sulfate. In the absence of the enzyme, sulfatides accumulate in the nervous system (e.g., myelin sheaths, neurons and glial cells) and to a lesser extent in visceral organs. The consequence of these molecular and cellular events is progressive demyelination and axonal loss within the CNS and PNS, which is accompanied clinically by severe motor and cognitive dysfunction.

[0173] A defining clinical feature of this disorder is central nervous system (CNS) degeneration, which results in cognitive impairment (e.g., mental retardation, nervous disorders, and blindness, among others).

[0174] MLD can manifest itself in young children (Late-infantile form), where affected children typically begin showing symptoms just after the first year of life (e.g., at about 15-24 months), and generally do not survive past the age of 5 years. MLD can manifest itself in children (Juvenile form), where affected children typically show cognitive impairment by about the age of 3-10 years, and life-span can vary (e.g., in the range of 10-15 years after onset of symptoms). MLD can manifest itself in adults (Adult-onset form) and can appear in individuals of any age (e.g., typically at age 16 and later) and the progression of the disease can vary greatly.

[0175] Compositions and methods of the present invention may be used to effectively treat individuals suffering from or susceptible to MLD. The terms, "treat" or "treatment," as used herein, refers to amelioration of one or more symptoms associated with the disease, prevention or delay of the onset of one or more symptoms of the disease, and/or lessening of the severity or frequency of one or more symptoms of the disease. Exemplary symptoms include, but are not limited to, intracranial pressure, hydrocephalus ex vacuo, accumulated sulfated glycolipids in the myelin sheaths in the central and peripheral nervous system and in visceral organs, progressive demyelination and axonal loss within the CNS and PNS, and/or motor and cognitive dysfunction.

[0176] In some embodiments, treatment refers to partially or complete alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of neurological impairment in an MLD patient. As used herein, the term "neurological impairment" includes various symptoms associated with impairment of the central nervous system (e.g., the brain and spinal cord). In some embodiments, various symptoms of MLD are associated with impairment of the peripheral nervous system (PNS). In some embodiments, neurological impairment in an MLD patient is characterized by decline in gross motor function. It will be appreciated that gross motor function may be assessed by any appropriate method. For example, in some embodiments, MISS motor function is measured as the change from a baseline in motor function using the Gross Motor Function Measure-88 (GMFM-88) total raw score.

[0177] In some embodiments, treatment refers to decreased sulfatide accumulation in various tissues. In some embodi-

ments, treatment refers to decreased sulfatide accumulation in brain target tissues, spinal cord neurons, and/or peripheral target tissues. In certain embodiments, sulfatide accumulation is decreased by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or more as compared to a control. In some embodiments, sulfatide accumulation is decreased by at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold as compared to a control. It will be appreciated that sulfatide storage may be assessed by any appropriate method. For example, in some embodiments, sulfatide storage is measured by alcian blue staining. In some embodiments, sulfatide storage is measured by LAMP-1 staining.

[0178] In some embodiments, treatment refers to reduced vacuolization in neurons (e.g., neurons containing Purkinje cells). In certain embodiments, vacuolization in neurons is decreased by about 5%, 10%, 15%, 10%, 15%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or more as compared to a control. In some embodiments, vacuolization is decreased by at least 1-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold as compared to a control.

[0179] In some embodiments, treatment refers to increased ASA enzyme activity in various tissues. In some embodiments, treatment refers to increased ASA enzyme activity in brain target tissues, spinal cord neurons and/or peripheral target tissues. In some embodiments, ASA enzyme activity is increased by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 49%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900% 1000% or more as compared to a control. In some embodiments, ASA enzyme activity is increased by at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold as compared to a control. In some embodiments, increased ASA enzymatic activity is at least approximately 10 nmol/hr/mg, 20 nmol/hr/mg, 40 nmol/hr/mg, 50 nmol/hr/mg, 60 nmol/hr/mg, 70 nmol/hr/mg, 80 nmol/hr/mg, 90 nmol/hr/mg, 100 nmol/hr/mg, 150 nmol/hr/mg, 200 nmol/hr/mg, 250 nmol/hr/mg, 300 nmol/hr/mg, 350 nmol/hr/mg, 400 nmol/hr/mg, 450 nmol/hr/mg, 500 nmol/hr/mg, 550 nmol/hr/mg, 600 nmol/hr/mg or more. In some embodiments, ASA enzymatic activity is increased in the lumbar region. In some embodiments, increased ASA enzymatic activity in the lumbar region is at least approximately 2000 nmol/hr/mg, 3000 nmol/hr/mg, 4000 nmol/hr/mg, 5000 nmol/hr/mg, 6000 nmol/hr/mg, 7000 nmol/hr/mg, 8000 nmol/hr/mg, 9000 nmol/hr/mg, 10,000 nmol/hr/mg, or more.

[0180] In some embodiments, treatment refers to decreased progression of loss of cognitive ability. In certain embodiments, progression of loss of cognitive ability is decreased by about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or more as compared to a control. In some embodiments, treatment refers to decreased developmental delay. In certain embodiments, developmental delay is decreased by about 5%, 19%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or more as compared to a control.

[0181] In some embodiments, treatment refers to increased survival (e.g., survival time). For example, treatment can result in an increased life expectancy of a patient. In some embodiments, treatment according to the present invention results in an increased life expectancy of a patient by more

than about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 105%, about 110%, about 115%, about 120%, about 125%, about 130%, about 135%, about 140%, about 145%, about 150%, about 155%, about 160%, about 165%, about 170%, about 0.175%, about 0.180%, about 185%, about 190%, about 195%, about 200% or more, as compared to the average life expectancy of one or more control individuals with similar disease without treatment. In some embodiments, treatment according to the present invention results in an increased life expectancy of a patient by more than about 6 month, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, about 10 years or more, as compared to the average life expectancy of one or more control individuals with similar disease without treatment. In some embodiments, treatment according to the present invention results in long term survival of a patient. As used herein, the term "long term survival" refers to a survival time or life expectancy longer than about 40 years, 45 years, 50 years, 55 years, 60 years or longer.

[0182] The terms, "improve," "increase" or "reduce," as used herein, indicate values that are relative to a control. In some embodiments, a suitable control is a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A "control individual" is an individual afflicted with the same form MLD (e.g., late-infantile, juvenile, or adult-onset form), who is about the same age and/or gender as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

[0183] The individual (also referred to as "patient" or "subject") being treated is an individual (fetus, infant, child, adolescent, or adult human) having, MLD or having the potential to develop WILD. The individual can have residual endogenous ASA expression and/or activity, or no measurable activity. For example, the individual having MLD may have ASA expression levels that are less than about 30-50%, less than about 25-30%, less than about 20-25%, less than about 15-20%, less than about 10-15%, less than about 5-10%, less than about 0.1-5% of normal ASA expression levels.

[0184] In some embodiments, the individual is an individual who has been recently diagnosed with the disease. Typically, early treatment (treatment commencing as soon as possible after diagnosis) is important to minimize the effects of the disease and to maximize the benefits of treatment.

[0185] Immune Tolerance

[0186] Generally, intrathecal administration of a therapeutic agent (e.g., replacement enzyme) according to the present invention does not result in severe adverse effects in the subject. As used herein, severe adverse effects induce, but are not limited to, substantial immune response, toxicity, or death. As used herein, the term "substantial immune response" refers to severe or serious immune responses, such as adaptive T-cell immune responses.

[0187] Thus, in many embodiments, inventive methods according to the present invention do not involve concurrent immunosuppressant therapy (i.e., any immunosuppressant therapy used as pre-treatment/pre-conditioning or in parallel

to the method). In some embodiments, inventive methods according to the present invention do not involve an immune tolerance induction in the subject being treated. In some embodiments, inventive methods according to the present invention do not involve a pre-treatment or preconditioning of the subject using T-cell immunosuppressive agent.

[0188] In some embodiments, intrathecal administration of therapeutic agents can mount an immune response against these agents. Thus, in some embodiments, it may be useful to render the subject receiving the replacement enzyme tolerant to the enzyme replacement therapy. Immune tolerance may be induced using various methods known in the art. For example, an initial 30-60 day regimen of a T-cell immunosuppressive agent such as cyclosporin A (CsA) and an anti-proliferative agent, such as, azathioprine (Aza), combined with weekly intrathecal infusions of low doses of a desired replacement enzyme may be used.

[0189] Any immunosuppressant agent known to the skilled artisan may be employed together with a combination therapy of the invention. Such immunosuppressant agents include but are not limited to cyclosporine, FK506, rapamycin, CTLA4-Ig, and anti-TNF agents such as etanercept (see e.g. Moder, 2000, *Ann. Allergy Asthma Immunol.* 84, 280-284; Nevins, 2000, *Curr. Opin. Pediatr.* 12, 146-150; Kurlberg et al., 2000, *Scand. J. Immunol.* 51, 224-230; Ideguchi et al., 2000, *Neuroscience* 95, 217-226; Potter et al., 1999, *Ann. N.Y. Acad. Sci.* 875, 159-174; Slavik et al., 1999, *Immunol. Res.* 19, 1-24; Gaziev et al., 1999, *Bone Marrow Transplant.* 25, 689-696; Henry, 1999, *Clin. Transplant.* 13, 209-2.20; Gummert et al 1999, *J. Am. Soc. Nephrol.* 10, 1366-1380; Qi et al., 2000, *Transplantation* 69, 1275-1283). The anti-IL2 receptor (α -subunit) antibody daclizumab (e.g. Zenapax™), which has been demonstrated effective in transplant patients, can also be used as an immunosuppressant agent (see e.g. Wiseman et al., 1999, *Drugs* 58, 1029-1042; Beniaminovitz et al., 2000, *N. Engl. J. Med.* 342, 613-619; Ponticelli et al., 1999, *Drugs R. D.* 1, 55-60; Berard et al, 1999, *Pharmacotherapy* 19, 1127-1137; Eckhoff et al., 2000, *Transplantation* 69, 1867-1872; Ekberg et al., 2000, *Transpl. Int.* 13, 151-159). Additional immunosuppressant agents include but are not limited to anti-CD2 (Branco et al., 1999, *Transplantation* 68, 1588-1596; Przepiorka et al., 1998, *Blood* 92, 4066-4071), anti-CD4 (Marinova-Mutafchieva et al., 2000, *Arthritis Rheum.* 43, 638-644; Fishwild et al., 1999, *Clin. Immunol.* 92, 138-152), and anti-CD40 ligand (Hong et al., 2000, *Semin. Nephrol.* 20, 108-125; Chirmule et. al., 2000, *J. Virol.* 74, 3345-3352; Ito et al., 2000, *J. Immunol.* 164, 1230-1235).

[0190] Administration

[0191] Inventive methods of the present invention contemplate single as well as multiple administrations of a therapeutically effective amount of the therapeutic agents (e.g., replacement enzymes) described herein. Therapeutic agents (e.g., replacement enzymes) can be administered at regular intervals, depending on the nature, severity and extent of the subject's condition (e.g., a lysosomal storage disease). In some embodiments, a therapeutically effective amount of the therapeutic agents (e.g., replacement enzymes) of the present invention may be administered intrathecally periodically at regular intervals (e.g., once every year, once every six months, once every five months, once every three months, bimonthly (once every two months), monthly (once every month), biweekly (once every two weeks), weekly).

[0192] In some embodiments, intrathecal administration may be used in conjunction with other routes of administra-

tion (e.g., intravenous, subcutaneously, intramuscularly, parenterally, transdermally or transmucosally (e.g., orally or nasally)). In some embodiments, those other routes of administration (e.g., intravenous administration) may be performed no more frequent than biweekly, monthly, once every two months, once every three months, once every four months, once every five months, once every six months, annually administration.

[0193] As used herein, the term "therapeutically effective amount" is largely determined base on the total amount of the therapeutic agent contained in the pharmaceutical compositions of the present invention. Generally, a therapeutically effective amount is sufficient to achieve a meaningful benefit to the subject (e.g., treating, modulating, curing, preventing and/or ameliorating the underlying disease or condition). For example, a therapeutically effective amount may be an amount sufficient to achieve a desired therapeutic and/or prophylactic effect, such as an amount sufficient to modulate lysosomal enzyme receptors or their activity to thereby treat such lysosomal storage disease or the symptoms thereof (e.g., a reduction in or elimination of the presence or incidence of "zebra bodies" or cellular vacuolization following the administration of the compositions of the present invention to a subject). Generally, the amount of a therapeutic agent (e.g., a recombinant lysosomal enzyme) administered to a subject in need thereof will depend upon the characteristics of the subject. Such characteristics include the condition, disease severity, general health, age, sex and body weight of the subject. One of ordinary skill in the art will be readily able to determine appropriate dosages depending on these and other related factors. In addition, both objective and subjective assays may optionally be employed to identify optimal dosage ranges.

[0194] A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular therapeutic protein, a therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. Also, the specific therapeutically effective amount (and/or unit dose) for any particular patient may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and/or rate of excretion or metabolism of the specific fusion protein employed; the duration of the treatment; and like factors as is well known in the medical arts.

[0195] In some embodiments, the therapeutically effective dose ranges from about 0.005 mg/kg brain weight to 500 mg/kg brain weight, e.g., from about 0.005 mg/kg brain weight to 400 mg/kg brain weight, from about 0.005 mg/kg brain weight to 300 mg/kg brain weight, from about 0.005 mg/kg brain weight to 200 mg/kg brain weight, from about 0.005 mg/kg brain weight to 100 mg/kg brain weight, from about 0.005 mg/kg brain weight to 90 mg/kg brain weight, from about 0.005 mg/kg brain weight to 80 mg/kg brain weight, from about 0.005 mg/kg brain weight to 70 mg/kg brain weight, from about 0.005 mg/kg brain weight to 60 mg/kg brain weight, from about 0.005 mg/kg brain weight to 50 mg/kg brain weight, from about 0.005 mg/kg brain weight to 40 mg/kg brain weight, from about 0.005 mg/kg brain

weight to 30 mg/kg brain weight, from about 0.005 mg/kg brain weight to 25 mg/kg brain weight, from about 0.005 mg/kg brain weight to 20 mg/kg brain weight, from about 0.005 mg/kg brain weight to 15 mg/kg brain weight, from about 0.005 mg/kg brain weight to 10 mg/kg brain weight.

[0196] In some embodiments, the therapeutically effective dose is greater than about 0.1 mg/kg brain weight, greater than about 0.5 mg/kg brain weight, greater than about 1.0 mg/kg brain weight, greater than about 3 mg/kg brain weight, greater than about 5 mg/kg brain weight, greater than about 10 mg/kg brain weight, greater than about 15 mg/kg brain weight, greater than about 20 mg/kg brain weight, greater than about 30 mg/kg brain weight, greater than about 40 mg/kg brain weight, greater than about 50 mg/kg brain weight, greater than about 60 mg/kg brain weight, greater than about 70 mg/kg brain weight, greater than about 80 mg/kg brain weight, greater than about 90 mg/kg brain weight, greater than about 100 mg/kg brain weight, greater than about 150 mg/kg brain weight, greater than about 200 mg/kg brain weight, greater than about 250 mg/kg brain weight, greater than about 300 mg/kg brain weight, greater than about 350 mg/kg brain weight, greater than about 400 mg/kg brain weight, greater than about 450 mg/kg brain weight, greater than about 500 mg/kg brain weight.

[0197] In some embodiments, the therapeutically effective dose may also be defined by mg/kg body weight. As one skilled in the art would appreciate, the brain weights and body weights can be correlated. Dekaban A S, "Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights," *Ann Neurol* 1978; 4:345-56. Thus, in some embodiments, the dosages can be converted, as shown in Table 6.

TABLE 6

| Correlation between Brain Weights, body weights and ages of males | | |
|---|-------------------|------------------|
| Age (year) | Brain weight (kg) | Body weight (kg) |
| 3 (31-43 months) | 1.27 | 15.55 |
| 4-5 | 1.30 | 19.46 |

[0198] In some embodiments, the therapeutically effective dose may also be defined by mg/15 cc of CSF. As one skilled in the art would appreciate, therapeutically effective doses based on brain weights and body weights can be converted to mg/15 cc of CSF. For example, the volume of CSF in adult humans is approximately 150 mL (Johanson C E, et al "Multiplicity of cerebrospinal fluid functions: New challenges in health and disease," *Cerebrospinal Fluid Res.* 2008 May 14; 5; 10). Therefore single dose injections of 0.1 mg to 50 mg protein to adults would be approximately 0.01 mg/15 cc of CSF (0.1 mg) to 5.0 mg/15 cc of CSF (50 mg) doses in adults.

[0199] It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the enzyme replacement therapy and that dosage ranges set forth herein are exemplary only and are not intended to hunt the scope or practice of the claimed invention.

Kits

[0200] The present invention further provides kits or other articles of manufacture which contains the formulation of the

present invention and provides instructions for its reconstitution (if lyophilized) and/or use. Kits or other articles of manufacture may include container, an IDDD, a catheter and any other articles, devices or equipment useful in intertheal administration and associated surgery. Suitable containers include, for example, bottles, vials, syringes (e.g., pre-filled syringes), ampules, cartridges, reservoirs, or lyo-jects. The container may be formed from a variety of materials such as glass or plastic. In some embodiments, a container is a pre-filled syringe. Suitable pre-filled syringes include, but are not limited to, borosilicate glass syringes with baked silicone coating, borosilicate glass syringes with sprayed silicone, or plastic resin syringes without silicone.

[0201] Typically, the container may hold formulations and a label on, or associated with, the container that may indicate directions for reconstitution and/or use. For example, the label may indicate that the formulation is reconstituted to protein concentrations as described above. The label may further indicate that the formulation is useful or intended for, for example, IT administration. In some embodiments, a container may contain a single dose of a stable formulation containing a therapeutic agent (e.g., a replacement enzyme). In various embodiments, a single dose of the stable formulation is present in a volume of less than about 15 ml, 10 ml, 5.0 ml, 4.0 ml, 3.5 ml, 3.0 ml, 2.5 ml, 2.0 ml, 1.5 ml, 1.0 ml, or 0.5 ml. Alternatively, a container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g., from 2-6 administrations) of the formulation. Kits or other articles of manufacture may further include a second container comprising a suitable diluent (e.g., BWFI, saline, buffered saline). Upon mixing of the diluent and the formulation, the final protein concentration in the reconstituted formulation will generally be at least 1 mg/ml (e.g., at least 5 mg/ml, at least 10 mg/ml, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml, at least 100 mg/ml). Kits or other articles of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, IDDDs, catheters, syringes, and package inserts with instructions for use.

[0202] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature citations are incorporated by reference.

EXAMPLES

Example 1

Evaluation of the Stability of Poloxamer Formulations

[0203] The purpose of this example was to evaluate the use of poloxamer in stabilizing recombinant human ASA and reducing the level of enzyme precipitation and/or aggregation. Three separate studies were performed, using different concentrations of a poloxamer in a saline or buffer based formulation, to examine its role in promoting enzyme stability. Each study were designed to simulate the agitation, pressure and fluctuation in temperature experienced by a protein during commercial manufacturing. Therefore, it will be appreciated by one skilled in the art, that the results presented in this example represent the effect of poloxamers on protein stability during the manufacture, purification, storage and/or re-formulation of a commercial product.

Agitation Studies

[0204] This study demonstrates that including a poloxamer in a saline or buffer based formulation significantly reduces enzyme precipitation including particulate (e.g., fibers and flakes, formation. For the study, a series of agitation experiments were designed to assess the aggregation of recombinantly-prepared human Arylsulfatase A (rhASA) in formulations containing various amounts of a poloxamer surfactant, P188, as compared to a control formulation with no surfactant. To achieve this, rhASA was prepared and formulated with 0.05% P188, 0.1% P188 or 0.15% P188. The rhASA formulations were agitated by shaking at room temperature for 48 hours.

[0205] The no-surfactant control developed a few fibers and flakes after 2 hrs of shaking. The number of the particulates increased over time, as the shaking study progressed to 48 hrs time-point (see FIG. 1). Formulations containing 0.05% P188 also developed small flakes after 2 hrs of shaking. After 6 hrs, fibers were also observed. The appearance of the solution worsened as the shaking was continued to 48 hrs (FIG. 1). In contrast, the samples containing 0.15% P188 did not develop any fibers, flakes or particular matter over the entire 48 hour period (FIG. 1). This data indicates the use of poloxamer P188 at a concentration close to 0.15% can increase stability and reduce the level of rhASA precipitation, following agitation at room temperature. Therefore, for the next study Poloxamer concentrations of 0.1% and 0.15% were selected.

Pumping and Shaking Studies

[0206] For the next study, the objective was to examine the effect of pumping followed by shaking on rhASA formulations in the presence of 0.1% and 0.15% of poloxamer 188. The process of high speed pumping followed by shaking is used to serve as an exaggeration of the rigors endured by a sample during a routine manufacturing process. In these experiments, 0.1% P188 or 0.15% P188 rhASA samples were first pumped through an IVEC pump (200 strokes a 700 rpm) and then shaken for up to 48 hours. Immediately after pumping, only a few white fibers were observed for the no-surfactant control samples. However, after pumping and shaking for ~6 hrs, more fibers as well as some particulate matter was observed within the no-surfactant control sample. As shown in FIG. 2, after 48 hrs of shaking of each pumped sample, the worse appearance belonged to the sample with no surfactant followed by the samples (n=3) with 0.1% P188. However, as can be seen in FIG. 3, the samples containing 0.15% P188 contained only a few fibers after shaking the pumped material for 48 hrs. At all time-points, the pumped but not shaken controls looked better than the pumped and shaken samples, regardless of the presence of the surfactant.

[0207] Therefore, 0.15% P188 clearly demonstrated protection for the rhASA protein against stressful conditions such as pumping and agitation, and/or combination of both. 0.1% may still be considered a viable option for the long term storage of rhASA at 2-8° C. under less stressful conditions.

Loop Term Stability Studies

[0208] Experiments were conducted to evaluate the effect of poloxamer on the long term stability of rhASA with 0.1% and 0.15% P188. Specifically, the following two formulations were used: 154 mM NaCl, with 0.1% poloxamer 188, at pH 6.0; and 154 mM NaCl, with 0.15% poloxamer 188, at pH 6.0.

The samples were placed in long term storage at a temperature of 2-8° C. for a period of 12 months. The samples were analyzed periodically for stability, by evaluating for the presence of precipitate or flocculent material, along with other analytical techniques including, but not limited to, SEC and SDS-PAGE. Based on the study results (data not shown), the data suggests that rhASA is able to remain soluble and stable at 2-8° C. for at least as period of 12 months, for both the 0.1% and 0.15% poloxamer formulation. Based on the results to-date, the data suggests that rhASA may be able to remain soluble and stable at 2-8° C. for a period greater than 12 months, for both poloxamer formulations.

[0209] In addition, the stability of long term storage of rhASA at 25° C. was also evaluated. Specifically, the above two formulations were placed at a temperature of 25° C. for a period of 6 months. The samples were analyzed periodically for stability, by evaluating for the presence of precipitate or flocculent material, and other analytical techniques (e.g., SEC and SDS-PAGE). The data (not shown) suggests that rhASA is able to remain soluble and stable at 25° C. for a period of 3 months, for both the 0.1% and 0.15% poloxamer formulation.

[0210] Therefore, the stability data demonstrate that P188 (e.g., at both 0.1% and 0.15%) facilitates rhASA long term stability.

Example 2

Lyophilization of Poloxamer-Contain rhASA Formulation

[0211] In this example, rhASA was lyophilized in the presence of poloxamer as a surfactant in saline-based formulations. The experiment was designed to evaluate the protective property of poloxamer towards rhASA in formulations pre and post lyophilized conditions. rhASA samples were prepared and formulated by dialysis using a 3K MWCO membrane in 110 mM sodium chloride solution. Post dialysis, poloxamer, sucrose, and saline were added to achieve a final formulation composition containing 25 mg/mL rhASA, 110 mM NaCl, 3% sucrose, 0.15% P188, at pH 6.0.

[0212] For lyophilization 2 mL of the formulated rhASA sample were filled in 5 cc glass vials, partially stoppered with lyophilization stoppers and placed on a pre-cooled lyophilizer shelf at 5° C. For the initial thermal treatment, the shelf was cooled down to 50° C. at a ramp rate of 0.25° C. per minute and held at the set temperature for 120 minutes. The shelf was then warmed to -30° C. at a ramp rate of 0.25° C. per minute and held for 360 minutes. The shelf was subsequently re-cooled to -50° C. at a ramp rate of 0.25° C. per minute and held at the set temperature for 120 minutes. During primary drying, the shelf was warmed to -34° C. at a ramp rate of 0.25° C. per minute and the chamber pressure was reduced to 0.133 mbar and held for 6,600 minutes. During secondary drying, the shelf was warmed to +25° C. at a ramp rate of 0.25° C. per minute and the chamber pressure was reduced to 0.133 mbar and held for 420 minutes. Once the lyophilization process has been completed, the vials were back-filled with nitrogen, fully stoppered and sealed with an aluminum cap.

[0213] Post lyophilization, the appearance was a white solid cake. The cake reconstitution time with water was 10 seconds. The reconstituted solution appearance determined, by visual observation was slightly opalescent with no particles similar to the pre-lyophilize sample. The pre and post

lyophilization pH measurements were 5.94 and 6.00, respectively. The pre and post lyophilization osmolality measurements were 329 and 321 mOsm/kg, respectively. The percentage main peak as measured by size exclusion chromatography for both pre and post lyophilization samples was 99%. The percentage main peak as measured by reverse-phase chromatography for both pre and post lyophilization samples was 100%. Protein concentration measurements by absorbance at 280 nm for pre and post lyophilization samples were 24.1 and 24.8 mg/mL, respectively. The protein sample attributes did not change pre and post lyophilization as determined by visual observation, pH, osmolality, size exclusion chromatography, reversed-phase chromatography, and protein concentration.

[0214] These data indicates that poloxamer-containing formulations preserve rhASA protein stability during the lyophilization process.

[0215] While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds of the invention and are not intended to limit the same.

[0216] The articles "a" and "an" as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product, or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed, from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising, particular elements, features, etc., certain embodiments of the invention or aspects of invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. The publications, websites and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

1-47. (canceled)

48. A stable formulation for intrathecal administration comprising an arylsulfatase A (ASA) protein and a polox-

amer, wherein the ASA protein is present at a concentration of at least approximately 0.1 mg/ml, and wherein less than 5% of the arylsulfatase A (ASA) protein exists in aggregated form.

49. The stable formulation of claim **48**, wherein the poloxamer is selected from the group consisting of Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, 407, and combination thereof.

50. The stable formulation of claim **49**, wherein the poloxamer is poloxamer 188.

51. The stable formulation of claim **48**, wherein the poloxamer is present at a concentration ranging from approximately 0.05-0.50%, ranging from approximately 0.05-0.20%, of approximately 0.15%, or of approximately 0.1%.

52. The stable formulation of claim **48**, wherein less than 4%, less than 3%, less than 2%, or less than 1% of the arylsulfatase A (ASA) protein exists in aggregated form.

53. The stable formulation of claim **48**, wherein the ASA protein is present at a concentration selected from about 1 mg/ml, 10 mg/ml, 30 mg/ml, 50 mg/ml, or 100 mg/ml.

54. The stable formulation of claim **48**, wherein the ASA protein comprises an amino acid sequence having at least 80% identity to SEQ ID NO:1 or the ASA protein comprises the amino acid sequence of SEQ ID NO:1.

55. The stable formulation of claim **48**, wherein the ASA protein is produced from a human cell line or from CHO cells.

56. The stable formulation of claim **48**, wherein the formulation further comprises salt.

57. The stable formulation of claim **56**, wherein the salt is NaCl and is present at a concentration ranging from approximately 0-300 mM, ranging from approximately 80-160 mM, or of approximately 154 mM.

58. The stable formulation of claim **48**, wherein the stable formulation further comprises a buffering agent selected from the group consisting of phosphate, acetate, histidine, succinate, citrate, Tris, and combinations thereof.

59. The stable formulation of claim **58**, wherein the buffering agent is phosphate.

60. The stable formulation of claim **48**, wherein the formulation has a pH of approximately 3-8.0, approximately 6.0-6.5, or approximately 6.0.

61. The stable formulation of claim **48**, wherein the formulation is a liquid formulation or is formulated as lyophilized dry powder.

62. The stable formulation of claim **48**, wherein the formulation further comprises a stabilizing agent selected from the group consisting of sucrose, glucose, mannitol, sorbitol, polyethylene glycol (PEG), histidine, arginine, lysine, phospholipids, trehalose and combination thereof.

63. A stable formulation for intrathecal administration comprising

an arylsulfatase A (ASA) protein at a concentration ranging from approximately 0.1-100 mg/ml, a poloxamer at a concentration ranging from approximately 0.05-0.50%, NaCl at a concentration ranging from approximately 0-300 mM, and a pH of 3-8.0, wherein less than 5% of the ASA protein exists in aggregated form.

64. A container comprising a single dosage form of a stable formulation according to claim **48**.

65. The container of claim **64**, wherein the container is selected from an ampule, a vial, a cartridge, a reservoir, a lyo-ject, or a pre-filled syringe.

66. The container of claim **64**, wherein the stable formulation is present in a volume of less than about 50.0 ml or less than about 5.0 ml.

67. A method of treating metachromatic leukodystrophy (MLD) disease comprising a step of administering intrathecally to a subject in need of treatment a formulation comprising an arylsulfatase A (ASA) protein at a concentration ranging from approximately 0.1-100 mg/ml, a poloxamer at a concentration ranging from approximately 0.05-0.50%, NaCl at a concentration ranging from approximately 0-300 mM, and a pH of 3-8.0, wherein less than 5% of the ASA protein exists in aggregated form.

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