



## (51) International Patent Classification:

A61K 31/137 (2006.01) A61K 48/00 (2006.01)  
A61K 38/47 (2006.01) C07H 19/20 (2006.01)

## (21) International Application Number:

PCT/US2016/025215

## (22) International Filing Date:

31 March 2016 (31.03.2016)

## (25) Filing Language:

English

## (26) Publication Language:

English

## (30) Priority Data:

62/140,808 31 March 2015 (31.03.2015) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LI, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

## Published:

— with international search report (Art. 21(3))

(54) Title: METHODS AND COMPOSITIONS FOR TREATING GLYCOGEN STORAGE DISEASES USING AGENTS THAT MIMIC OR ELEVATE CYCLIC AMP

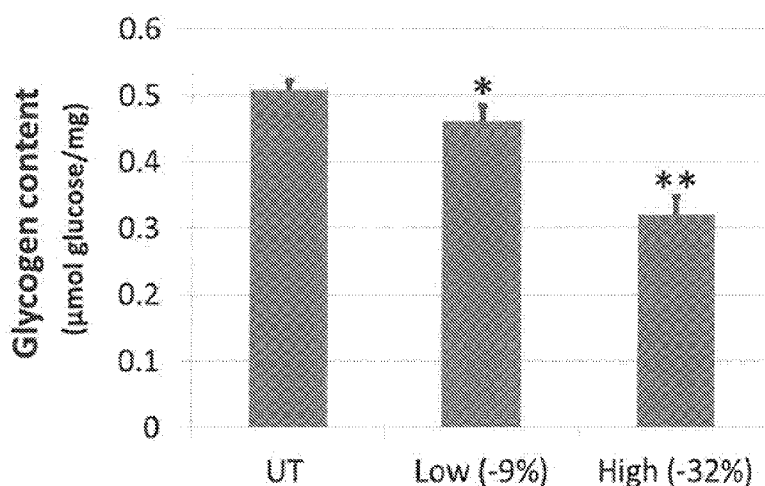


Figure 2. Forskolin reduced glycogen content in primary muscle cells from patient with GSD IIIa. UT, untreated; Low, 1 μM Forskolin; High, 10 μM Forskolin. \*, p<0.05 vs UT; \*\*, p<0.001 vs UT.

(57) Abstract: The invention provides compositions and methods for treating glycogen storage diseases or conditions with a buildup of glycogen. Cyclic AMP elevator compositions are shown to reduce glycogen storage in affected cells and therefore can be used to treat or reduce symptoms in subjects having glycogen storage diseases or conditions with a buildup of glycogen.

## METHODS AND COMPOSITIONS FOR TREATING GLYCOGEN STORAGE DISEASES USING AGENTS THAT MIMIC OR ELEVATE CYCLIC AMP

### PRIORITY

5           This application claims the benefit of U.S. 62/140,808, filed on March 31, 2015, the contents of which are incorporated by reference in their entirety.

### BACKGROUND

          Glycogen is a branched polymer of glucose that serves as a form of energy storage in human and animals. The two major sites of glycogen storage are liver and muscle. The primary function of glycogen varies in different tissues. In liver, glycogen serves as a glucose reserve for the maintenance of blood-glucose levels; in muscle, glycogen provides energy for muscle contraction [1,2]. Glycogen metabolism is a complex process involving many different enzymes that directly or indirectly regulate glycogen synthesis and degradation. In mammalian cells, glycogen is synthesized in the cytosol by the two enzymes, glycogen synthase (GS) and glycogen branching enzyme (GBE). Most glycogen is degraded in the cytoplasm (glycogenolysis) by a combined action of glycogen phosphorylase (GP) (cleaves the  $\alpha$ -1,4-glycosidic bonds) and glycogen debranching enzyme (GDE) (cleaves  $\alpha$ -1,6-glycosidic bonds at the branch points), but a very small amount of glycogen is transported into lysosomes and digested into glucose by the enzyme acid alpha-glucosidase (GAA) [2,3].

          Glycogen synthesis and degradation involve numerous enzymes. Mutations in genes encoding these enzymes cause a partial or complete loss of the enzyme activities in glycogen storage diseases (GSDs), a group of genetic disorders with abnormal metabolism of glycogen primarily in liver, muscle, and the brain. Most of the GSDs are inherited in an autosomal recessive manner, some are X linked or inherited in an autosomal dominant manner. The overall frequency of all forms of glycogen storage diseases is approximately 1 in 10,000 live births. There are over 13 forms of GSD presently identified, and a wide spectrum of clinical presentations is seen. GSD types I, II, III, VI, and IX are currently recognized as the most common forms, accounting for over 90 percent of all cases. Some deficiencies affect only one tissue (liver or muscle); others can affect several tissues (liver,

muscle, and other tissues) [4,5,6]. Among these diseases, GSD II (Pompe disease), a deficiency in the lysosomal enzyme acid alpha-glucosidase (GAA), is the only GSD that has glycogen storage in lysosomes and therefore is also classified as a lysosomal storage disease. All other GSDs, except for  
5 GSD 0 (no glycogen is synthesized in patients with GSD 0 due to the deficiency of glycogen synthase), have glycogen accumulation in the cytoplasm (cytoplasmic GSDs) and possibly also in the lysosomes.

Enzyme replacement therapy (ERT) would be an ideal treatment for genetic diseases with singly-gene deficiency. ERT has been effective in  
10 diseases in which the responsible enzymes/proteins exert their functions in extracellular fluids, such as adenosine deaminase deficiency, hemophilia, and alpha 1-antitrypsin deficiency, or in a lysosomal location such as lysosomal storage diseases including Pompe disease in which the therapeutic enzyme could be efficiently delivered into the lysosome of diseased cells via a  
15 mannose-6-phosphate receptor (M6PR or IGF2)-mediated uptake. ERT with recombinant human GAA (rhGAA, Myozyme, Lumizyme, Alglucosidase Alfa) is the only approved therapy for Pompe disease. ERT, however, has been successful only in some patients as challenges with low targeting efficiency in skeletal muscle and immunogenicity among others. There is therefore an  
20 urgent need to develop new therapies for the treatment of other GSDs especially a therapy that is suitable for most, if not all, GSDs.

### SUMMARY

Methods and compositions for treating glycogen storage diseases are provided herein. In particular, administration of cyclic AMP elevators is shown  
25 to reduce glycogen storage in affected cells and thus can be used to treat or reduce symptoms in subjects with glycogen storage diseases or other conditions where there is a build of glycogen. The methods of treating a glycogen storage disease or other conditions where there is a build of glycogen in a subject provided herein include administering a therapeutically  
30 effective amount of a cyclic AMP elevator to the subject in need of treatment. The methods and compositions can further include administering an enzyme replacement therapy, a gene replacement therapy, a chaperone therapy, or a substrate reduction therapy using RNAi-based treatment approaches, antisense therapies, or small molecule or peptide drugs to the subject.

One embodiment of the invention provides a method of treating a glycogen storage disease or other conditions where there is a build of glycogen in a subject in need of such treatment comprising administering a therapeutically effective amount of a cyclic AMP elevator to the subject in  
5 need of treatment for a glycogen storage disease, or a condition where there is a buildup of glycogen from other disorders. The glycogen storage disease or other condition where there is a build of glycogen can be selected from the group consisting of GSD I, GSD II (Pompe disease), GSD III, GSD IV, GSD V, GSD VI, GSD VII, GSD IX, GSD XI, GSD XII, GSD XIII, GSD XIV, Danon  
10 disease, Fanconi-Bickel disease, Lafora disease, cardiac/muscle glycogenosis due to AMP-activated protein kinase gamma subunit 2-deficiency (PRKAG2 cardiac syndrome), or other disorders where there is secondary accumulation of glycogen, such as Niemann-Pick Disease, GSD X, phosphoglycerate kinase deficiency, RBCK1 deficiency, and GSD XV.

15 The cyclic AMP elevator can be an adenylate cyclase activator, a phosphodiesterase (PDE) inhibitor, a Toll-like receptor ligand, a calcium ionophore, a protein kinase A (PKA) activator, a protein kinase C (PKC) activator, a beta2-adrenergic receptor agonist, an adenylate cyclase toxin, or combinations thereof.

20 The adenylate cyclase activator can be a labdane diterpene, a G-protein coupled receptor agonist, a G-protein activator, the pyrazole derivative A02011-1, benzyloxybenzaldehyde and analogs thereof, or combinations thereof.

The labdane diterpene can be labdane, forskolin, a forskolin derivative,  
25 6-acetyl-7-deacetyl-forskolin, 7-deacetyl-forskolin, 7-deacetyl-6-(N-acetylglycyl)-forskolin, 7-deacetyl-7-O-hemisuccinyl-forskolin, 7-deacetyl-7-(O-N-methylpiperazino)-γ-butyryl-dihydrochloride-forskolin, 7-HPP-forskolin, 6-HPP-forskolin, colforsin daropate hydrochloride (NKH477), or combinations thereof.

30 The G-protein coupled receptor agonist can be catecholamine, dopamine, dobutamine, isoproterenol, adenosine, carbacyclin, endothelin, epinephrine, glucagon, octopamine, pituitary adenylate cyclase-activating peptide (PACAP), parathyroid hormone, prostaglandin, vasopressin, or combinations thereof.

The G-protein activator can be cholera toxin or a subunit thereof.

The PDE inhibitor can be a PDE3, PDE4, PDE7, or PDE8 inhibitor.

The Toll-like receptor ligand can be lipopolysaccharide (LPS), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (pLPC), lipoteichoic acid (LTA), flagellin, or combinations thereof.

The calcium ionophore can be an ionomycin calcium salt, A23187, or combinations thereof.

The PKA activator can be 6-Bnz-cAMP, 8-CPT-2'-O-Me-cAMP, 8-CPT-cAMP, 8-Bromo-cAMP, Dibutyl-cAMP, Dioctanoyl-cAMP, Sp-8-Br-cAMPS, Sp-cAMPS, cAMP, a PKA subunit, or combinations thereof.

The PKC activator can be phorbol myristate acetate (PMA), a PKC purified enzyme, or combinations thereof.

The beta2-adrenergic receptor agonist can be bitolterol, fenoterol, isoprenaline, levosalbutamol, orciprenaline, pirbuterol, procaterol, ritodrine, salbutamol, terbutaline, arformoterol, bambuterol, clenbuterol, formoterol, salmeterol, indacaterol, olodaterol, vilanterol, vilanterol with umeclidinium bromide, vilanterol with fluticasone furoate, zilpaterol, or combinations thereof.

The methods of the invention can further comprise administering a therapeutically effective amount of an enzyme replacement therapy, a gene therapy, a substrate reduction therapy, a chaperone therapy, or combinations thereof to the subject with a glycogen storage disease (primary or a secondary accumulation of glycogen due to other primary diseases).

Two or more cyclic AMP elevators can administered to the subject. The cyclic AMP elevator can be administered by a route selected from oral, parenteral, intramuscular, intravenous, intraperitoneal and subcutaneous.

Another embodiment of the invention provides a pharmaceutical composition comprising at least one cyclic AMP elevator and an enzyme replacement therapy, gene therapy, substrate reduction therapy, chaperone therapy specific for a glycogen storage disease, or combinations thereof.

The enzyme replacement therapy or gene therapy can be selected from the group consisting of administration of the following therapeutic enzymes or genes encoding these enzymes: acid alpha-glucosidase, glucose-6-phosphatase, glycogen debranching enzyme, glycogen branching enzyme, muscle glycogen phosphorylase, liver glycogen phosphorylase, muscle

phosphofructokinase, phosphorylase kinase, lactate dehydrogenase, glucose transporter 2, Aldolase A, phosphoglucomutase, Laforin or Malin, LAMP-2, and  $\beta$ -enolase.

The substrate reduction therapy can be selected from the group  
5 consisting of methods of inhibiting glycogen synthase to reduce glycogen accumulation, siRNA-based therapies, shRNA-based therapies, antisense therapies, and therapies using small molecules or peptide drugs.

Yet another embodiment of the invention provides a kit comprising at least one cyclic AMP elevator, an enzyme replacement therapy or gene  
10 therapy and instructions for administering the cyclic AMP elevator and the enzyme replacement therapy or gene therapy to a subject with a glycogen storage disease or other conditions where there is a build of glycogen, wherein the enzyme replacement therapy or gene therapy replaces an enzyme or gene encoding the enzyme deficient in the glycogen storage  
15 disease. The kit can further comprise one or more chaperone therapies.

Another embodiment of the invention provides a kit comprising at least one cyclic AMP elevator, a substrate reduction therapy, and instructions for administering the cyclic AMP elevator and the substrate reduction therapy to a  
20 subject with a glycogen storage disease or other condition where there is a build of glycogen, wherein the substrate reduction therapy reduces glycogen synthase activity and prevents glycogen accumulation in the glycogen storage disease.

#### BRIEF DESCRIPTION OF THE DRAWINGS

25 Figure 1 is a schematic showing the role of cyclic AMP in stimulating glycogen breakdown and inhibiting glycogen synthesis through activation of cAMP-dependent protein kinase A (PKA). Inactive forms are phosphorylase b, phosphorylase kinase b, and glycogen synthase b. Active forms are phosphorylase kinase a, phosphorylase a, and glycogen synthase a. Adapted  
30 from Berridge, M.J., 2014.

Figure 2 is a graph showing that Forskolin reduced glycogen content in primary muscle cells from patients with GSD IIIa. UT, untreated; Low, 1  $\mu$ M Forskolin; High, 10  $\mu$ M Forskolin. \*,  $p < 0.05$  vs. UT; \*\*,  $p < 0.001$  vs. UT.

Figure 3 is a graph showing that glycogen content was decreased in GSD IV patient fibroblasts by Forskolin treatment. UT, untreated; Low, 1  $\mu$ M Forskolin; High, 10  $\mu$ M Forskolin. \*,  $p < 0.05$  vs. UT; \*\*,  $p < 0.001$  vs. UT.

5

## DETAILED DESCRIPTION

This invention relates to methods and compositions of treating or preventing glycogen storage diseases (GSD) type III (Forbes-Cori disease, Debranching enzyme deficiency) and IV (Andersen disease, Branching Enzyme Deficiency), by administering one or more cAMP elevators or agents that mimic cAMP, for example Forskolin or its derivatives or analogs thereof in a therapeutically effective amount. The invention also relates to use of the same method as an adjunctive therapy in combination with other therapies, such as enzyme replacement therapy, gene therapy, substrate reduction, chaperone therapy, and other therapeutic approaches. The invention further relates to use of the same method, either alone or as an adjunctive therapy, for treating other GSDs and other disorders where there is a buildup of glycogen including, but not limited to, type I (von Gierke disease, glucose-6-phosphatase deficiency), II (Pompe disease, acid-alpha-glucosidase deficiency), V (McArdle disease, Muscle Phosphorylase Deficiency), VI (Hers disease, Liver phosphorylase enzyme), VII (Tarui disease, Muscle Phosphofructokinase Deficiency), IX (Phosphorylase Kinase Deficiency), XI (Lactate dehydrogenase deficiency), XII (Aldolase A deficiency), XIII ( $\beta$ -enolase deficiency), GSD XIV (Phosphoglucomutase deficiency); Fanconi-Bickel disease (deficiency in glucose transporter GLUT2), Lafora disease (Laforin/Malin deficiency), Danon disease (LAMP2 deficiency), cardiac glycogenosis due to AMP-activated protein kinase gamma subunit 2-deficiency (PRKAG2 cardiac syndrome), Niemann-Pick Disease Types A-C), GSD X (phosphoglycerate mutase deficiency; increased glycogen in muscle); phosphoglycerate kinase deficiency (increased glycogen in muscle); RBCK1 deficiency (polyglucosan body myopathy caused by deficiency of ubiquitin ligase RBCK1; polyglucosan body); and GSD XV (Glycogenin-1 deficiency; polyglucosan body).

Glycogen synthesis and degradation are reciprocally regulated by hormonal signals. Insulin and glucagon (or epinephrine) are the major

hormones that regulate glycogen storage and mobilization. Glycogen synthesis is triggered when blood-glucose levels are high, through insulin-induced activation of protein phosphatase-1 (PP1). PP1 activates GS (b→ a) and inactivates GP and glycogen phosphorylase kinase (GPK) (a→ b) by dephosphorylating these enzymes (Figure 1). In contrast, glycogen breakdown is initiated in a starved state by glucagon (in liver) or epinephrine (in muscle) triggered cyclic AMP (cAMP) cascade acting through cAMP-dependent protein kinase A (PKA) (Figure 1). PKA stimulates glycogen degradation by the following actions: 1) phosphorylates and activates GPK (b → a), which subsequently phosphorylates and activates GP (b → a); 2) phosphorylates GS (a→ b), which leads to a decrease in enzymatic activity to prevent glycogen being synthesized at the same time that it is being broken down; and 3) disables PP1 function by dissociating it from glycogen through phosphorylation of the glycogen-binding subunit G<sub>M</sub> (Figure 1) [3,7,8,9].

#### 15 **GSD III (Glycogen debranching enzyme deficiency, Cori Disease)**

Genetic deficiency of glycogen debranching enzyme (GDE) causes an incomplete glycogenolysis in GSD III, resulting in accumulation of abnormal glycogen with short outer chain in various organs, mostly liver and muscle. The disease is characterized by hepatomegaly, hypoglycemia, short stature, variable myopathy and cardiomyopathy. Most patients have diseases involving both liver and muscle (type IIIa), while some patients (~15%) have only liver involvement (type IIIb). Liver symptoms normally occur in childhood. Liver cirrhosis and hepatocellular carcinoma have been reported in some cases [4,10]. Muscle weakness is present during childhood. It becomes more prevalent in adults with onset in the third or fourth decade. There is significant morbidity from progressive muscle weakness and patients in later stages can become wheel chair bound. Patients can also develop cardiomyopathy. There is significant clinical variability in the severity of the symptoms that these patients develop. The progressive myopathy and/or cardiomyopathy and/or peripheral neuropathy are major causes of morbidity in adults [10,11,12]. Current treatment is symptomatic, and there is no effective therapy for the disease. Hypoglycemia can be controlled by frequent meals high in carbohydrates with cornstarch supplements or nocturnal gastric drip feedings. Patients with myopathy have been treated with a diet high in protein during

the daytime plus overnight enteral infusion. In some patients transient improvement in symptoms has been documented, but there are no systemic studies or long-term data demonstrating that the high protein diet prevents or treats the progressive myopathy [10]. These approaches do little to alter the long term course and morbidity of these diseases.

Curly coated retrievers (CCR) with a phenotype mimicking type IIIa disease have been identified. These dogs have hepatomegaly, hypoglycemia, and elevated liver enzymes and creatine phosphokinase. The clinical signs in these affected dogs appear to be mild in the first year of life, becoming more prominent with age and leading to lethargy, exercise intolerance, and episodic hypoglycemia with collapse/unresponsiveness. These dogs are homozygous for the c.4223delA mutation [13]. Rapamycin, a specific inhibitor of mTOR, can significantly reduce glycogen content in both liver and skeletal muscle of affected CCR dogs [14]. This suggests suppression of glycogen synthesis with Rapamycin is a potential useful therapy for GSD III. In addition, a mouse model of GSD IIIa has been developed recently. Histology revealed massive glycogen accumulation in the liver, muscle, and heart of the homozygous affected mice. Hepatomegaly and progressive liver fibrosis were also found in the affected mice[15]. These animal models provide excellent tools for testing novel therapies in pre-clinical studies.

#### **GSD IV (Glycogen branching enzyme deficiency; Andersen Disease)**

GSD IV is a rare autosomal recessive disorder caused by deficiency of glycogen branching enzyme (GBE), a key enzyme involved in glycogen synthesis. Patients with GSD IV have accumulation of insoluble, amylopectin-like polysaccharide in multiple tissues, including liver, skeletal muscle, heart and central and peripheral nervous system [4,16,17]. GSD IV is clinically variable. The classical form of GSD IV is characterized by failure to thrive, hepatosplenomegaly, and progressive liver cirrhosis which normally lead to death by age 5 years. Some patients can develop hepatic adenomas and hepatocellular carcinoma. In addition to the hepatic presentation, four neuromuscular forms can be distinguished based on the ages at onset. The perinatal form is characterized by multiple congenital contractures, hydrops fetalis, and perinatal death. The congenital form includes hypotonia, muscle wasting, neuronal involvement, inconsistent cardiomyopathy, and death in

early infancy. In the childhood form, patients present predominantly with a myopathy or cardiomyopathy. The adult form can present as an isolated myopathy or as a multisystem disorder with central and peripheral nervous system dysfunction accompanied by accumulation of polyglucosan material in the nervous system (so-called adult polyglucosan body disease) [18,19,20]. There is no specific treatment for GSD IV. Maintenance of normoglycemia and adequate nutrient intake improve liver function and muscle strength in some patients. For progressive hepatic failure, liver transplantation is the only treatment option [4].

Norwegian forest cats (NFC) are a naturally occurring animal model of GSD IV, caused by an inherited, recessive mutation in the branching enzyme gene [21]. Most homozygous affected kittens die at or soon after birth due to hypoglycemia. The surviving cats appear clinically normal until 5 month of age, when skeletal muscle, heart, and CNS degeneration become obvious, accompanied by elevated body temperature, but cirrhosis and liver failure are absent. A mouse model of GSD IV is available. The homozygous mice (Gbe1(neo/neo)) exhibit a phenotype similar to juvenile onset GSD IV, with wide spread accumulation of polyglucosan bodies [22]. Other mice models of GSDs are also available.

Similarly, there are no effective treatments for other cytoplasmic GSDs, including GSD I (von Gierke's disease, glucose-6-phosphatase deficiency, 1b translocase deficiency), GSD V (McArdle's disease, a deficiency in muscle phosphorylase), GSD VI (Hers' disease, a deficiency in liver phosphorylase), GSD VII (a deficiency in muscle phosphofructokinase; Tarui's disease), GSD IX (phosphorylase kinase deficiency), GSD XI (Lactate dehydrogenase deficiency), GSD XII (Aldolase A deficiency), GSD XIII (a deficiency in  $\beta$ -enolase); GSD 0 (A deficiency in glycogen synthase), Fanconi-Bickel disease (deficiency in glucose transporter GLUT2), Lafora disease (laforin/malin deficiency), cardiac/muscle glycogenosis due to AMP-activated protein kinase gamma subunit 2-deficiency (PRKAG2 cardiac syndrome), GSD XIV due to phosphoglucomutase deficiency; Danon disease (GSD 2B) due to LAMP-2 deficiency, and conditions where there is a secondary buildup of glycogen, such as Niemann-Pick Disease [4], GSD X (phosphoglycerate mutase deficiency; increased glycogen in muscle); phosphoglycerate kinase

deficiency (increased glycogen in muscle); RBCK1 deficiency (polyglucosan body myopathy caused by deficiency of ubiquitin ligase RBCK1; polyglucosan body); and GSD XV (Glycogenin-1 deficiency; polyglucosan body).

In all the GSDs, other than Pompe disease treatment is symptomatic and primarily with diet modifications.

#### **Cyclic AMP elevators**

The term "cAMP elevator" as used herein refers to an agent that increases intracellular levels of cAMP beyond the background physiological intracellular level.

Intracellular levels of cAMP can be measured by, for example, assays that measure cAMP levels through protein kinase A (PKA), which is activated upon release of its regulatory subunits after binding to cAMP (e.g., cAMP-GLO™ Max assay). cAMP levels can be increased by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 percent or more (or any range between about 5 and 100 percent).

cAMP is synthesized from ATP by the enzyme adenylate cyclase and is degraded into AMP by cAMP phosphodiesterases. cAMP elevators therefore include agents that activate or enhance the activity of adenylate cyclase (hereinafter referred to as "adenylate cyclase activators"), agents that increase the availability of adenylate cyclase, and agents that inhibit or block the activity of cAMP and/or cGMP phosphodiesterases (hereinafter referred to as "PDE inhibitors").

The activity of adenylate cyclase can be measured by, for example, ELISA. The activity of adenylate cyclase can be increased by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 percent or more (or any range between about 5 and 100 percent).

The availability of adenylate cyclase can be measured by, for example, ELISA. The availability of adenylate cyclase can be increased by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 percent or more (or any range between about 5 and 100 percent).

The activity of cAMP phosphodiesterases or cGMP phosphodiesterases can be measured by, for example, a PDELight™ Assay Kit (Lonza, Basel, Switzerland). The activity of cAMP phosphodiesterases or cGMP phosphodiesterases can be decreased by about 5, 10, 20, 30, 40, 50,

60, 70, 80, 90, 100 percent or more (or any range between about 5 and 100 percent).

Other representative cAMP elevators include, but are not limited to, the Toll-like receptor ligands, calcium channel activators or calcium activators, beta2-adrenergic receptor agonists, protein kinase C (PKC) activators, and adenylyl cyclase toxin. These classes of cAMP elevators are described in more detail herein below. In a particular embodiment, the cAMP elevator is an adenylyl cyclase activator, more particularly, a labdane diterpene such as Forskolin or a derivative or analog thereof.

The term "agent that mimics cAMP" as used herein refers to an agent that produces physiological effects similar to endogenous cAMP such as, for example, activating protein kinase A (PKA) (also known as cAMP-dependent enzyme). Accordingly, agents that mimic cAMP include, for example, PKA activators. The activity of PKA activators can be determined using an ELISA (for example, an ELISA that utilizes a synthetic peptide as a substrate for PKA and a polyclonal antibody that recognizes the phosphorylated form of the substrate). A PKA activator can increase protein kinase A activity by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 percent or more (or any range between about 5 and 100 percent).

#### **Forskolin**

Forskolin (also called Coleonol) is a chemical that is extracted from the roots of the Indian Coleus plant (*Coleus forskohlii*). Forskolin is commonly used as a cAMP elevator to raise levels of cAMP in the research of cell physiology. Forskolin activates the enzyme adenylyl cyclase and increases intracellular levels of cAMP [23,24,25,26].

It has been shown that Forskolin could induce glycogenolysis and reduce glycogen levels in both cultured cells and experimental animals [27] [28] [29]. However, the role of cyclic AMP in GSD is unknown and the ability of forskolin or another cyclic AMP elevator to treat GSD has not been suggested and would not be predicted to overcome enzyme deficiencies. The ability of some cyclic AMP elevators to cross the blood brain barrier can also make these compounds useful to treat central or peripheral nervous system aspects of disease that are not amenable to treatment with ERT.

Forskolin is already in use as a pharmaceutical to treat a number of other unrelated conditions and diseases. A Forskolin dosage can be about 5, 10, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100 or more mg in one day and the dose is divided into twice or thrice a day. Forskolin can be administered by  
5 intravenously (IV), intramuscularly (IM), subcutaneously (SC), inhalation, oral dosages, eye drops, or any other suitable methods.

The effectiveness of Forskolin to clear the massive glycogen accumulation in GSDs has not been tested or contemplated prior to this invention, likely due to the consideration that this treatment would not  
10 theoretically work for these diseases. For example, deficiency of GDE in GSD III results in accumulation of abnormal glycogen with short outer chains, in the presence of normal GP function. Increased activity of GP by Forskolin will not help degrade the stored glycogen (because the cells are still unable to cleave the  $\alpha$ -1, 6-glycosidic bonds). In GSD IV, deficiency of GBE causes the  
15 formation of less-branched, insoluble glycogen molecules that cannot be broken down by the normal GP and GDE activities. The deficiencies in GP (GSD V & VI) and GPK (GSD IX) obviously will interrupt the Forskolin-induced glycogen degradation through the cAMP/PKA-GPK-GP pathway.

Forskolin, however, unexpectedly can significantly reduce glycogen  
20 levels in GSD III patient muscle cells and GSD IV patient fibroblast cells (see Examples; Figure 2 and Figure 3), likely through the inhibition of glycogen synthesis. This treatment approach can be extremely effective for the patients with, for example, GSD V/VI and GSD IX who have residual enzyme activities, but the approach can be effective in patients with various GSDs, including but  
25 not limited to GSD type I, II, III, IV, V, VI, VII, IX, XI, XII, XIII, XIV, Lafora disease, Fanconi-Bickel disease, Danon disease, PRKAG2 cardiac syndrome, and conditions where there is a secondary buildup of glycogen, such as Niemann-Pick Disease, GSD X (phosphoglycerate mutase deficiency; increased glycogen in muscle); phosphoglycerate kinase deficiency  
30 (increased glycogen in muscle); RBCK1 deficiency (polyglucosan body myopathy caused by deficiency of ubiquitin ligase RBCK1; polyglucosan body); and GSD XV (Glycogenin-1 deficiency; polyglucosan body).

The invention provides methods of use and compositions comprising cAMP elevators, alone or in combination with one or more enzyme

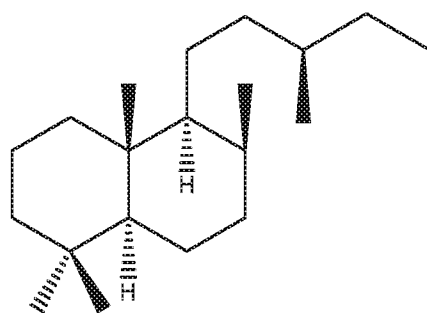
replacement therapies, which can be from other classes of therapeutic agents, one or more gene replacement therapies, one or more substrate reduction therapies, or one or more chaperone therapies. Suitable cAMP elevators include, but are not limited to, the following types of therapeutic agents: an adenylate cyclase activator, a PDE inhibitor, a Toll-like receptor ligand, a calcium activator, a protein kinase A activator, a protein kinase C activator, a beta2-adrenergic receptor agonist, or a adenylate cyclase toxin. Each of these types of cAMP elevators are described in more detail below.

#### **Adenylate Cyclase Activity**

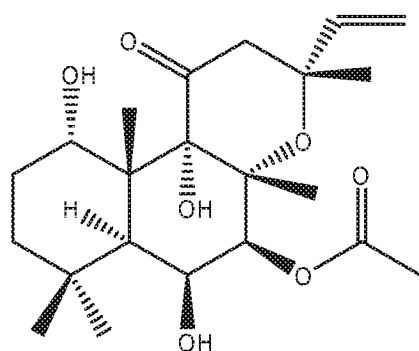
Adenylate cyclase is an enzyme that synthesizes cAMP from ATP. There are at least nine isoforms of adenylate cyclase, which differ considerably in regulatory properties and are differentially expressed among tissues [47, 48]. Early studies indicated that cyclase activity was regulated primarily by interactions with alpha subunits of heterotrimeric G proteins, which are activated through G protein-coupled receptors. More recently, it has become clear that cyclase activity is regulated by multiple effectors, which include not only the alpha subunits of G<sub>s</sub> and G<sub>i</sub> proteins, but also the beta-gamma subunits of G proteins and protein kinase C. Five of the adenylate cyclases known are regulated by calcium [49, 50]. All known adenylate cyclases are stimulated by exposure of cells to forskolin.

Any compound or agent that enhances adenylate cyclase activity *in vivo* to elevate intracellular levels of cAMP can be used in methods and compositions of the invention. Exemplary adenylate cyclase activators include, but are not limited to, the labdane diterpenes, such as forskolin or a derivative or analog thereof, pyrazole derivatives, benzyloxybenzaldehyde analog, G-protein coupled receptor agonists, and G-protein activators.

Thus, in one embodiment, the cAMP elevator for use within the methods and compositions of the invention is a labdane diterpene such as labdane, forskolin, or a forskolin derivative or analog. The chemical structure for labdane is depicted below:



The chemical structure of forskolin is depicted below:



5        Other labdane diterpenes are known to those of skill in the art and can also be used as cyclic AMP elevators within the scope used herein and include those disclosed in U.S. Patent No. 5,268,471 to de Souza, U.S. Patent No. 5,789,439 to Hosono, U.S. Patent No. 4,517,200 to Kreutner, U.S. Patent No. 5,869,523 to de Souza, U.S. Patent No. 5,350,864 to Seamon,  
 10    U.S. Patent No. 4,871,764 to Schutske. Pharmaceutically acceptable salts of the labdane diterpenes are also included herein.

Other adenylate cyclase activators for use in the methods and compositions of the invention include, but are not limited to, G-protein coupled receptor agonists and G-protein activators. Adenylate cyclase in mammalian  
 15    cells is normally activated by the stimulatory regulatory protein  $G_s$  and guanosine triphosphate (GTP); however, the activation is normally brief because an inhibitory regulatory protein ( $G_i$ ) hydrolyzes the GTP. Cholera toxin and pertussis toxin catalyze the covalent incorporation of ADP-ribose into the G-protein  $\alpha$ -subunit [51-54]. The pertussis toxin A subunit catalyzes  
 20    the ADP-ribosylation of  $G_i$  at a site that impairs the ability of this heterotrimeric G-protein to interact with receptors, thereby blocking the inhibitory effects of

$G_i$  on adenylate cyclase. In this manner, the conversion of ATP to cAMP is stimulated. The cholera toxin A subunit catalyzes the attachment of ADP-ribose to  $G_s$  in a manner that stabilizes the GTP-bound form resulting in persistent activation of adenylate cyclase. Purified subunits of these toxins  
5 (e.g., cholera toxin A subunit) have also been shown to activate adenylate cyclase.

Accordingly, suitable G-protein coupled receptor agonists for use in the methods and compositions of the invention include, but are not limited to, a catecholamine, dopamine, dobutamine, isoproterenol, adenosine, carbacyclin,  
10 endothelin, epinephrine, glucagon, octopamine, pituitary adenylate cyclase-activating peptide (PACAP), parathyroid hormone, prostaglandin, and vasopressin. Exemplary G-protein activators for use in the methods and compositions of the invention include, but are not limited to, cholera toxin or a subunit thereof and pertussis toxin or a subunit thereof.

15 Still further adenylate cyclase activators for use in the methods and compositions of the invention include the pyrazole derivative A02011-1 [55] and benzyloxybenzaldehyde and analogs thereof [56].

### PDE Inhibitors

20 A cAMP elevator for use in the methods and compositions of the present invention can be a PDE inhibitor. Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that regulate the cellular levels of the second messengers, cAMP and cGMP, by controlling their rates of degradation. There are 11 different PDE families, with each family having  
25 different selectivities for cyclic nucleotide substrates as follows: PDE1 (cAMP/cGMP), PDE2 (cAMP/cGMP), PDE3 (cAMP>>cGMP), PDE4 (cAMP), PDE5 (cGMP), PDE6 (cGMP), PDE7 (cAMP), PDE8 (cAMP), PDE9 (cGMP), PDE10 (cAMP/cGMP), and PDE11 (cAMP/cGMP).

Both nonspecific and selective or partially selective PDE inhibitors are  
30 known and can be used within the methods and compositions of the present invention. For example, the non-specific PDE inhibitor, 3-Isobutyl-1-methylxanthine (IBMX), has been shown to significantly increase intracellular cAMP levels in human bladder epithelial cells compared to untreated controls [57]. Other non-specific PDE inhibitors include, but are not limited to,

theophylline, theobromine, aminophylline, pentoxifylline, and caffeine and other methyl xanthine and non-xanthine derivatives.

Selective or partially selective PDE inhibitors for use in the methods and compositions of the invention include, but are not limited to, Vinpocetine (e.g., INTELECTOL®) (available from, e.g., Covex Pharma Inc., Miami, Florida); Nicardipine HCl (available from, e.g., Par Pharmaceutical Companies, Inc., Spring Valley, New York); 8-MeOM-IBMX (8-methoxymethyl-3-isobutyl-1-methylxanthine) (available from Biomol International LP, Plymouth Meeting, Pennsylvania); EHNA (erythro-9-(2-hydroxy-3-nonyl)adenine) (available from, e.g., A.G. Scientific, San Diego, California); IC933 (see, e.g., [58]); 2-(3,4-Dimethoxybenzyl)-7-[(1R)-1-[(1R)-1-hydroxyethyl]-4-phenylbutyl]-5-methylimidazo[5,1-f][1,2,4]triazin-4(3H)-one (Bay 60-7550) (available from, e.g., Axxora, LLC, San Diego, California); Lixazinone (available from Syntex Corporation, Palo Alto, California); Cilostamide (available from, e.g., Sigma-Aldrich, Co., St. Louis, Missouri); Milrinone (e.g., PRIMACOR®, discontinued by Sanofi-Aventis, Bridgewater, New Jersey) (available from, e.g., Haorui Pharma-Chem, Inc., Edison, New Jersey); Cilostazol (available from, e.g., Mylan Pharmaceuticals, Inc., Morgantown, West Virginia); OPC-33540 (6-[3-[3-cyclooctyl-3-[(1R\*,2R\*)-2-hydroxycyclohexyl]ureido]-propoxy]-2(1H)-quinolinone) (see [59]); Dihydropyridazinone (for representative derivatives thereof, see U.S. Patent No. 4,921,856 to Schickaneder et al.); Sildenafil citrate (e.g., VIAGRA®, available from Pfizer, Inc., New York, New York); Zaprinas (available from, e.g., A.G. Scientific, San Diego, California); Dipyridamole (e.g., PERSANTINE®, available from Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut); ARIFLO® (cilomilast) (available from GlaxoSmithKline, Research Triangle Park, North Carolina); Vardenafil HCl (LEVITRA®) (available from Schering Corporation, Kenilworth, New Jersey); Tadalafil (CIALIS®) (available from Lilly ICOS LLC, Indianapolis, Indiana); E4021 (sodium 1-[6-chloro-4-(3,4-methylenedioxybenzyl)-aminoquinazolin-2-yl] piperidine-4-carboxylate sesquihydrate) (available from Eisai Co., Ltd., Tokyo, Japan); DMPPO (1,3-dimethyl-6-(2-propoxy-5-methanesulfonylamidophenyl)pyrazol[3,4d]-pyrimidin-4-(5H)-one) (available from GlaxoSmithKline, Les Ulis, France); 3-(N,N-dimethylsulfonamido)-4-

methyl-nitrobenzene (BRL 50481) Biomol International LP, Plymouth Meeting, Pennsylvania); IC242 (available from Lilly ICOS LLC, Indianapolis, Indiana); BMS-586353 (available from Bristol-Myers Squibb Company, New York, New York); Thiadiazoles; SCH 51866 (cis-5,6a,7,8,9,9a-hexahydro-2-(4-  
 5 (trifluoromethyl)phenylmethyl)-5-methyl-cyclopent(4,5)imidazo(2,1-b)purin-4(3H)-one) (available from Schering-Plough Corporation, Kenilworth, New Jersey); and Papaverine (available under several brand names, depending on which salt is desired) (available from, e.g., MP Biomedicals, Inc., Irvine, California). A summary of the selectivity profiles of these compounds for  
 10 different members of the PDE family is provided in Table 1.

Table 1. Selective or Partially-Selective PDE Inhibitors.

| <u>PDE Family</u> | <u>Compounds</u>                                                                                                 |
|-------------------|------------------------------------------------------------------------------------------------------------------|
| PDE1              | Vinpocetine, Nicardipine, 8-MeOM-IBMX, nimodipine, IC224, IC295, dioclein                                        |
| PDE2              | EHNA, IC933, Bay 60-7550, PDP, oxindole, ND7011                                                                  |
| PDE3              | Lixazinone, Cilostamide, Milrinone, Cilostazol, OPC-33540, Dihydropyridazinone, siguazodan,                      |
| PDE4              | Rolipram, Ro 20-1724, Denbufylline, Cilomilast, Roflumilast, SCH 351591, V11294A, AWD 12-281, L-826,141, NCS 613 |
| PDE5              | Sildenafil, Zaprinast, Dipyridamole, Vardenafil, Tadalafil, E4021, DMPPO                                         |
| PDE6              | Zaprinast, Dipyridamole, Vardenafil, Tadalafil, E4021, DMPPO                                                     |
| PDE7              | Dipyridamole, BRL 50481, IC242, BMS-586353, Thiadiazoles, ASB16165                                               |
| PDE8              | Dipyridamole, PF-04957325                                                                                        |
| PDE9              | Zaprinast, SCH 51866, BAY-73-6691, PF-044447943                                                                  |
| PDE10             | Dipyridamole, Papaverine, TP-10, MP-10                                                                           |
| PDE11             | Tadalafil, Zaprinast, Dipyridamole                                                                               |

In another embodiment, a PDE inhibitor for is a cAMP-specific PDE  
 15 inhibitor. In one embodiment, the cAMP-specific inhibitor is a PDE3 inhibitor, a PDE4 inhibitor, a PDE7 inhibitor, or a PDE8 inhibitor, including, but not limited to, compounds described above and summarized in Table 1.

PDE3 is significantly expressed in cardiac and vascular myocytes, brain, and liver but not in skeletal muscle [30,31]. PDE4 is expressed widely in  
 20 most tissues but is the predominant PDE isoenzymes in skeletal muscle and

most immune cells. PDE4 is also present at relatively high levels in liver, heart, brain, smooth muscle, and vascular endothelium cells [30,31,32,33,34,35]. PDE7 is highly expressed in immune system, skeletal muscle, heart, liver, and also found in brain, kidney, and lung [30,31]. PDE8 is expressed in testes, cardiomyocytes, thyroid gland, brain, and adrenal gland [36].

The cAMP-specific PDE inhibitor can be a PDE4 inhibitor. The PDE4 family encompasses four subtypes, which are designated PDE4 A, PDE4 B, PDE4 C, and PDE4 D and differ in their regulatory behavior and tissue expression patterns. PDE4 inhibitors exhibit structural diversity and include compounds as described above in Table 1, as well as xanthine derivatives, such as arofylline (available from Almirall Prodesfarma, S.A.) and cipamfylline (GlaxoSmithKline, Research Triangle Park, North Carolina); catechol derivatives, such as rolipram (EMD Biosciences, San Diego, California), Ro 20-1724 (A.G. Scientific, Inc., San Diego, California), piclamilast, cilomilast (ARIFLO®, GlaxoSmithKline, Research Triangle Park, North Carolina), roflumilast (Altana Pharma, Germany), and atizoram; indole derivatives, such as AWD 12-281 (Elbion AG, Germany); and thalidomide derivatives, such as CC-10004 (Celgene Corporation, Summit, New Jersey). Such PDE4 inhibitors are described, for example, in [60], which is incorporated herein by reference in its entirety.

Still further PDE4 inhibitors that can be used within the methods and compositions of the invention include CC-10015 (available from Celgene Corporation), 4AZA-PDE4i (available from Elbion NV), ELB353 (available from Elbion NV), ELB326 (available from Elbion NV), GRC 4039 (available from Glenmark Pharmaceuticals Limited), GRC 4039 (available from Glenmark Pharmaceuticals Limited), IPL4088 (available from Inflazyme Pharmaceuticals Ltd.), MEM 1917 (available from Memory Pharmaceuticals Corp), PLX369 / PDE 4 Inhibitor (available from Plexxikon Inc.), AVE8112 (available from Sanofi-Aventis), Theophylline (available from SCOLR Pharma Inc), Oglemilast (available from Teijin Pharma Limited), Oglemilast / GRC 3886 (available from Teijin Pharma Limited), Z15370A (available from Zambon Group), LAS 37779 (available from Almirall Prodesfarma, S.A.), Atopik (available from Barrier Therapeutics Inc), CC-11050 (available from

Celgene Corporation), 256066 (available from GlaxoSmithKline plc), NIK-616  
 (available from Kowa Co., Ltd.), MEM 1414 (available from Memory  
 Pharmaceuticals Corp), AWD 12-281 / GW842470 (available from Elbion NV),  
 Oglemilast (available from Forest Laboratories Inc), 256066 (available from  
 5 GlaxoSmithKline plc), GW842470 / AWD 12-281 (available from  
 GlaxoSmithKline plc), Oglemilast (available from Glenmark Pharmaceuticals  
 Limited), IPL455,903 / HT-0712 (available from Helicon Therapeutics, Inc),  
 IPL455,903 / HT-0712 (available from Inflazyme Pharmaceuticals Ltd.), MN-  
 166 (ibudilast) (available from MediciNova Inc), OPC-6535 (available from  
 10 Otsuka America Pharmaceutical, Inc.), Tofimilast (available from Pfizer Inc),  
 Daxas (roflumilast) / APTA-2217 (available from Nycomed), OPC-6535  
 (available from Otsuka America Pharmaceutical, Inc.), Daxas (roflumilast) /  
 APTA-2217 (available from Tanabe Seiyaku Co., Ltd.), Theolair (theophylline)  
 (available from 3M Company), Dot (drotaverine hydrochloride) (available from  
 15 Acme Laboratories Ltd.), Thenglate (theophylline) (available from Acme  
 Laboratories Ltd.), Pulmophyllin (theophylline) (available from Adcock Ingram  
 Limited), Solphylllex (theophylline, etofylline, diphenylpyraline hydrochloride,  
 ammonium chloride and sodium citrate) (available from Adcock Ingram  
 Limited), Solphyllin (theophylline and etofylline) (available from Adcock  
 20 Ingram Limited), Baladex (theophylline, guaifenesin) (available from  
 AFLOFARM), Taverine (drotaverine) (available from Ajanta Pharma Limited),  
 Etafin (acepifylline) (available from Aleppo Pharmaceutical Industries, L.L.C.),  
 Theo-dur (theophylline) (available from Almirall Prodesfarma, S.A.), No-spa  
 (drotaverine hydrochloride) (available from Ambee Pharmaceuticals Ltd.),  
 25 Contine (theophylline) (available from Aristopharma Ltd.), Etophylline plus  
 Theophylline (available from Arvind Remedies Ltd), Bitophyllin (theophylline  
 and guaifenesin) (available from BARAKAT Pharmaceutical Industries),  
 Theophylline (available from Barr Pharmaceuticals Inc), Theolin (theophylline  
 anhydrous) (available from Beacons Pharmaceuticals Pte Ltd), Theophylline  
 30 (theophylline anhydrous) (available from Beacons Pharmaceuticals Pte Ltd),  
 Dyphylin Injection (etophylline and theophylline) (available from BELCO  
 Pharma.), Theospirex (theophylline anhydrous) (available from BIOFARM Sp.  
 z o.o.), Asima (doxofylline) (available from Bukwang Pharmaceutical  
 Company Limited), Theobid Tablets (theophylline) (available from Cipla Ltd.),

Theoday Tablets (theophylline) (available from Cipla Ltd.), Theoped Syrup (theophylline) (available from Cipla Ltd.), Bronchipret (theophylline) (available from Darya-Varia), Frivent (theophylline) (available from Dompe S.p.A.), Trospa (available from Dr Reddys Laboratories Ltd), Theo-Dur (theophylline)  
 5 (available from Elan Corp Plc), Dotarin (drotaverine hydrochloride) (available from Elder), Gulamyl (theophylline and guaiphenesin) (available from ELPEN Pharmaceutical Co. Inc.), Drotaverine hydrochloride (available from ELSaad Pharmaceutical Industries), Theophylline (available from Eurand), Puroxan (doxofylline) (available from Eurodrug Laboratories), Choledyl (choline theophyllinate, theophylline) (available from Galenica s.a.), Drotaverine-Grindeks (drotaverine) (available from Grindeks), Tromphylin (theophylline) (available from Grupo Ferrer), Hesotanol (etophylline nicotinate) (available from HanAll Pharmaceutical), Neophin (diethylaminoethyl theophylline) (available from HanAll Pharmaceutical), Arutopa (acepifylline) (available from  
 15 Hawon Pharmaceutical Corporation), Theophylline (available from Indchemie Health Specialities Pvt. Ltd), Theophylline and Etophylline (available from Indchemie Health Specialities Pvt. Ltd), Doverin (drotaverine) (available from Intas Pharmaceuticals Ltd.), Euphyllinum-N (theophylline) (available from JSC Farmak International), Theophar (theophylline) (available from Julphar), Draw  
 20 (drotaverine) (available from Kamron Laboratories Ltd.), Quibron-T (theophylline) (available from King Pharmaceuticals Inc), Quibron-T/SR (theophylline anhydrous) (available from King Pharmaceuticals Inc), Theodur (theophylline) / Theodrip (available from Kowa Co., Ltd.), Teotard (theophylline) (available from Krka, d. d.), Theolan-B SR (theophylline)  
 25 (available from KunWha Pharmaceutical Co., Ltd.), Hespil (acepifylline) (available from Kyung Dong Pharma. Co., Ltd.), Theophylline monohydrate (available from Laboratoires SMB SA), Sedacris (theophylline, guaifenesin) (available from Laboratorio Elea SACIFYA), Aminofilin (theophyllin) (available from Laboratorios Phoenix), Dexa aminofilin (dexamethasone and  
 30 theophylline) (available from Laboratorios Phoenix), Dexa teosona (dexamethasone and theophylline) (available from Laboratorios Phoenix), Inastmol (ketotifen and theophylline) (available from Laboratorios Phoenix), Teosona (theophylline) (available from Laboratorios Phoenix), Theodur (theophylline) (available from Lavipharma Group), Spacovin Injection

(drotaverine) (available from M.J. Group), Drotikind (drotaverine hydrochloride) (available from Mankind Pharma Ltd.), Ranispas-DV (drotaverine, omeprazole hydrochloride) (available from Mankind Pharma Ltd.), Drot (drotaverine hydrochloride) (available from Mapra Laboratories Pvt. Ltd.), Theodur (theophylline) (available from Mitsubishi Pharma Corporation), Uniphyllin continus (theophylline) (available from Mundipharma International Limited), Unicon / Uniphyl (theophylline) (available from Mundipharma K.K.), Uniphyllin Continus (theophylline) (available from Napp Pharmaceuticals Limited), Theonat (theophylline) (available from Natco Pharma Limited), Theophylline (available from Natco Pharma Limited), Xtma (theophylline and etophylline) (available from Neon Laboratories Ltd.), Unicon (theophylline) (available from Nichi-iko Pharmaceutical Co., Ltd (formerly Nihon Iyakuin Kogyo Co., Ltd)), Teokap SR (theophylline) (available from Nobel Ilac Sanayii ve Ticaret A.S.), Compound theophylline (available from North China Pharmaceutical Group Corp), Euphyllin/Euphyllong (theophylline) (available from Nycomed), Orophil (etofyllin, theophyllin) (available from Ortin Laboratories Limited), Teosona SOL (theophylline) (available from Osmotica Pharmaceutical Corp), Synaclyn (theophylline) (available from Otsuka Pharmaceutical Co., Ltd.), Uniphyl (theophylline) (available from Otsuka Pharmaceutical Co., Ltd.), Cholelyl SA (oxtriphylline) (available from Pfizer Inc), Farcophylline (piperazine theophylline ethanoate) (available from Pharco Pharmaceuticals Inc.), Farcosolvin (ambroxol hydrochloric acid, guaiphenesin and theophylline anhydrous) (available from Pharco Pharmaceuticals Inc.), Remind (hexobendine dihydrochloride, etofylline and ethamivan) (available from Pharco Pharmaceuticals Inc.), Theofar S.R (anhydrous theophylline) (available from Pharco Pharmaceuticals Inc.), Pharmaniaga theophylline (theophylline) (available from Pharmaniaga Berhad), pms-Oxytriphylline (oxytriphylline) (available from Pharmascience Inc.), Retaphyl (theophylline) (available from PT Kimia Farma Tbk), T-phyl (theophylline) (available from Purdue Pharma L.P), Uniphyl (theophylline) (available from Purdue Pharma L.P), Teofilina (theophylline) (available from Ranbaxy Laboratories Ltd.), Theostan-CR (theophylline) (available from Ranbaxy Laboratories Ltd.), Theodur (theophylline) (available from Recordati SpA), Glyphillin (theophylline sodium glycinate) (available from Rekah Pharmaceutical Industry Ltd.), No

Spa (drotaverine) (available from Sanofi-Aventis), Relispa (drotaverine hydrochloride) (available from Searle Pakistan Pvt. LTD.), Respro SR (theophylline) (available from Searle Pakistan Pvt. LTD.), Theotard (theophylline) (available from Sopharma JSCo.), Asmany SR (theophylline) (available from Square Pharmaceuticals Ltd.), Espa (drotaverine hydrochloride) (available from Square Pharmaceuticals Ltd.), Broncolin (guaicol and theophylline) (available from Standard Chem. & Pharm.), TR Phyllin (theophylline) (available from Sun Pharmaceutical Industries Ltd.), Theophylline (available from Themis Laboratories Private Ltd), Teofurmate L (theophylline) (available from Towa Pharmaceutical Co., Ltd.), Teofurmate Dry Syrup (theophylline) (available from Towa Pharmaceutical Co., Ltd.), Theophylline (available from United Research Laboratories and Mutual Pharmaceutical Company), E.T.phyllin (etophylline, theophylline) (available from Vanguard Therapeutics), Vero-Drotaverine (drotaverine) (available from Veropharm), Lungfyl SR Tablet (available from Yash Pharma Laboratories Ltd.), Deoprin retard (theophylline) (available from Yooyoung Pharmaceutical Co., Ltd.), Soluphin (diethylaminoethyl theophylline hydrochloride) (available from YOOYOUNG Pharmaceutical Co., Ltd.), Green (guaicol glyceryl ether, theophylline sodium glycinate) (available from Yung shin Pharmaceutical), Sentin (diprophylline) (available from Yung shin Pharmaceutical), Spophyllin retard (theophylline) (available from Zentiva, a.s. (formerly Leciva a.s.)), Theolate Liquid (theophylline and guaifenesin) (available from Alpharma Inc), Theophylline Elixir (theophylline) (available from Alpharma Inc), IC485 (available from Array BioPharma Inc), Lirimilast (available from Bayer Ag), Mesopram (available from Bayer Schering Pharma AG), CC 7085 (available from Celgene Corporation), CC-10004 (available from Celgene Corporation), CC-1088 (available from Celgene Corporation), CC-1088 (available from Celgene Corporation), CDC-998 (available from Celgene Corporation), AWD 12-281 / GW842470 (available from Elbion NV), IC485 (available from Eli Lilly & Co), Ariflo (available from GlaxoSmithKline plc), GW842470 / AWD 12-281 (available from GlaxoSmithKline plc), GRC 3015 (available from Glenmark Pharmaceuticals Limited), GRC 3566 (available from Glenmark Pharmaceuticals Limited), GRC 3590 (available from Glenmark Pharmaceuticals Limited), GRC-3785 (available from Glenmark

Pharmaceuticals Limited), KW-4490 (available from Kyowa Hakko Kogyo Co., Ltd.), MEM 1414 (available from Memory Pharmaceuticals Corp), CDP 840 (available from Merck & Co Inc), (MRK)ND1251 (available from Neuro3d), ONO-6126 (available from Ono Pharmaceutical Co., Ltd.), Daxas (roflumilast) 5 (available from Pfizer Inc), MEM 1414 (available from Roche Holdings Ltd), MEM 1917 (available from Roche Holdings Ltd), CDP-840 (available from UCB S.A.), CT-5357 (available from UCB S.A.), ONO-6126 (available from Ono Pharmaceutical Co., Ltd.), and ONO-6126 (available from Santen Pharmaceutical Co., Ltd.).

10       Based on the predominant tissue distribution pattern of each PDE, a combination of PDE4 inhibitors and PDE7 inhibitors can be used for correcting glycogen storage in skeletal muscle and the brain. Similarly, a combination of PDE4 and PDE3 inhibitors can be combined to enhance the therapeutic efficacy in the liver and heart.

#### 15   **Toll-Like Receptor Ligands**

          In another embodiment, the cAMP elevator for use in the methods and compositions of the present invention is a Toll-like receptor ligand. Toll-like receptors are a class of single membrane-spanning non-catalytic receptors that recognize structurally conserved molecules derived from microbes once 20 they have breached physical barriers such as the skin or urinary tract mucosa and activate immune cell responses. The Toll-like receptor family has been described as type I transmembrane pattern recognition receptors that possess varying numbers of extracellular N-terminal leucine-rich repeat motifs, followed by a cysteine-rich region, a TM domain, and an intracellular Toll/IL-1 25 R (TIR) motif. [61-67]. The leucine-rich repeat domain is important for ligand binding and associated signaling and the TIR domain is important in protein-protein interactions and is typically associated with innate immunity. [68-71]. The human TLR family is composed of at least 10 members, each of which is specific in its expression patterns and pathogen-associated molecular pattern 30 sensitivities. [72-73].

          Toll-like receptor ligands that activate the TLR pathway thus represent other cAMP elevators useful in the present invention. Exemplary Toll-like receptor ligands for use within the methods and compositions of the invention include, but are not limited to, lipopolysaccharide (LPS), 1-palmitoyl-2-

linoleoyl-sn-glycero-3-phosphocholine (pLPC), lipoteichoic acid (LTA), and flagellin.

#### **Calcium Ionophores**

In some embodiments, the cAMP elevator for use within the methods  
5 and compositions of the invention is a calcium ionophore. Calcium ionophores act as calcium activators and include, but are not limited to, ionomycin calcium salts (Sigma) or A23187 (Sigma) (see also, [74-75]).

#### **Activators of Protein Kinase A and Protein Kinase C**

In other embodiments, the cAMP elevator for use within the methods  
10 and compositions of the invention is an activator of protein kinase A. Suitable protein kinase A (PKA) activators include, but are not limited to, 6-Bnz-cAMP, 8-CPT-2'-O-Me-cAMP, 8-CPT-cAMP, 8-Bromo-cAMP, Dibutyryl-cAMP, Dioctanoyl-cAMP, Sp-8-Br-cAMPS, Sp-cAMPS, cAMP, and a PKA subunit.

Another suitable cAMP elevator for use in the methods and  
15 compositions of the present invention is an activator of protein kinase C (PKC). Protein kinase C is a ubiquitous phospholipid-dependent enzyme that is involved in signal transduction associated with cell proliferation, differentiation, and apoptosis. At least eleven closely related PKC isozymes have been reported that differ in their structure, biochemical properties, tissue  
20 distribution, subcellular localization, and substrate specificity [76-83]. They are classified as conventional, novel, and atypical isozymes. Conventional PKC isozymes are  $\text{Ca}^{2+}$ -dependent, while novel and atypical isozymes do not require  $\text{Ca}^{2+}$  for their activation. All but the atypical PKC isozymes are activated by diacylglycerol (DAG). Membrane receptor binding of a hormone  
25 or other effector molecule results in activation of phospholipase C (PLC) or phospholipase  $\text{A}_2$  ( $\text{PLA}_2$ ) via a G-protein-dependent phenomenon. The activated PLC hydrolyzes phosphatidylinositol-4, 5-bisphosphate ( $\text{PIP}_2$ ) to produce DAG and inositol-1,4,5-trisphosphate ( $\text{IP}_3$ ). The  $\text{IP}_3$  causes the release of endogenous  $\text{Ca}^{2+}$  that binds to the cytosolic PKC and exposes the  
30 phospholipid-binding site. The binding of  $\text{Ca}^{2+}$  translocates PKC to the membrane, where it interacts with DAG and is transformed into a fully active enzyme.

In particular, PKC activators potentiate forskolin-induced cAMP formation. In some embodiments, the PKC activator for use within the

methods and compositions of the invention is phorbol myristate acetate (PMA) or a PKC purified enzyme.

#### **Beta2-Adrenergic Receptor Agonists**

Beta2-adrenergic agonists, also known as beta2-adrenergic receptor agonists, act on beta2-adrenergic receptors.  $\beta$  adrenergic receptors are coupled to a stimulatory G protein of adenylyl cyclase. This enzyme produces the second messenger cyclic adenosine monophosphate(cAMP). beta2-adrenergic agonists therefore can increase cAMP production

Examples of beta2-adrenergic agonists include, for example, bitolterol, fenoterol, isoprenaline, levosalbutamol, orciprenaline, pirbuterol, procaterol, ritodrine, salbutamol, terbutaline, arformoterol, bambuterol, clenbuterol, formoterol, salmeterol, indacaterol, olodaterol, vilanterol, vilanterol with umecclidinium bromide, vilanterol with fluticasone furoate, zilpaterol.

#### **Adenylate Cyclase Toxin**

Adenylate cyclase toxin represents another type of cAMP elevator for use in the methods and compositions of the present invention. Adenylate cyclase toxin is a single polypeptide A/B-type bacterial toxin that has the ability to interact with target cells, insert into the cytoplasmic membrane, and deliver its adenylate cyclase enzymatic domain to the cell interior [84-85]. Once entry has occurred, the enzymatic activity of the toxin produces cAMP from host cell ATP [86]. Accordingly, a further cAMP elevator that can be used in the methods and compositions of the present invention is adenylate cyclase toxin.

#### **Methods of Treating GSD and Conditions with Accumulation of Glycogen**

The terms, "treat" and "treatment," as used herein, refer to the application or administration of one or more cAMP elevators or agents that mimic cAMP to an individual having one of the GSDs or other conditions where there is a build of glycogen. The terms, "treat" and "treatment," as used herein, also refer to amelioration of one or more symptoms associated with the diseases, prevention or delay of the onset of one or more symptoms of the diseases, and/or lessening of the severity or frequency of one or more symptoms of the diseases. For example, treatment can refer to improvement of liver (e.g., improvement of liver enzymes, prevention of the progressive

fibrosis, reduction of liver size, and stabilization of the disease); improvement of muscle function (e.g., prevention of progressive myopathy, increase in muscle strength, increase in function and activities of daily living); improvement of cardiac status (e.g., prevention of ventricular hypertrophy, cardiomyopathy, and rhythm disturbances); improvement in neurodevelopment and/or motor skills (e.g., increase in AIMS score and functional measures such as 6MWT); reduction of glycogen levels in tissue of the individual affected by the diseases; or any combination of these effects. The terms, "improve," "increase" or "reduce," as used herein, 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 type/form and stage of GSD 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). A value relative to baseline can improve or increase by about 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% or more. A value relative to baseline can improve or decrease by about 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% or more.

The individual being treated can be an individual (fetus, child, adolescent, or adult human) having a certain type of GSDs (i.e., type I, II, III, IV, V, VI, VII, IX, XI, XII, XIII, XIV, Lafora disease, Fanconi-Bickel disease, Danon disease, PRKAG2 cardiac syndrome, etc.). An individual can also have a condition where there is a secondary build-up of glycogen. Such conditions include, for example, Niemann-Pick Disease [87]. Niemann-Pick Disease encompasses a group of lysosomal storage diseases that affect metabolism and are caused by genetic mutations. The most common forms of Niemann-Pick Types A and B (ASMD or Acid Sphingomyelinase Deficiency) and Niemann-Pick Disease Type C (NPC). Other conditions with a secondary buildup of glycogen include, for example, GSD X (phosphoglycerate mutase deficiency; increased glycogen in muscle); phosphoglycerate kinase deficiency (increased glycogen in muscle); RBCK1 deficiency (polyglucosan

body myopathy caused by deficiency of ubiquitin ligase RBCK1; polyglucosan body); and GSD XV (Glycogenin-1 deficiency; polyglucosan body).

The individual can have residual (partial loss) enzyme activity, or no measurable (complete loss) enzyme activity. The term "subject" refers to any  
5 organism to which the presently disclosed treatment methods and pharmaceutical compositions can be administered. In specific embodiments, a subject is a mammal. In other embodiments, a subject is a primate, a human, a domestic animal, or an agricultural animal. A subject can include a human subject for medical purposes, such as treatment of a condition or  
10 disease, or an animal subject for medical, veterinary purposes, or developmental purposes. Suitable animal subjects include mammals and avians. The term "avian" as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys, and pheasants. The term "mammal" as used herein includes, but is not limited to, primates, e.g., humans, monkeys,  
15 apes, and the like; bovines, e.g., cattle, oxen, and the like; ovines, e.g., sheep and the like; caprines, e.g., goats and the like; porcines, e.g., pigs, hogs, and the like; equines, e.g., horses, donkeys, zebras, and the like; felines, including wild and domestic cats; canines, including dogs; lagomorphs, including rabbits, hares, and the like; and rodents, including mice, rats, and the like.  
20 Thus, in one embodiment the subject is a mammal such as a domestic cat or dog. In another embodiment the subject is a human. The term subject and patient are used interchangeably herein.

The therapeutically effective amount of a composition or medicament can be administered at regular intervals, depending on the nature and extent  
25 of the disease's effects, and on an ongoing basis. Administration at a "regular interval," as used herein, indicates that the therapeutically effective amount is administered periodically (as distinguished from a one-time dose). The interval can be determined by standard clinical techniques. A cyclic AMP elevator can be administered monthly, every two weeks, weekly, twice  
30 weekly, daily, twice daily, three times daily or more. The administration interval for a single individual need not be a fixed interval, but can be varied over time, depending on the needs of the individual.

#### **Pharmaceutical Compositions and Kits**

The cyclic AMP elevators can be used to make pharmaceutical compositions. Pharmaceutical compositions comprising the one or more (e.g., 1, 2, 3, 4, 5, 6, or more) of the cAMP elevators described above are provided and can include a pharmaceutically acceptable carrier. A  
5 pharmaceutically acceptable carrier is any carrier suitable for *in vivo* administration. Examples of pharmaceutically acceptable carriers suitable for use in the composition include, but are not limited to, water, buffered solutions, glucose solutions, oil-based or bacterial culture fluids. Additional components of the compositions can suitably include, for example, excipients  
10 such as stabilizers, preservatives, diluents, emulsifiers and lubricants. Examples of pharmaceutically acceptable carriers or diluents include stabilizers such as carbohydrates (e.g., sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin or casein, protein-containing agents such as bovine serum or skimmed milk and buffers (e.g., phosphate  
15 buffer). Especially when such stabilizers are added to the compositions, the composition is suitable for freeze-drying or spray-drying. The composition can also be emulsified.

Pharmaceutical compositions including two or more cAMP elevators (e.g., 2, 3, 4, 5, 6, or more) are provided herein. The two or more cAMP  
20 elevators within the pharmaceutical composition can be from the same class (or type) of cAMP elevators; in other embodiments, the two or more cAMP elevators can be from two or more classes of cAMP elevators. The two or more cAMP elevators within the pharmaceutical composition are thus selected from one or more of the following non-limiting examples of classes of  
25 cAMP elevators: adenylate cyclase activators, PDE inhibitors, Toll-like receptor ligands, calcium ionophores, beta2-adrenergic receptor agonists, protein kinase A activators, protein kinase C activators, and adenylate cyclase toxin.

Where the composition comprising two or more cAMP elevators  
30 comprises a combination of two or more adenylate cyclase activators, the adenylate cyclase activators can be selected from the group consisting of the labdane diterpenes in one embodiment. In another embodiment, at least one of the adenylate cyclase activators is a labdane diterpene, and the remaining adenylate cyclase activator(s) is (are) selected from the group consisting of a

G-protein coupled receptor agonist, a G-protein activator, the pyrazole derivative A02011-1 [48], and benzyloxybenzaldehyde and analogs thereof such as those disclosed in [49].

In another embodiment, the composition comprises two or more cAMP  
5 elevators in therapeutically effective amounts for treating a GSD, and at least one of the cAMP elevators is an adenylate cyclase activator, and at least one of the remaining cAMP elevator(s) is a PDE inhibitor. In further embodiments, where the composition comprises two or more cAMP elevators, at least one of the cAMP elevators is an adenylate cyclase activator, and at least one of the  
10 remaining cAMP elevator(s) is a Toll-like receptor ligand, a calcium ionophore, a protein kinase A activator, a protein kinase C activator, a beta2-adrenergic receptor agonists, or adenylate cyclase toxin.

One or more cAMP elevators can also be administered with one or more enzyme replacement therapies, gene therapies, chaperone therapies,  
15 or substrate reduction therapies using siRNA/shRNA, anti-sense oligonucleotides, or small molecule or peptide drugs. An enzyme replacement therapy is any therapy with the purpose of replacing or overcoming an enzyme deficiency in a subject. Protein based therapies in which one or more enzymes are provided to the subject or to cells in the subject directly is an  
20 enzyme replacement therapy. GSD II can be treated by administration of acid alpha-glucosidase. The enzyme replacement therapy can be or can include, but is not limited to, administration of acid alpha-glucosidase, glucose-6-phosphatase, glycogen debranching enzyme, glycogen branching enzyme, muscle glycogen phosphorylase, liver glycogen phosphorylase, muscle  
25 phosphofructokinase, phosphorylase kinase, glucose transporter, Aldolase A, phosphoglucomutase deficiency, LAMP-2,  $\beta$ -enolase,  $\beta$ -glucuronidase, imiglucerase, agalsidase alpha, agalsidase beta, aglucosidase alpha, laronidase, idursulphase, galsulphase, or combinations thereof. The enzyme replacement therapy and the cAMP elevator can be delivered in a single  
30 pharmaceutical composition or can be administered in separate compositions. A chaperone therapy can be added into the single pharmaceutical composition or can be administered as a separate composition.

Also included are RNA or DNA based gene therapies to restore the missing or deficient enzyme in the affected cells. Gene therapies include, for

example, administration of a therapeutic nucleic acid encoding a functional GAA (for GSD II), G6Pase (for GSD I), GBE (for GSD IV), GDE (for GSD III), or a deficient enzyme or protein in other types of GSDs. The therapeutic nucleic acid can be delivered, for example, by a viral vector, such as  
5 adenoviruses, adeno-associated viruses (AAVs), lentiviruses, herpes virus, pox virus, human foamy virus (HFV), or retroviruses, or by a nonviral delivery platform, such as naked DNA vector, lipid/polymer- or nanoparticle-based nucleic acid delivery systems, etc.

Methods of inhibiting glycogen synthase (substrate reduction therapy)  
10 include, but are not limited to an RNAi-based therapy using small interference RNA (siRNA) or short hairpin RNA (shRNA), an antisense therapy using an anti-sense oligonucleotide (ASO), or therapies using small molecules or peptides. For example, inhibition of glycogen synthesis by shRNA mediated gene silencing of glycogen synthase (GYS) and glycogenin (GYG), the two  
15 major enzymes involved in glycogen synthesis, led to a decrease in cytoplasmic and lysosomal glycogen accumulation, and a strong reduction in the lysosomal size in primary muscle cells from Pompe disease (GAA-KO) mice. Intramuscular injection of an AAV vector expressing GYS-shRNA into newborn GAA-KO mice significantly reduced muscle glycogen accumulation,  
20 demonstrating the in vivo therapeutic efficacy [37]. In other examples, Rapamycin (Sirolimus), a small molecule drug and specific inhibitor of mTOR (mammalian target of rapamycin), effectively reduced glycogen accumulation in GAA-KO (GSD II) mice and GSD III dogs through inhibition of glycogen synthase [14,38].

25 Small molecule inhibitors, such as small molecule inhibitors of glycosyltransferases can be used in treatment of type 1 Gaucher disease. Inhibitors of glucosylceramide biosynthesis (Zavesca®) can be used to treat Gaucher disease. siRNAs, shRNAs, anti-sense oligonucleotides, small molecules and peptides that target glycogen synthase are known in the art.

30 A chaperone therapy provides molecules that can assist in the folding of enzymes or proteins (e.g., enzymes and proteins provided as part of an enzyme replacement therapy or gene therapy). The chaperone therapy molecules can also help the enzymes or proteins retain their catalytic activity, prevent their recognition by quality control systems in cells that can destroy

the enzymes or proteins, and provide improved trafficking of the enzymes or proteins to their final destination. Examples of chaperone therapy include, for example, the use of 1-deoxy-galactonojirimycin (DGJ), to enhance, for example, alpha-galactosidase activity in Fabry disease. Other chaperone  
5 therapies include, for example, dimethyl sulfoxide and trimethylamine *N*-oxide, galactose, *N*-(*n*-nonyl)deoxynojirimycin, *N*-(*n*-butyl)deoxynojirimycin, deoxynojirimycin, N370S and G202R GC pharmacologic chaperones, *N*-octyl-isofagomine, *N*-octyl-2,5-dideoxy-2,5-imino-D-glucitol,  $\alpha$ -1-C-nonyl-1,5-dideoxy-1,5-imino-D-xylitol, isofagomine, adamantyl terminated *N*-alkyl  
10 isofagomines, 2,5-anhydro-2,5-imino-D-glucitol derivatives, *N*-adamantyl-4-((3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)piperidin-1-yl)-butanamide, *N*-octyl-4-epi- $\beta$ -valienamine, pyrimethamine, hexosaminidase inhibitors, CpGH89, pyrimethamine, and galactostatin bisulphite.

A cAMP elevator (or more than one cAMP elevator) and an enzyme  
15 replacement therapy or a gene therapy, or chaperone therapy, a substrate reduction therapy can take many forms. The compositions can be administered in any order, at the same time or as part of a unitary composition. The two or more compositions can be administered such that one composition is administered before the other with a difference in  
20 administration time of 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 4 days, 7 days, 2 weeks, 4 weeks or more.

The pharmaceutical compositions for treating GSD or other conditions where there is a build of glycogen in a subject in need thereof that include two or more cAMP elevators in therapeutically effective amounts for treating a  
25 GSD or other condition where there is a build of glycogen, and can include a pharmaceutically acceptable carrier. In some embodiments, this pharmaceutical composition further comprises one or more additional enzyme replacement therapies or chaperone therapies. In another embodiment, the present invention relates to a pharmaceutical composition for treating a  
30 disease or condition in a subject in need thereof that includes at least one cAMP elevator and one or more enzyme replacement therapies or chaperone therapies each of which is present in a therapeutically effective amount for treating a disease or condition in a subject in need thereof, and a pharmaceutically acceptable carrier. As used herein the term

“pharmaceutically acceptable carrier” includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

5 An effective amount or a therapeutically effective amount as used herein means the amount of a compound that, when administered to a subject for treating GSD or other conditions where there is a build of glycogen is sufficient to effect a treatment (as defined above). The therapeutically effective amount will vary depending on the compounds, formulation or composition, the disease and its severity and the age, weight, physical  
10 condition and responsiveness of the subject to be treated. One of skill in the art can determine a therapeutically effective amount.

The compositions described herein can be administered by any means known to those skilled in the art, including, but not limited to, oral, topical, intranasal, intradermal, intraperitoneal, parenteral, intravenous, intramuscular,  
15 subcutaneous, intrathecal, transcutaneous, nasopharyngeal, or transmucosal absorption. Therefore, the compounds can be formulated as an ingestible, injectable, topical or suppository formulation. The compounds can also be delivered with in a liposomal or time-release vehicle. Administration of the compounds to a subject in accordance with the invention can exhibit  
20 beneficial effects in a dose-dependent manner. Thus, within broad limits, administration of larger quantities of the compounds is expected to achieve increased beneficial biological effects than administration of a smaller amount. Moreover, efficacy is also contemplated at dosages below the level at which toxicity is seen.

25 It will be appreciated that the specific dosage administered in any given case will be adjusted in accordance with the compound or compounds being administered, the disease to be treated or inhibited, the condition of the subject, and other relevant medical factors that can modify the activity of the compound or the response of the subject, as is well known by those skilled in  
30 the art. For example, the specific dose for a particular subject depends on age, body weight, general state of health, diet, the timing and mode of administration, the rate of excretion, medicaments used in combination and the severity of the particular disorder to which the therapy is applied. Dosages for a given patient can be determined using conventional

considerations, e.g., by customary comparison of the differential activities of the compound of the invention and of a known agent, such as by means of an appropriate conventional pharmacological or prophylactic protocol.

5 The maximal dosage for a subject is the highest dosage that does not cause undesirable or intolerable side effects. The number of variables in regard to an individual prophylactic or treatment regimen is large, and a considerable range of doses is expected. The route of administration will also impact the dosage requirements. It is anticipated that dosages of the compound will reduce symptoms of the condition at least 5%, 10%, 20%, 30%,  
10 40%, 50%, 60%, 70%, 80%, 90% or 100% compared to pre-treatment symptoms or symptoms is left untreated. It is specifically contemplated that pharmaceutical preparations and compositions can palliate or alleviate symptoms of the disease without providing a cure, or, in some embodiments, can be used to cure the disease or disorder.

15 A therapeutically effective amount of a cAMP elevator or additional active compound within the methods and compositions of the present invention typically ranges from about 1 µg/kg to about 500 mg/kg, about 10 µg/kg to about 500 mg/kg, about 100 µg/kg to about 500 mg/kg, about 1 mg/kg to about 500 mg /kg, about 1 mg/kg to about 400 mg/kg, about 1 mg/kg  
20 to about 300 mg/kg, about 1 mg/kg to about 200 mg/kg, about 1 mg/kg to about 100 mg/kg, about 1 mg/kg to about 75 mg/kg, about 1 mg/kg to about 50 mg/kg, or about 1 mg/kg to about 25 mg/kg. In another embodiment, the therapeutically effective dose of a cAMP elevator or additional active compound is an amount of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg,  
25 about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 65 mg/kg, about 70 mg/kg, about 75 mg/kg, about 80 mg/kg, about 85 mg/kg, about 90 mg/kg,  
30 about 95 mg/kg, about 100 mg/kg, about 110 mg/kg, about 120 mg/kg, about 125 mg/kg, about 130 mg/kg, about 140 mg/kg, about 150 mg/kg, about 160 mg/kg, about 170 mg/kg, about 175 mg/kg, about 180 mg/kg, about 190 mg/kg, about 200 mg/kg, about 225 mg/kg, about 250 mg/kg, about 275 mg/kg, about 300 mg/kg, about 325 mg/kg, about 350 mg/kg, about 375

mg/kg, about 400 mg/kg, about 425 mg/kg, about 450 mg/kg, about 475 mg/kg, to about 500 mg/kg. For particular agents with greater toxicity profiles, one of skill in the art will appreciate that the therapeutically effective amount can be even lower, for example from about 1 ng/kg to about 1 mg/kg, about  
5 50 ng/kg to about 1 mg/kg, about 100 ng/kg to about 1 mg/kg, about 500 ng/kg to about 1 mg/kg, about 1 µg/kg to about 1 mg/kg, about 50 µg/kg to about 1 mg/kg, about 100 µg/kg to about 1 mg/kg, or about 500 µg/kg to about 1 mg/kg. In such embodiments, the therapeutically effective dose of a cAMP elevator or additional active compound is an amount of about 1 ng/kg,  
10 about 5 ng/kg, about 10 ng/kg, about 20 ng/kg, about 30 ng/kg, about 40 ng/kg, about 50 ng/kg, about 100 ng/kg, about 200 ng/kg, about 300 ng/kg, about 400 ng/kg, about 500 ng/kg, about 600 ng/kg, about 700 ng/kg, about 800 ng/kg, about 900 ng/kg, about 1 µg/kg, about 5 µg/kg, about 10 µg/kg, about 20 µg/kg, about 30 µg/kg, about 40 µg/kg, about 50 µg/kg, about 100  
15 µg/kg, about 200 µg/kg, about 300 µg/kg, about 400 µg/kg, about 500 µg/kg, about 600 µg/kg, about 700 µg/kg, about 800 µg/kg, about 900 µg/kg, about 1 mg/kg, and other such values between about 1 ng/kg and about 1 mg/kg.

Kits comprising one or more cyclic AMP elevators (e.g., about 1, 2, 3, 4, 5, 6, or more), one or more enzyme replacement therapies or gene  
20 therapies (e.g., 1, 2, 3, 4, 5, 6, or more) and instructions for administering the cyclic AMP elevator and the enzyme replacement therapy to a subject with a glycogen storage disease or condition with a buildup of glycogen are also provided. The kits can additionally include one or more chaperone therapies (e.g., 1, 2, 3, 4, 5, 6, or more). The enzyme replacement therapy included in  
25 these kits replaces an enzyme deficient in the glycogen storage disease.

Kits comprising at least one cyclic AMP elevator (e.g., about 1, 2, 3, 4, 5, 6, or more), one or more substrate reduction therapies (e.g., about 1, 2, 3, 4, 5, 6, or more), and instructions for administering the cyclic AMP elevator and the substrate reduction therapy to a subject with a glycogen storage  
30 disease or condition with a buildup of glycogen are also provided.

The present disclosure is not limited to the specific details of construction, arrangement of components, or method steps set forth herein. The compositions and methods disclosed herein are capable of being made, practiced, used, carried out and/or formed in various ways that will be

apparent to one of skill in the art in light of the disclosure that follows. The phraseology and terminology used herein is for the purpose of description only and should not be regarded as limiting to the scope of the claims. Ordinal indicators, such as first, second, and third, as used in the description and the claims to refer to various structures or method steps, are not meant to be construed to indicate any specific structures or steps, or any particular order or configuration to such structures or steps. As used herein, the singular forms "a," "an", and "the" include plural referents unless the context clearly dictates otherwise. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to facilitate the disclosure and does not imply any limitation on the scope of the disclosure unless otherwise claimed. No language in the specification, and no structures shown in the drawings, should be construed as indicating that any non-claimed element is essential to the practice of the disclosed subject matter. The use herein of the terms "including," "comprising," or "having," and variations thereof, is meant to encompass the elements listed thereafter and equivalents thereof, as well as additional elements. Embodiments recited as "including," "comprising," or "having" certain elements are also contemplated as "consisting essentially of" and "consisting of" those certain elements.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure. Use of the word "about" to describe a particular recited amount or range of amounts is meant to indicate that values very near to the recited amount are included in that amount, such as values that could or naturally would be accounted for due to

manufacturing tolerances, instrument and human error in forming measurements, and the like. The term "about" in association with a numerical value can mean that the value varies up or down by 5%. For example, for a value of about 100, means 95 to 105 (or any value between 95 and 105).

5           No admission is made that any reference, including any non-patent or patent document cited in this specification, constitutes prior art. In particular, it will be understood that, unless otherwise stated, reference to any document herein does not constitute an admission that any of these documents forms part of the common general knowledge in the art in the United States or in any  
10   other country. Any discussion of the references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinence of any of the documents cited herein. All references cited herein are fully incorporated by reference, unless explicitly indicated otherwise. The present disclosure shall control in the event there are any disparities between  
15   any definitions and/or description found in the cited references.

The following examples are meant only to be illustrative and are not meant as limitations on the scope of the invention or of the appended claims.

## EXAMPLES

### 20   **Example 1: Forskolin treatment for GSD III**

Primary muscle cells isolated from skeletal muscle biopsy of a patient with GSD IIIa were used to evaluate the *in vitro* efficacy of Forskolin treatment for GSD III [39]. Myoblasts were isolated from skeletal muscle biopsy of a patient with GSD IIIa and maintained in high-serum growth medium as  
25   described, and differentiation of myoblasts into mature myotubes was induced in low-serum differentiation medium [39]. Forskolin (Sigma), dissolved in DMSO at a concentration of 1 mM, was added to the culture medium at a final concentration of 1  $\mu$ M (low-dose) or 10  $\mu$ M (high-dose). After 18h, cells were washed three times with cold phosphate buffered saline and then collected  
30   with a scraper. Glycogen content was determined in the cell lysates as previously described [39,40].

Forskolin significantly reduced glycogen levels by 9% at low-dose and 32% at high-dose in the myotubes derived from a GSD IIIa patient (Figure 2). This result indicates that Forskolin was capable of reducing glycogen in

myotubes from a patient with GSD IIIa. The results also indicate that a higher dose of Forskolin treatment can further reduce glycogen in GSD III.

**Example 2: Forskolin treatment for GSD IV.**

Fibroblasts from a patient with GSD IV were used to evaluate the *in vitro* efficacy of Forskolin treatment. Fibroblast cells were grown to confluency in DMEM containing 10% FBS [41]. Forskolin was added to the culture medium at a final concentration of 1  $\mu$ M (low-dose) or 10  $\mu$ M (high-dose). After 18 h, the cells were washed three times with cold phosphate buffered saline and then collected with a scraper. Glycogen content was determined in the cell lysates.

Forskolin significantly reduced glycogen levels by 28% at low-dose and 15% at high-dose in the GSD IV patient fibroblasts (Figure 3). This result indicates that lower doses of Forskolin treatment might be favored for reducing glycogen accumulation in GSD IV.

**Example 3: *In vitro* screening of cAMP elevator drugs**

Administration of a cAMP activator drug will induce glycogen degradation and inhibit glycogen synthesis via PKA activation, and hence reduce cytoplasmic glycogen accumulation in the affected tissues of, for example, GSD III and IV patients. PDE inhibitor drugs can be evaluated for treatment of GSD IV and GSD III using cellular and animal disease models.

Mouse C2C12 or rat L6 muscle cells and human HepG2 or mouse AML12 liver cells can be used as *in vitro* platforms for screening cAMP elevator by assessing cAMP changes in muscle and liver, respectively. A broad concentration curve for each compound can be determined and cAMP levels can rapidly be quantified in the cells or media (or both) at different time points.

For example, candidate PDE inhibitor drugs (i.e., PDE4 inhibitors Crisaborole, E6005 (RVT 501), roflumilast (Daliresp), apremilast (Otezla), etc; PDE3 inhibitors Cilostazol, Pletal (cilostazol), Perfan I.V. (enoximone), Primacor (minnone lactate) etc.; PDE7 inhibitors BRL50481, IC242, ABS16165, etc.) can be screened *in vitro* using C2C12 or HepG2 cells seeded in 12-well plates.

To determine the ability of PDE inhibitors to elevate cAMP in cultured cells, up to 5 doses (from low to high) of each compound can be tested in

C2C12 myoblast cells (for screening of PDE4 and PDE7 inhibitors) or HepG2 liver cells (for screening of PDE3 and PDE4 inhibitors). For each treatment (n=3 wells), both media and cells can be collected at different time points (i.e. 30 min, 2 h, 6 hr, 12 hr, and 24 hr) to quantify cAMP levels using standard colorimetric cAMP kits. Forskolin can be used as positive control. Data from the experiment can be used to determine the most effective dose and responsive time of each compound. Based upon the ability of each candidate drug to induce cAMP elevation, several compounds from each class of PDE4, PDE3, and PDE7 inhibitors can be selected for further evaluation.

10 **Example 4: Examination of the ability of PDE4 inhibitors to elevate cAMP and reduce glycogen storage in primary GSD patient fibroblast cells**

It has been reported that PDE4s, but not PDE3 and PDE7, are the dominant isoforms in human fibroblasts [42]. Primary fibroblast cells derived from patients with GSD IV, GSD III, and other GSDs can be tested.

For each selected PDE4 inhibitor, the most effective dose to elevate cellular cAMP, as determined in Example 3, can be used for treatment of GSD IV, GSD III, or other types of GSD patient fibroblasts. This experiment can include 3 groups (n= 3 dishes per group):

Group 1. Untreated group – no treatment (negative control);  
Group 2. Forskolin treatment group – add 1  $\mu$ M Forskolin (positive control);  
Group 3. PDE4 inhibitor at the most effective dose.

GSD patient fibroblast cells can be grown to confluency in DMEM containing 10% FBS. The tested drug can be added to culture medium at the indicated concentration for each group. After 12, 24, or 48 hr, cells can be washed three times with cold PBS buffer and collected with a scraper. Glycogen content and cAMP level can be assayed in cell lysate.

Additionally, primary GSD IIIa patient myoblast cells can be used to test the ability of PDE4 inhibitor or PDE7 inhibitor (or both) to reduce glycogen content.

**Example 5: Efficacy of PDE4 inhibitor treatment in GSD IV mice**

*In vivo* experiments can be conducted in a GSD animal model. Mouse models are available for several GSDs including GSD I, II, III, and IV to those

of skill in the art. 3 groups (n=8 mice each group) can be used for testing each PDE4 inhibitor drug in GSD III or GSD IV mice:

- Group 1. No treatment group—untreated control
- Group 2. Forskolin treatment group – positive control;
- 5 Group 3. PDE4 inhibitor treatment group - human equivalent dose.

Treatment can start at the age of about 2 months. All mice can be euthanized at age 5 months following overnight fasting, to collect urine, blood, and tissues. Dosage regimen and administration route will be determined based on human use of each drug. Glycogen content can be analyzed in different tissues including liver, heart, skeletal muscles, brain, and diaphragm, as described in, for example, [40,43]. Tissue histology can be analyzed in a pathology laboratory. Urine can be used for testing urinary Hex4, a biomarker for several GSDs, by stable isotope-dilution electrospray tandem mass spectrometry as previously described in [44]. Blood chemistry including AST, ALT, ALP, CPK, GLU, etc. can be analyzed [45]. Behavioral and muscle function can be tested at about ages 2, 3.5, and 5 months, to assess reversal of neuromuscular involvement by treadmill, Rota-rod, wire-hang tests as described in, for example, [43,46] and.

20 Combined therapy with inhibitors of PDE4 + PDE3 (for liver) or PDE4 + PDE7 (for muscle) can also be tested in GSD IV or GSD III mice.

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## CLAIMS

We claim:

1. A method of treating a glycogen storage disease or a condition with a buildup of glycogen in a subject in need of such treatment comprising administering a therapeutically effective amount of a cyclic AMP  
 5 elevator to the subject in need of treatment for a glycogen storage disease.
2. The method of claim 1, wherein the glycogen storage disease or a condition with a buildup of glycogen is selected from the group  
 10 consisting of GSD I, GSD II, GSD III, GSD IV, GSD V, GSD VI, GSD VII, GSD IX, GSD XI, GSD XII, GSD XIII, GSD XIV, Fanconi-Bickel disease, Danon disease, Lafora disease, PRKAG2 cardiac syndrome, Niemann-Pick Disease, GSD X, phosphoglycerate kinase deficiency, RBC1 deficiency, and GSD XV, and other conditions with a  
 15 secondary buildup of glycogen.
3. The method of claim 1 or 2, wherein the cyclic AMP elevator is an adenylate cyclase activator, a phosphodiesterase (PDE) inhibitor, a Toll-like receptor ligand, a calcium ionophore, a protein kinase A (PKA) activator, a protein kinase C (PKC) activator, a beta2-adrenergic  
 20 receptor agonist, an adenylate cyclase toxin, or combinations thereof.
4. The method of claim 3, wherein the adenylate cyclase activator is a labdane diterpene, a G-protein coupled receptor agonist, a G-protein activator, the pyrazole derivative A02011-1, benzyloxybenzaldehyde and analogs thereof, or combinations thereof.
- 25 5. The method of claim 4, wherein the labdane diterpene is labdane, forskolin, a forskolin derivative, 6-acetyl-7-deacetyl-forskolin, 7-deacetyl-forskolin, 7-deacetyl-6-(N-acetylglycyl)-forskolin, 7-deacetyl-7-O-hemisuccinyl-forskolin, 7-deacetyl-7-(O-N-methylpiperazino)-γ-butryl-dihydrochloride-forskolin, 7-HPP-forskolin, 6-HPP-forskolin,  
 30 colforsin daropate hydrochloride (NKH477), or combinations thereof.
6. The method of claim 4, wherein the G-protein coupled receptor agonist is catecholamine, dopamine, dobutamine, isoproterenol, adenosine, carbacyclin, endothelin, epinephrine, glucagon, octopamine, pituitary

adenylate cyclase-activating peptide (PACAP), parathyroid hormone, prostaglandin, vasopressin, or combinations thereof.

7. The method of claim 4, wherein the G-protein activator is cholera toxin or a subunit thereof.
- 5 8. The method of claim 3, wherein the PDE inhibitor is a PDE3, PDE4, PDE7, or PDE8 inhibitor.
9. The method of claim 3, wherein the Toll-like receptor ligand is lipopolysaccharide (LPS), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (pLPC), lipoteichoic acid (LTA), flagellin, or combinations thereof.
- 10 10. The method of claim 3, wherein the calcium ionophore is an ionomycin calcium salt, A23187, or combinations thereof.
11. The method of claim 3, wherein the PKA activator is 6-Bnz-cAMP, 8-CPT-2'-O-Me-cAMP, 8-CPT-cAMP, 8-Bromo-cAMP, Dibutyryl-cAMP, 15 Diocanoyl-cAMP, Sp-8-Br-cAMPS, Sp-cAMPS, cAMP, a PKA subunit, or combinations thereof.
12. The method of claim 3, wherein the PKC activator is phorbol myristate acetate (PMA), a PKC purified enzyme, or combinations thereof.
13. The method of claim 3, wherein the beta2-adrenergic receptor agonist 20 is bitolterol, fenoterol, isoprenaline, levosalbutamol, orciprenaline, pirbuterol, procaterol, ritodrine, salbutamol, terbutaline, arformoterol, bambuterol, clenbuterol, formoterol, salmeterol, indacaterol, olodaterol, vilanterol, vilanterol with umeclidinium bromide, vilanterol with fluticasone furoate, zilpaterol, or combinations thereof.
- 25 14. The method of any one of the preceding claims, further comprising administering a therapeutically effective amount of an enzyme replacement therapy, a gene therapy, a chaperone therapy, a substrate reduction therapy, or combinations thereof to the subject with a glycogen storage disease or a condition with a buildup of glycogen.
- 30 15. The method of any one of the preceding claims, wherein two or more cyclic AMP elevators are administered to the subject.
16. The method of any one of the preceding claims, wherein the cyclic AMP elevator is administered by a route selected from oral, parenteral, intramuscular, intravenous, intraperitoneal and subcutaneous.

17. A pharmaceutical composition comprising at least one cyclic AMP elevator and an enzyme replacement therapy, a gene therapy, a substrate reduction therapy, a chaperone therapy, or combinations thereof.
- 5 18. The pharmaceutical composition of claim 17, wherein the enzyme replacement therapy or gene therapy is selected from the group consisting of acid alpha-glucosidase, glucose-6-phosphatase, glycogen debranching enzyme, glycogen branching enzyme, muscle glycogen phosphorylase, liver glycogen phosphorylase, muscle phosphofructokinase, phosphorylase kinase, glucose transporter, Aldolase A, phosphoglucomutase deficiency, Laforin or Malin, LAMP-2, and  $\beta$ -enolase.
- 10 19. The pharmaceutical composition of claim 17, wherein the substrate reduction therapy is selected from the group consisting of methods of inhibiting glycogen synthase to reduce glycogen accumulation, siRNA-based therapies, shRNA-based therapies, antisense therapies, and therapies using small molecule or peptide drugs.
- 15 20. The pharmaceutical composition of claim 17, wherein the cyclic AMP elevator is selected from the group consisting of an adenylate cyclase activator, a phosphodiesterase (PDE) inhibitor, a Toll-like receptor ligand, a calcium ionophore, a protein kinase A activator, a protein kinase C activator, a beta2-adrenergic receptor agonist, an adenylate cyclase toxin and combinations thereof.
- 20 21. A kit comprising at least one cyclic AMP elevator, an enzyme replacement therapy or gene therapy and instructions for administering the cyclic AMP elevator and the enzyme replacement therapy or gene therapy to a subject with a glycogen storage disease or a condition with a buildup of glycogen, wherein the enzyme replacement therapy or gene therapy replaces an enzyme or gene encoding the enzyme
- 25 22. The kit of claim 21, wherein the enzyme or the gene encoding the enzyme is selected from the group consisting of acid alpha-glucosidase, glucose-6-phosphatase, glycogen debranching enzyme,
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glycogen branching enzyme, muscle glycogen phosphorylase, liver glycogen phosphorylase, muscle phosphofructokinase, phosphorylase kinase, glucose transporter, Aldolase A, phosphoglucomutase deficiency, Laforin or Malin, LAMP-2, and  $\beta$ -enolase.

- 5 23. A kit comprising at least one cyclic AMP elevator, one or more substrate reduction therapies, and instructions for administering the cyclic AMP elevator and the substrate reduction therapy to a subject with a glycogen storage disease or a condition with a buildup of glycogen, wherein the substrate reduction therapy reduces glycogen  
10 synthase activity and prevents glycogen accumulation in the glycogen storage disease or the condition with a buildup of glycogen.
24. The kit of claim 23, wherein the substrate reduction therapy is selected from the group consisting of methods of inhibiting glycogen synthase to reduce glycogen accumulation, siRNA-based therapies, shRNA  
15 therapies, antisense therapies, or therapies using small molecule or peptide drugs.
25. The kit of claim 21 or 23, wherein the cyclic AMP elevator is selected from the group consisting of an adenylate cyclase activator, a phosphodiesterase (PDE) inhibitor, a Toll-like receptor ligand, a  
20 calcium ionophore, a protein kinase A activator, a protein kinase C activator, a beta2-adrenergic receptor agonist, an adenylate cyclase toxin and combinations thereof.
26. The kit of claim 21, further comprising one or more chaperone therapies.

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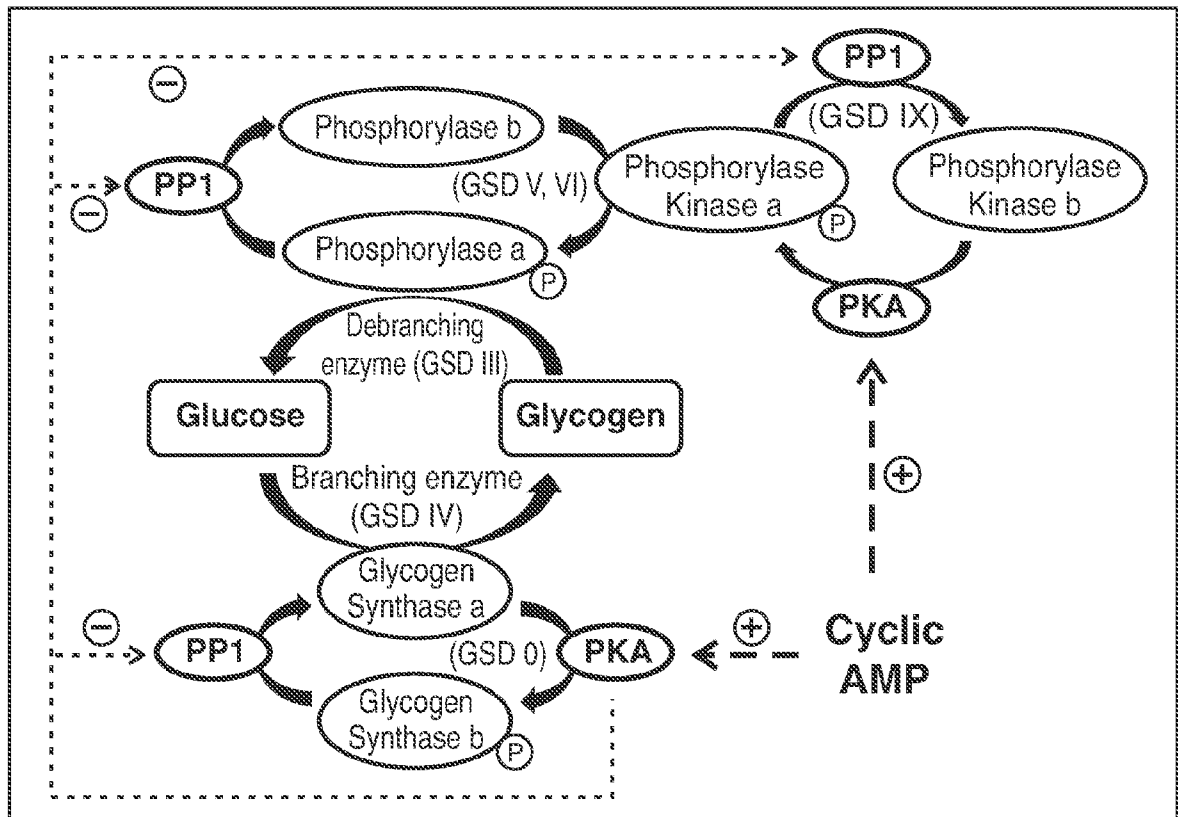
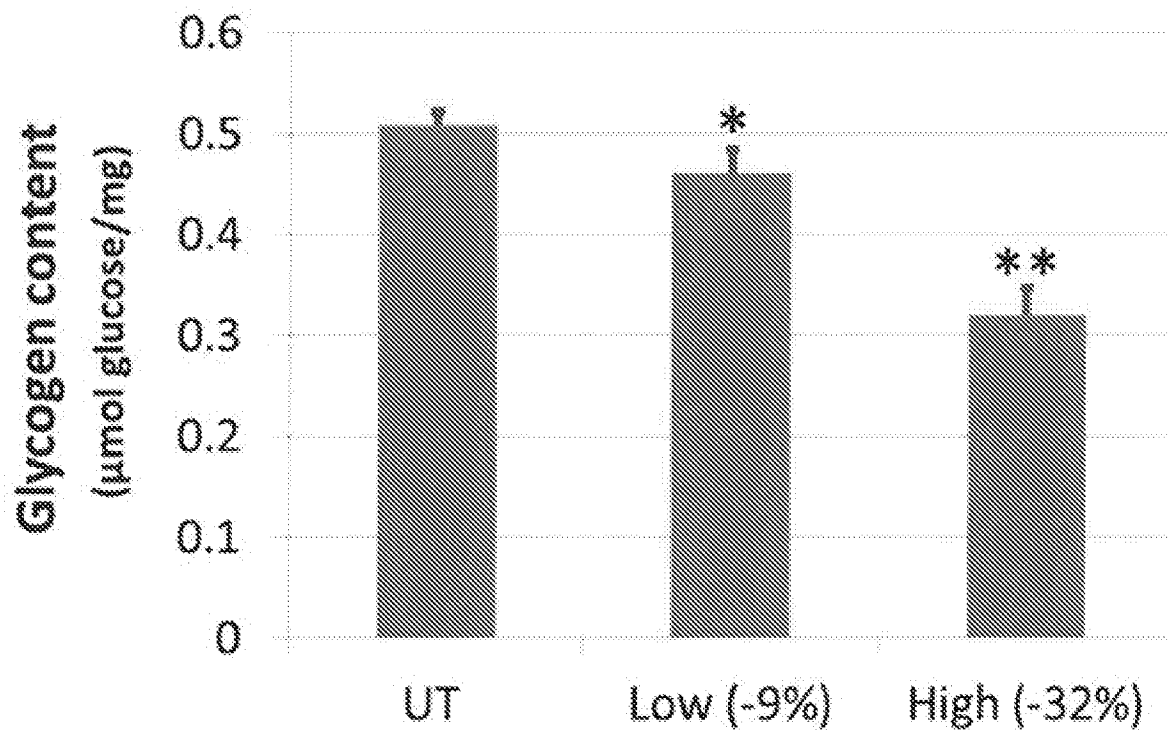
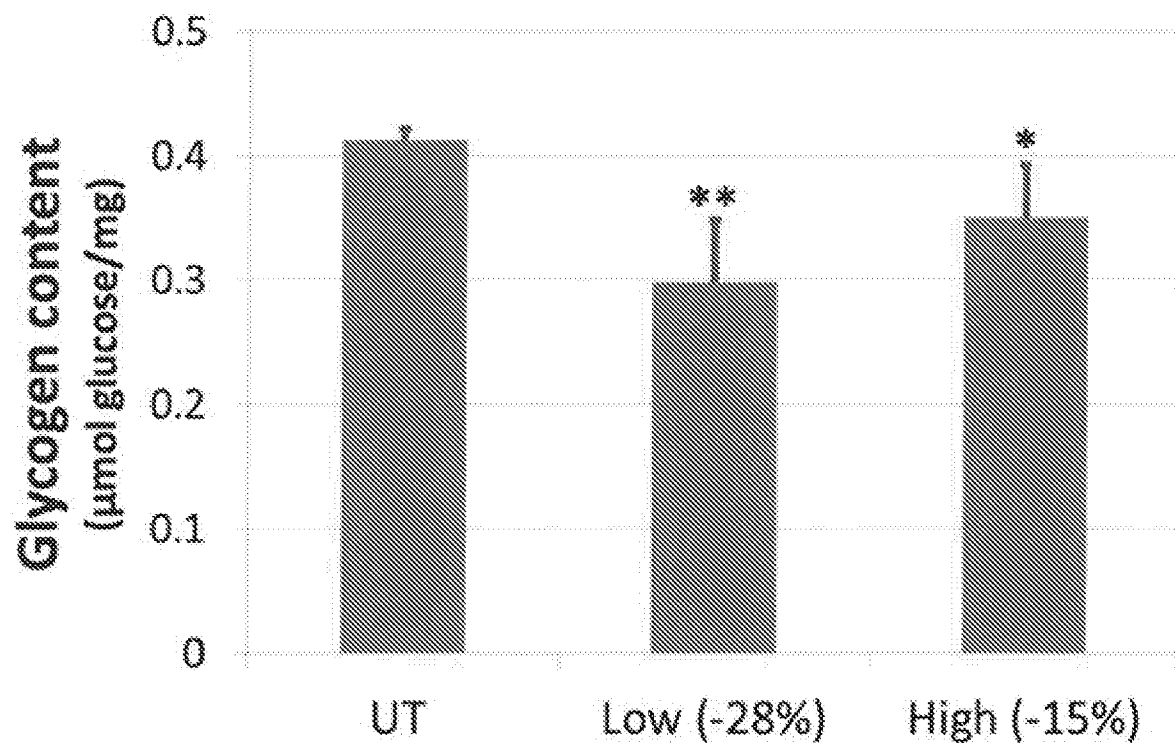


Fig. 1. Cyclic AMP stimulates glycogen breakdown and inhibits glycogen synthesis through activation of cAMP-dependent protein kinase A (PKA). (adapted from Cell Signalling Biology. Berridge, M.J., 2014)



**Figure 2.** Forskolin reduced glycogen content in primary muscle cells from patient with GSD IIIa. UT, untreated; Low, 1 μM Forskolin; High, 10 μM Forskolin. \*,  $p < 0.05$  vs UT; \*\*,  $p < 0.001$  vs UT.



**Figure 3.** Glycogen content was decreased in GSD IV patient fibroblasts by the Forskolin treatment. UT, untreated; Low, 1 μM Forskolin; High, 10 μM Forskolin. \*,  $p<0.05$  vs UT; \*\*,  $p<0.001$  vs UT.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/25215

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/137, 38/47, 48/00; C07H 19/20 (2016.01)

CPC - A61K 31/137, 31/155, 38/47; C07H 19/20

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (8) - A61K 31/137, 31/355, 38/11, 38/47, 45/06, 48/00; C07H 19/20, 19/167; A61P 3/00, 9/00, 21/00 (2016.01);

CPC - A61K 31/137, 31/155, 38/11, 38/47, 45/06, 48/00; C07H 19/20, 19/167; C07K 7/00; USPC - 424/94.1; 514/54, 60, 893

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

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

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH), EBSCO, Google/Google Scholar, PubMed; glycogen storage disease, glycogen, cyclic AMP, GSD1, GSDII, GSDIV, GSDX, Fanconi-Bickel disease, Niemann-Pick Disease, adenylate cycloase activator, PDE inhibitor, calcium inophore, protein, kinase A, beta2-adrenergic

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*     | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                  | Relevant to claim No.                                                                                                                                   |
|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| X<br>---<br>Y | WO 2014/130723 A1 (VALERION THERAPEUTICS, LLC) 28 August 2014; page 2, line 19; page 4, lines 7-10; page 50, lines 22-28; page 65, lines 21-25; page 66, lines 8-15; page 67, lines 26-30; page 68, lines 5-18; page 69, lines 1-12, 19-22; page 70, lines 17-20; page 76, lines 11-18; page 84, lines 1-6, 14-17; claim 96         | 1-2, 3/1-2, 13/3/1-2, 17, 19-24, 25/21, 25/23, 26<br>---<br>4/3/1-2, 5/4/3/1-2, 6/5/4/3/1-2, 7/4/3/1-2, 8/3/1-2, 9/3/1-2, 10/3/1-2, 11/3/1-2, 12/3/1-2, |
| X             | US 8,679,478 B2 (KOEBERL, DD) 25 March 2014; column 2, lines 53-57; column 3, lines 19-22, 54-59; column 6, lines 6, 66-67; column 7, lines 1-2; column 8, lines 17-20, 41-47, 61-67; column 9, lines 60-65; column 10 lines 5-12, 38-50, 65-67; column 12, lines 17-22; claim 8                                                    | 1-2, 3/1-2, 13/3/1-2, 17-18, 20                                                                                                                         |
| X             | (KOEBERL, DD et al.) Enhanced Efficacy of Enzyme Replacement Therapy in Pompe Disease Through Mannose-6-Phosphate Receptor Expression in Skeletal Muscle. Molecular Genetics and Metabolism. 2011. vol. 103, no. 2; pages 107-112 (pages 1-15); page 2, first paragraph; page 4, fourth paragraph; page 5, second-fourth paragraphs | 1-2, 3/1-2, 13/3/1-2                                                                                                                                    |
| Y             | WO 2008/157205 A2 (DUKE UNIVERSITY) 24 December 2008; page 2, lines 9-11; page 7, lines 5-13; page 8, lines 8-15; page 35, lines 23-25; page 36, lines 8-10; page 38, lines 17-19; page 46, lines 2-5; claims 7-11, 14, 16, 18                                                                                                      | 4/3/1-2, 5/4/3/1-2, 6/5/4/3/1-2, 7/4/3/1-2, 8/3/1-2, 9/3/1-2, 10/3/1-2, 11/3/1-2, 12/3/1-2                                                              |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 May 2016 (22.05.2016)

Date of mailing of the international search report

24 JUN 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

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Authorized officer

Shane Thomas

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PCT OSP: 571-272-7774

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/25215

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

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

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 14-16  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.