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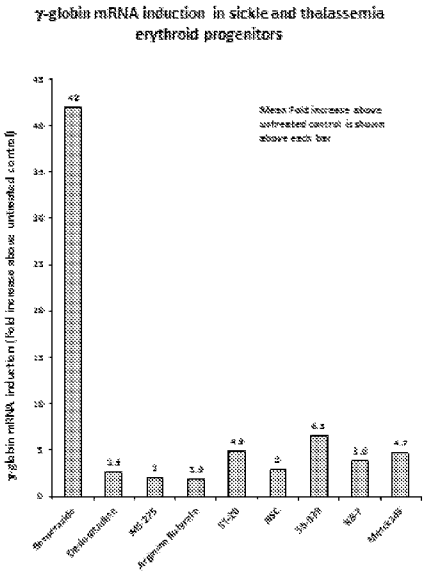

FIGURE 2

(57) Abstract: The present invention generally relates to compositions containing chemical compounds and compositions and pharmaceutical formulations of the compounds which increase total hemoglobin or globin protein such as embryonic or fetal globin, or the proliferation of hemoglobin expressing cells. Preferred compounds include benserazide, a benzamide histone deacetylase inhibitor such as entinostat, or one or more of ambroxol, desloratadine, resveratrol or NSC-95397. These compositions can be used to treat or prevent the symptoms associated with sickle cell diseases, beta thalassemias and other blood cell deficiencies and blood disorders. The invention also relates to methods for administering these compositions to subjects and for use as medical aids for the treatment and prevention of anemias caused by globin gene mutations and other red blood cell disorders.

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CHEMICAL INDUCERS OF FETAL HEMOGLOBIN

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Reference to Related Applications

This application claims priority to U.S. Provisional Application No. 61/867,965 entitled "*Chemical Inducers of Fetal Hemoglobin (HbF) for Treatment of Sickle Cell Disease and Beta-Thalassemias*" filed August 20, 2013, the entirety of which is specifically incorporated by reference.

Background

1. Field of the Invention

The present invention is directed to methods and compositions for the treatment of the beta-globin blood disorders sickle cell disease and beta-thalassemia and, in particular, compositions and methods comprising ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, and/or MS-275 (entinostat).

2. Background of the Invention

Inherited disorders of production of the beta-chain of adult hemoglobin A (beta-thalassemia) or mutations affecting the structure of the beta-globin chain (sickle cell disease) are the most common genetic diseases in the world, afflicting millions of individuals worldwide, and are designated by WHO as a global health burden. Disorders of hemoglobin synthesis include deficiencies of globin synthesis such as thalassemia syndromes and structural abnormalities of globin such as sickle cell syndromes and syndromes associated with unstable hemoglobins.

The beta hemoglobin disorders are characterized by hemolytic anemia, shortened red blood cell lifespan, which reduces oxygen transport throughout the body and multi-organ damage. Pharmacological augmentation of fetal hemoglobin (beta-globin chain) production, to replace the defective or missing beta-globin chains, is a promising therapeutic approach.

Fetal globin (also known as gamma globin) normally combines with alpha globin chains prenatally to form fetal hemoglobin (HbF). Fetal globin is replaced by beta globin after birth, which then combines with alpha globin to form adult hemoglobin A. Fetal globin performs the same function as beta globin, and can combine with the alpha chains to generate a healthy form of hemoglobin thereby reducing high concentrations of unmatched alpha globin chains.

The various types of beta-thalassemias are syndromes resulting from mutations which produce a deficiency of globin chains. In beta-thalassemia, the unmatched alpha globin chains aggregate inside red blood cells (RBCs) and their progenitors, causing the premature destruction of RBCs and RBC progenitors, which results in anemia, transfusion-dependence, iron overload, organ failure, and early death.

In sickle cell disease (SCD), one amino acid substitution in the beta globin chain results in the generation of sickling hemoglobin (HbS), which allows polymerization with repeated cycles of deoxygenation. Polymerization results in "sickling" of RBCs. The sickled RBCs undergo hemolysis, while adhesive sickled RBCs occlude the microcirculation, provoking widespread tissue ischemia and organ infarction. The natural history of SCD is marked by painful crises and acute chest syndrome, and eventual potentially life-threatening sequelae, including renal insufficiency, retinitis, osteonecrosis, osteomyelitis, aplastic crises, functional asplenism, stroke, priapism, and severe pulmonary hypertension.

Many efforts to stimulate HbF production have accordingly been undertaken, but pharmacologic reactivation of high-level HbF expression with non-toxic and tolerable therapeutic agents that are orally available (for worldwide therapeutic application) has been an elusive therapeutic goal for many years. In sickle cell disease, average HbF levels in adult patients are 5-7%; but levels of HbF greater than 15-20% in 70% or more of red blood cells expressing HbF (F-cells) are typically required to ameliorate most of the clinical complications. One HbF stimulant therapeutic, hydroxyurea (HU), is FDA-approved for treatment of sickle cell disease and benefits approximately 40% of subjects, with most benefit occurring those who attain absolute HbF levels greater than 0.5 g/dl or 20%. HbF levels achieved are often not sufficiently high to completely ameliorate all complications. Additional therapeutics, especially non-cytotoxic agents which can be used in combinations with HU, could provide additional benefit. There are no therapeutic agents approved for the beta-thalassemia syndromes.
Fetal hemoglobin (HbF, gamma2alpha2) produced by the gamma-globin gene *(HBG),* is the major human hemoglobin *in utero,* and is replaced by beta-globin expression in infancy, even when the beta-globin genes are mutated. Fetal globin when present in even small amounts prevents sickling. In beta-thalassemia, increasing synthesis of fetal globin chains reduces the globin chain imbalance, caused by excess of alpha globin and deficiency of beta globin, and consequent anemia. Fetal globin is the major modulator of disease severity and a generally-

accepted target for drugs to ameliorate these diseases. A major unmet medical need exists for high-potency HbF inducers, particularly for patients with lower baseline levels of HbF, and for the many who do not have a robust response to HU alone. A combination chemotherapy approach is needed to increase response rates in sickle disease. Baseline HbF expression is influenced by genetic modifiers. The 3 most influential quantitative trait loci (QTLs) are responsible for 50% of variability in HbF levels. Polymorphisms in these QTLs are associated with higher baseline HbF levels. One QTL, BCL-11A, is a repressor of fetal globin synthesis. The inventions have been identified to suppress the expression of this repressor at the mRNA and protein levels. Several of these drug candidates act, in part, through suppression of repressors of fetal globin (Bcl-11A, LSD-1, HDAC-3, HDAC-2, and KLF-1).

The thalassemia syndromes are a heterogeneous group of disorders all characterized by a lack of or a decreased synthesis of the globin chains of HbA. Deficiencies of alpha-globin expression are referred to as alpha-thalassemias and deficiencies of beta-globin, beta-thalassemias. The hemolytic consequences of deficient globin chain synthesis result from decreased synthesis of one chain and also an excess of the complementary chain. Free chains tend to aggregate into insoluble inclusions within erythrocytes causing premature destruction of maturing erythrocytes and their precursors, ineffective erythropoiesis, and the hemolysis of mature red blood cells. The underlying defects of hemoglobin synthesis have been elucidated over the years and largely reside in the nucleic acid sequences which express or control the expression of alpha or beta-globin protein.

A small number of therapeutic agents of different chemical classes can induce HbF experimentally, with only a few are orally-active or currently in clinical testing. Three general classes of therapeutic agents have been shown to induce HbF significantly in subjects with sickle cell disease and beta-thalassemia, include: cytotoxic chemotherapeutic agents (such as Hydroxyurea (HU), 5-azacytidine, and decitabine), erythropoietin (EPO) preparations, and short chain fatty acids (SCFAs) and derivatives (SCFADs), which include some HDAC inhibitors. Additionally, there are a variety of small molecules have been shown to effect hemoglobin or fetal globin expression. Early experiments demonstrated that acetate (CH_3COOH), propionate (CH_3CH_2COOH), butyrate ($CH_3CH_2CH_2COOH$) and isobutyrate ($CH_3CH(CH_3)COOH$) all induced hemoglobin synthesis in cultured Friend leukemia cells (E. Takahashi et al., Gann 66:577-80, 1977).

Additional studies showed that polar compounds, such as acid amides, and fatty acids could stimulate the expression of both fetal and adult globin genes in murine erythroleukemia cells (U. Nudel et al., Proc. Natl. Acad. Sci. USA 74:1100-4, 1977). Hydroxyurea ($H_2NCONHOH$), another relatively small molecule, was found to stimulate globin expression (N.L. Letvin et al., N. Engl. J. Med. 310:869-73, 1984). Stimulation, however, did not appear to be very specific to fetal globin (S. Charache et al., Blood 69:109-16, 1987). Hydroxyurea (HU) is also a well-known carcinogen making its widespread and long term use as a pharmaceutical impractical. One of the major breakthroughs in the treatment of hemoglobinopathies was made when it was discovered that butyric acid (butanoic acid; $CH_3CH_2CH_2COOH$) accurately and specifically stimulated transcription of the human fetal (gamma) globin gene (G.A. Partington et al., EMBO J. 3:2787-92, 1984). Some of these have shown proof-of-principle, but, except for HU, has required parenteral administration or large doses, which were not suitable for broad application.

While three SCFA agents have been reported to induce gamma-globin expression and to increase hemoglobin levels in subjects with beta-thalassemia, rendering some beta-thalassemia subjects transfusion- independent, these prior generations of SCFAs, including arginine butyrate (AB) and sodium phenylbutyrate (SPB), have limited utility as a therapeutic agent *in vivo,* as they are either rapidly metabolized, required intravenous (IV) infusions, or required large doses which were difficult for subjects to tolerate long-term. Furthermore, these first generation SCFAs are also known to inhibit erythroid cell proliferation, and therefore require titration and intermittent dosing, complicating their use in conditions of anemia where compensatory erythroid cell proliferation is desirable. There, thus remains an unmet clinical need for a therapeutic agent that induces gamma-globin gene expression and does not inhibit erythroid cell proliferation (i.e., is not cytotoxic), and which is more applicable for wide application in these genetic diseases.

Summary of the Invention

The present invention relates to agents that function as fetal globin-inducing agents, for the treatment of beta-globin disorders, such as sickle cell anemia and beta-thalassemias, with higher efficacy than prior generation agents in predictive in vivo models and which do not have the limitations of prior generation candidates for long term use.

Lead candidates were investigated in in vivo non-primate models. While some identified

compounds are already FDA-approved for other medical indications, their safety profiles are benign and they are not known to increase fetal-globin expression. Accordingly, subjects in need of increased fetal-globin expression can be selected and administered the compounds identified herein for treatment of beta-globin diseases, such as Sickle Cell Disease and the diverse variations of beta-thalassemia syndromes.

The disclosure herein provides novel methods and compositions for increasing the amount of fetal hemoglobin in a subject with a blood disorder, including beta-thalassemias and sickle cell disease. Fetal hemoglobin (HbF: alpha2, gamma2) is an endogenous type of hemoglobin which is present in all humans, but is normally suppressed in infancy to levels below 2%. Decades of biochemical, clinical, and epidemiologic research have shown that any increase in HbF and F-cell levels reduce the severity of sickle cell disease, or alleviate the anemia of beta-thalassemia. It is well-established that fetal globin (gamma-globin) chains interfere with the polymerization of sickle hemoglobin, preventing many pathologic consequences of sickling, and also that adequate levels of fetal hemoglobin (also referred to as hemoglobin F or HbF), correlate with mild or benign courses in sickle cell disease (SCD).

Accordingly, pharmacologic augmentation of fetal hemoglobin (HbF, gamma-globin) production replaces diminished beta-globin chains in the beta-thalassemias and inhibits HbS polymerization in sickle cell disease. Despite long-term efforts, regulatory approval has been obtained for only one chemotherapeutic agent. Pharmacologic reactivation of high-level HbF expression with non-cytotoxic, tolerable therapeutics is still an unmet medical need for this global health burden. To investigate potential therapeutic libraries for unrecognized HbF inducers, a high-throughput screening (HTS) program was developed to interrogate diverse chemical libraries, including a library of FDA-approved and clinical stage drugs. Using this assay, unexpected new and highly potent HbF-inducing drugs were identified, some of which are already in clinical use for other medical indications and have established safety profiles.

The invention overcomes the problems and disadvantages associated with current strategies and designs, and provides new compositions, methods, and aids for the treatment and prevention of blood disorders. In particular, the present invention generally relates to increasing the percentage of fetal hemoglobin (HbF or gamma-globin) in the blood of a subject without decreasing proliferation of cells, the method comprising administering to the subject a composition comprising at least one of, or any combination of HbF-inducing drugs, which

include, ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275.

In some embodiments, the composition comprises benserazide or desloratadine or MS-275, or any pharmaceutically acceptable salt, polymorph or ester thereof.

One embodiment of the invention is directed to compositions that comprise one or more compounds which stimulate the proliferation of hemoglobin-producing cells, the expression of hemoglobin or the expression of embryonic or fetal globin in mammalian cells. Chemical compounds, or HbF-inducing agents include, ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275.

Another embodiment of the invention is directed to compositions comprising at least one or any combination of HbF-inducing agents selected from the group of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide, desloratadine or MS-275 that stimulate the proliferation of hemoglobin producing and other types of cells, the expression of hemoglobin or the expression of embryonic or fetal globin in mammalian cells, but do not decrease or otherwise adversely affect cell viability. Such HbF-inducing agents include benserazide, desloratadine or MS-275. Cell viability may be assayed by DNA fragmentation assays or cell division assays, or by measuring the amount of nucleic acid or protein synthesis which occurred in treated cells as compared to untreated cells. Cells tested may be normal healthy cells, subject cells to be treated or cells in tissue culture.

Another embodiment of the invention is directed to methods for the treatment of blood disorders. Compositions containing an effective amount of one or more agents selected from, at least one or any combination of HbF-inducing agents selected from the group including, ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide, desloratadine or MS-275 which stimulate the proliferation of hemoglobin producing cells or the expression of embryonic or fetal globin from cells are administered to patients. Patients may be any mammal such as a human. Administration may be by parenteral or nonparenteral means, but is preferably oral or intravenous. Treatment may be for short periods of time, e.g., pulsed or continuous throughout the lifetime of the patient.

Another embodiment of the invention is directed to methods for the treatment of blood disorders comprising the administration of compositions containing therapeutically effective amounts of an HbF-inducing agent selected from at least one or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide,

desloratadine or MS-275 which increases the proportion or number of reticulocytes that express embryonic or fetal globin and the amount of embryonic or fetal globin expressed per cell.

Another embodiment of the invention is directed to methods for regulating the expression of a globin gene such as an embryonic or fetal globin gene or an at least partially functional pseudo-globin gene in mammalian cells. In some embodiments, treated cells, e.g., ex vivo, or products expressed from these cells, can be harvested and introduced or reintroduced to a subject to treat or prevent a blood disorder.

Another embodiment of the invention is directed to methods for regulating the proliferation of hemoglobin expressing cells. Cells in culture or in patients are exposed to compositions of HbF-inducing agents selected from the group including, at least one or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide, desloratadine or MS-275 and induced to proliferate.. Another embodiment of the invention is directed to methods for the prevention of the clinical manifestations of the hemoglobinopathy or beta thalassemic disorders . Compositions containing an effective amount of agents as disclosed herein, e.g., at least one or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide, desloratadine or MS-275 which stimulate the proliferation of hemoglobin producing cells or the expression of embryonic or fetal globin are administered to patients suspected of having a blood disorder. The subject may be any mammal such as a human and is preferably an adolescent, child, or infant. Administration may be by any route including parenteral and nonparenteral routes, but is preferably oral or intravenous. Treatment may be for short periods of time or continuous throughout the lifetime of the patient.

Other embodiments and advantages of the invention are set forth in part in the description, which follows, and in part, may be obvious from this description, or may be learned from the practice of the invention.

Description of the Drawings

Figure 1A is a schematic showing an illustration of the high throughput screening assay (HTS) with the cell construct comprising a locus control region (LCR) linked to the gamma-globin gene promoter and enhanced GFP (EGFP).

Figure 1B depicts a small panel of approved therapeutics were found to induce γ-globin expression from the library of compounds and US and EMA approved medicinal products tested.

Figure 2 shows a table of the fold increase fetal globin mRNA in erythroid progenitors in response to benserazide and MS-275 and other compounds identified in the high-throughput screen.

Figure 3 shows gamma-globin mRNA expression in the blood of baboons administered fetal hemoglobin inducing agent benserazide. Benserazide was compared to 2 other candidates (i) desloratadine (DLT), which was administered orally (0.5 mg/kg/dose), three times a week over two weeks, and (ii) MS-275, which was administered orally three times a week for two weeks at 0.2 mg/kg/dose. Doses of Benserazide are shown in the bars above the graph, with Benserazide administered at 1 mg/kg (Open squares) and 2 mg/kg (dark squares). Benserazide resulted in up to 33-fold induction of gamma-globin mRNA in vivo (**Figure 3A**) and an increase in total hemoglobin (Hb) (by 1.5g/dL) (**Figure 3B**).

Figure 4 shows the mean induction of fetal globin with Benserazide (BEN) and Desloratidine (DLT) as compared to other inducers in the Anaemic Baboon model. Benserazide induced fetal globin mRNA by a mean of 27-fold; Desloratidine (DLT) induced a 11-fold increase above baseline.

Figure 5 shows the mean increase of induction of F-Reticulocytes during and after administration of Benserazide (BEN) in the anemic baboon model.

Figure 6 shows the effect of benserazide compared to a known Hb inducer (Hydoxyurea (HU) in a transgenic mice model comprising the entire human non-alpha (gamma delta beta-globin) gene locus. Benserazide induced a mean increase in total hemoglobin (Hb) at 5 weeks (**Figure 6A**) and induced an absolute 13% mean increase in the number of F-cells (from 0.1% to 9% (mice #1), 0.4% to 18% (mice #2), and 0.13% to 12% (mice #3); and 10 to 33-fold increase in mean fluorescence intensity (MFI) (**Figure 6B**).

Figure 7. The structures of the Compounds named in this application**.**

Figure 8. Various derivative forms of MS-275 as structures I, II and III.

Figure 9 depicts a comparison of the magnitude of fetal globin mRNA induction over baseline levels in anemic baboons by new drugs compared to hydroxyurea (HU) (**Figure 9A**), and proportions of new red blood cells with fetal globin protein, F-reticulocytes, (**Figure 9B**).

Figures 10A-F depict Benserazide reduction of binding of two repressors, LSD1 and HDAC3, to the fetal globin gene promoter in erythroid progenitors from sources as indicated.

Figure 11 depicts the suppression of repressor proteins BCL11A and KLF1 in different erythroid progenitors treated with new drugs as compared with untreated control levels by Western blot analysis.

Description of the Invention

The present invention is directed to compounds, compositions and methods that increase gamma-globin expression *in vivo*, and in particular, compounds, compositions and methods for the treatment of gamma-globin disorders such as, but not limited to Sickle Cell anemia and beta-thalassemia. In particular, the present invention generally relates to increasing the percentage of fetal hemoglobin (HbF or gamma-globin) in the blood of a subject, the method comprising administering to the subject a composition comprising at least one of, or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275. In some embodiments, the composition comprises benserazide or desloratadine or MS-275, or any pharmaceutically acceptable salt, polymorph or ester thereof.

For convenience, certain terms employed herein, in the specification, examples and appended claims are collected here. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. Unless explicitly stated otherwise, or apparent from context, the terms and phrases below do not exclude the meaning that the term or phrase has acquired in the art to which it pertains. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The term "pharmaceutically acceptable excipient", as used herein, refers to carriers and vehicles that are compatible with the active ingredient (for example, a compound of the invention) of a pharmaceutical composition of the invention (and preferably capable of stabilizing it) and not deleterious to the subject to be treated. For example, solubilizing agents that form specific, more soluble complexes with the compounds of the invention can be utilized as pharmaceutical excipients for delivery of the compounds. Suitable carriers and vehicles are known to those of extraordinary skill in the art. The term "excipient" as used herein will encompass all such carriers, adjuvants, diluents, solvents, or other inactive additives. Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, etc. The

pharmaceutical compositions of the invention can also be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like, which do not deleteriously react with the active compounds of the invention.

Thus, as used herein, the term "pharmaceutically acceptable salt," is a salt formed from an acid and a basic group of a compound of the invention. Illustrative salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutarnate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, and pamoate salts.

The term "pharmaceutically acceptable salt" also refers to a salt prepared from a compound of the invention having an acidic functional group, such as a carboxylic acid functional group, and a pharmaceutically acceptable inorganic or organic base. Suitable bases include, but are not limited to, hydroxides of alkali metals such as sodium, potassium, and lithium; hydroxides of alkaline earth metal such as calcium and magnesium; hydroxides of other metals, such as aluminum and zinc; ammonia, and organic amines, such as unsubstituted or hydroxy-substituted mono-, di-, or trialkylamines; dicyclohexylamine; tributyl amine; pyridine; N-methyl, N-ethylamine; diethylamine; triethylamine; mono-, his-, or tris-(2-hydroxy-lower alkyl amines), such as mono-, his-, or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine, or tris-(hydroxymethyl)methylamine, N,N,-di-lower alkyl-N-(hydroxy lower alkyl)-amines, such as N,N- dimethyl-N-(2-hydroxyethyl)amine, or tri-(2-hydroxyethyl)amine; N-methyl-D-glucamine; and amino acids such as arginine, lysine, and the like. Other pharmaceutically acceptable salts are described in the Handbook of Pharmaceutical Salts. Properties, Selection, and Use (P. Heinrich Stahl and C. Wermuth, Eds., Verlag Helvetica Chica Acta, Zurich, Switzerland (2002)).

As used herein, a "prodrug" refers to compounds that can be converted via some chemical or physiological process (e.g., enzymatic processes and metabolic hydrolysis). The prodrug compound often offers advantages of solubility, or delayed release in an organism. The term "prodrug" is also meant to include any covalently bonded carriers, which release the active compound *in vivo* when such prodrug is administered to a subject. Prodrugs of an active

compound may be prepared by modifying functional groups present in the active compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo,* to the parent active compound. Prodrugs include compounds wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the active compound is administered to a subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of an alcohol or acetamide, formamide and benzamide derivatives of an amine functional group in the active compound and the like.

The term "subject" is used interchangeably herein with "patient" and refers to a vertebrate, preferably a mammal, more preferably a primate, still more preferably a human. Mammals include, without limitation, humans, primates, wild animals, rodents, feral animals, farm animals, sports animals, and pets. Primates include chimpanzees, cynomologous monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g., trout, catfish and salmon. Patient or subject includes any subset of the foregoing, e.g., all of the above, but excluding one or more groups or species such as humans, primates or rodents. In certain embodiments of the aspects described herein, the subject is a mammal, e.g., a primate, e.g., a human. A subject can be male or female.

The term "therapeutically effective amount" as used herein refers to an amount sufficient to affect a beneficial or desired clinical result upon treatment. Alternatively, a "therapeutically effective amount" is an amount of a compound of this invention sufficient to confer a therapeutic or prophylactic effect on the treated subject a hemoglobinopathy and/or thalassemia. Therapeutically effective amounts will vary, as recognized by those skilled in the art, depending on the specific disease treated, the route of administration, the excipient selected, and the possibility of combination therapy. Generally, a therapeutically effective amount can vary with the subject's history, age, condition, sex, as well as the severity and type of the medical condition in the subject, and administration of other pharmaceutically active agents.

The terms "increased" ,"increase" or "enhance" or "activate" are all used herein to generally mean an increase by a statistically significant amount; for the avoidance of any doubt, the terms "increased", "increase" or "enhance" or "activate" means an increase of at least 2% as

compared to a reference level

The term "statistically significant" or "significantly" refers to statistical significance and generally means a two standard deviation (2 SD) below normal, or lower, concentration of the marker. The term refers to statistical evidence that there is a difference. It is defined as the probability of making a decision to reject the null hypothesis when the null hypothesis is actually true. The decision is often made using the p-value.

The term "substantially" as used herein means a proportion of at least about 60%, or preferably at least about 70% or at least about 80%, or at least about 90%, at least about 95%, at least about 97% or at least about 99% or more or any integer between 70% and 100%.

As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the invention, yet open to the inclusion of unspecified elements, whether essential or not.

As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages can mean ±1%.

In this application and the claims, the use of the singular includes the plural unless specifically stated otherwise. In addition, use of "or" means "and/or" unless stated otherwise. Moreover, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both

elements and components comprising one unit and elements and components that comprise more than one unit unless specifically stated otherwise.

HbF-inducing agent

In particular, the present invention generally relates to increasing the percentage of fetal hemoglobin (HbF or gamma-globin) in the blood of a subject, the method comprising administering to the subject a composition comprising at least one of, or any combination of HbF-inducing agents, selected from the group comprising; ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275.

In some embodiments, an HbF-inducing agent is benserazide or desloratadine or MS-275, or any pharmaceutically acceptable salt, polymorph or ester thereof.

In certain embodiments, the amount of fetal globin in the blood of the subject increases with administration of an HbF-inducing agent as disclosed herein to the subject. In some embodiments, the number of F-cells in the blood of the subject increases with administration of one or more HbF-inducing agent as disclosed herein. In certain embodiments, the number of F-reticulocytes in the blood of the subject increases on administration of an HbF-inducing agent as disclosed herein. In some embodiments, the amount of total fetal hemoglobin in the blood of the subject increases. In certain embodiments, the amount of total hemoglobin in the blood of the subject increases. In some embodiments, hematocrit increases. In certain embodiments, red blood cell production increases.

In some embodiments, a composition comprising at least one HbF-inducing agent as disclosed herein is administered in an effective amount to increase the expression of gamma-globin in the blood by a statistically significant increase as compared to in the absence of a compound. In some embodiments, a composition comprising at least one HbF-inducing agent as disclosed herein is administered to increase the expression of gamma-globin in the blood by a statistically significant increase as compared to in the presence of a control agent such as, for example, hydroxyurea.

In some embodiments, a composition comprising at least one HbF-inducing agent as disclosed herein, for example, any one, or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, or in particular, benserazide, desloratadine, or MS-275 is administered in an effective amount to increase the level of gamma-goblin expression in blood by at least about 2%, as compared to either in the absence of the

composition, or as compared to a positive control agent, such ST20.

In some embodiments, a composition comprising at least one HbF-inducing agent as disclosed herein, for example, any one, or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, or in particular, benserazide, desloratadine, or MS-275 is administered in an effective amount to increase the total hemoglobin in the blood above the baseline.

In some embodiments, administration of one or more HbF-inducing agent is a pulsed administration. In certain embodiments, a pulsed administration comprises administering one or more HbF-inducing agent for about 8 weeks, followed by not administering an HbF-inducing agent for about 4 weeks. In some embodiments, the pulsed administration comprises administering at least one HbF-inducing agent for about 6 weeks, followed by not administering an HbF-inducing agent for about 2 weeks. In certain embodiments, the pulsed administration comprises administering at least one HbF-inducing agent for about 4 weeks, followed by not administering an HbF-inducing agent for about 2 weeks. In some embodiments, the pulsed administration comprises administering at least one HbF-inducing agent for about 4 days a week or for about 2 weeks or 4 weeks, followed by not administering an HbF-inducing agent for about 1 week or 2 weeks. In some embodiments, pulsed administration comprises pulses of administering at least one HbF-inducing agent for about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 10 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 9 months, about 12 months. In certain embodiments, pulsed administration comprises intervals of not administering an HbF-inducing agent of about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 10 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 9 months, about 12 months. In some embodiments, administration is continuous. In certain embodiments, administration is for the lifetime of the subject.

In some embodiments, a subject is a mammal. In certain embodiments, a mammal is an animal. In some embodiments, an animal is a horse or a dog. In certain embodiments, the mammal is a human. In some embodiments, the human is a child. In certain embodiments, a

human is under the age of 18. In some embodiments, a human is under the age of 10. In some embodiments, a human is under the age of 2.

Further provided herein are methods for increasing the percentage of fetal hemoglobin in the blood of a subject diagnosed with a beta-thalassemia comprising administering to the subject a composition comprising at least one HbF-inducing agent or a pharmaceutically acceptable salt, or ester thereof, wherein after the administration the percentage of fetal hemoglobin in the blood of the subject increases by a statistically significant amount as compared to the absence of administration of the HbF-inducing agent, or a control agent, such as, for example, ST20.

In some embodiments, provided herein are methods for increasing the percentage of fetal hemoglobin in the blood of a subject diagnosed with a beta-thalassemia comprising administering to the subject a composition comprising one or more HbF-inducing agents, as the free acid, a pharmaceutically acceptable salt, or ester thereof.

In some embodiments, the percentage of fetal hemoglobin in the blood of a subject increases after one week of administering as compared to baseline. In other embodiments the percentage of fetal hemoglobin in the blood of a subject increases after two weeks of administering as compared to baseline. In certain embodiments, the percentage of fetal hemoglobin in the blood of a subject increases after four weeks of administering as compared to baseline. In some embodiments, the percentage of fetal hemoglobin in the blood of a subject increases after one day of administering as compared to baseline. In certain embodiments the percentage of fetal hemoglobin in the blood of a subject increases after 3 days of administering as compared to baseline.

In some embodiments, the methods for increasing the percentage of fetal hemoglobin further comprises administering to the subject at least one other therapeutic agent with at least one HbF-inducing agent, wherein the therapeutic agent can be selected from the group consisting of; hydroxyurea, decitabine, an HDAC inhibitor, sodium 2,2 dimethylbutyrate, ST20 or any combination thereof.

Benserazide

Benserazide (also called SERAZIDE™ or Ro 4-4602) is a peripherally-acting aromatic L-amino acid decarboxylase (AAAD) or DOPA decarboxylase inhibitor, which is unable to cross the blood-brain barrier (**Figure 7**). Benserazide has a systematic (IUPAC) name (RS)-2-amino-3-hydroxy-N'-(2,3,4- trihydroxybenzyl)propanehydrazide, and CAS number 14919-77-8 and

formula: C10H15N3O5, and Molecular mass of 257.243 g/mol.

Desloratadine

Desloratadine, known as 8-chloro-6,11-dihydro-11-(4-piperidylidene)-5H-benzo[5,6]cyclohepta[1,2-b-]pyridine (**Figure 7**). Desloratadine is currently marketed as CLARINEX™ in the United States. CLARINEX™ is prescribed as an antihistamine for prevention or treatment of allergenic reactions, which may result in symptoms such as sneezing, itchy eyes and hives U.S. Pat. No. 4,659,716, which is incorporated herein in its entirety by reference discloses descarbonylethoxyloratadine (also known as Desloratadine), which possesses antihistaminic properties with substantially no sedative properties. The 4,659,716 patent describes a process for the preparation of Desloratadine by dissolving loratadine in water and basifying with dilute solution of potassium carbonate to obtain a pink-colored oil. The organic material is extracted with chloroform, washed with water and triturated with hexane. Desloratadine is obtained by recrystallisation of the extracted organic material with large volume of hexane after charcolisation.

U.S. Pat. No. 6,506,767 (hereinafter '767) which is incorporated herein in its entirety by reference, discloses two polymorphic forms of desloratadine, labeled Forms I and II. The XRPD peaks and the FTIR spectrum for the forms are also disclosed in the '767 patent. According to this patent '767 patent, discloses certain alcoholic solvents, e.g., hexanol and methanol produce 100% polymorph form 1, but others, e. g., 3-methyl-1-butanol and cyclohexanol produce significant amounts of form 2. Chlorinated solvents, e. g., dichloromethane produce form 1 substantially free of form 2. Ether solvents such as dioxane produced form 1 substantially free of form 2 but other alkane ethers, e.g., di-isopropyl ether produced form 1 with significant amounts of form 2 and di-n-butyl ether favored formation of form 2. Ketones such as methyl isobutyl ketone produced crystalline polymorph form 1 essentially free of form 2 but methyl butyl ketone produced 8:1 ratio of form 1 to form 2. Use of methyl isobutyl ketone is preferred to produce crystalline polymorph form 1 essentially free of form 2. Only ethyl acetate and di-n-butyl ether were found to produce crystalline polymorph form 2 substantially free of form 1. Use of di-n-butyl ether is preferred for producing crystalline form 2 substantially free of form 1. According to this patent the polymorph form obtained from U.S. Pat. No. 4,659,716 is a mixture of form I and form II.

Teva International Patent Application No. W02004/080461, which is incorporated herein

in its entirety by reference, claims a pharmaceutical composition of desloratadine comprising of a mixture of crystalline Desloratadine of form I and II in a weight to weight ratio of about 25% to about 75% of either form to the other and a pharmaceutically acceptable excipient.

Desloratadine or its pharmaceutically acceptable salts thereof can be used in the methods and compositions as disclosed herein, and are well known in the art and is disclosed and can be manufactured as taught in the following U.S. Patent Applications, 2010/0129310; 2010/0216831; 2010/0069402; 2010/0022576; 2010/0021542; 2008/0118555; 2007/0244144; 2007/0135472; 2007/0060756; 2007/0053974; 2007/0014855; 2007/0004671; 2006/0276495; 2006/0223841; 2006/0154948; 2006/0100435, which are incorporated herein in their entirety by reference.

MS-275

MS-275 is also called MS-27-275 or N-(2-arninophenyl)-4-[N-(pyridin-3-yl-methoxycarbonyl)arninomethyl]benzarnide (Figure 7). MS-275 is also commonly known in the art as Entinostat, and is an orally bioavailable, highly selective, class I histone deacetylase (HDAC) inhibitor with a long half-life that allows for weekly or every- other-week dosing. Entinostat is currently being investigated in multiple phase 2 clinical studies in cancer in combination with other agents.

MS-275 is a synthetic benzamide derivative that has been shown to inhibit cellular histone deacetylase activity and to block growth in a variety of human tumor cell lines (A. Saito, et al., 1999, Proc. Natl. Acad. Sci. USA, A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors, 96:4592-7). The chemical structure of MS-275 (structure I in **Figure 8**). MS-275 is chemically synthesized using methods known in the art. One such method is described in T. Suzuki et al., 1999, J. Med. Chem., Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives, 42:3001-3. Additional information relating to synthesis of MS-275 and related compounds is found in Japanese Unexamined Patent Publication Hei No. 10-152462. MS-275 is also available from various sources. One such source is Nihon Schering K.K. Another source is the National Cancer Institute (MS-275 is NSC No. 706995). Two benzamide derivatives closely related to MS-275 (structures II and III in **Figure 8**). MS-275 can have derivatives as shown as structures I, II and III, in U.S. Patent 6,841,565, which is incorporated herein in its entirety by reference. A polymorph of MS-275 is disclosed in GB patent GB0907347.9 entitled N-(2-aminophenyl)-4-[N-(pyridine-3-YL)-methoxycarbonyl-aminomethyl]-benzamide (MS-275) polymorph B, which

is incorporated herein in its entirety by reference.

Treatment of diseases

In one embodiment, the invention relates to compositions for the treatment and prevention of blood disorders such as anemia, thalassemia, and sickle cell disease. Compositions stimulate the specific expression of a gamma-globin protein, without inhibiting cell proliferation, and can increase the development of hemoglobin-expressing or other myeloid cells.

Blood Disorders

In another embodiment, the invention relates to methods and medical aids which utilize these compositions to treat blood disorders and/or to ameliorate symptoms associated with blood disorders. The term "blood disorders" as used herein includes hemoglobinopathies and thalassemias. Blood disorders include disorders that can be treated, prevented, or otherwise ameliorated by the administration of a compound of the invention. Treatable blood disorders also include syndromes such as hemoglobin C, D and E disease, hemoglobin lepore disease, and HbH and HbS diseases. Treatment ameliorates one or more symptoms associated with the disorder. Compositions provided to the subject may include any combination of the proteins or chemical compounds of the invention or known to those of ordinary skill in the art. Administration of the composition may be short term, continuous or sporadic as necessary. Patients with a suspected or diagnosed with a blood disorder may only require composition treatment for short periods of time or until symptoms have abated or have been effectively eliminated.

Another embodiment of the invention is directed to methods for the treatment of patients with blood disorders comprising the administration of one or more compositions of the invention. Compositions to be administered contain a therapeutically effective amount of a chemical compound. A therapeutically effective amount is that amount which has a beneficial effect to the subject by alleviating one or more symptoms of the disorder or by simply reducing premature mortality. For example, a beneficial effect may be a decrease in pain on an annual or daily basis, a decrease in duration, frequency or intensity of pain crises, an increased hematocrit, an improved erythropoiesis with decreased hemolysis, decrease in fatigue or an increased endurance or stamina or walking ability to walk a certain distance without shortness of breath, or increased strength. Preferably, a therapeutic amount is that amount of chemical compound or agent that stimulates or enhances the expression of non-adult globin such as embryonic or fetal

globin (HbF), or the proliferation of embryonic, fetal globin expressing cells. In some embodiments, a therapeutic amount is that amount of chemical compound or agent as disclosed herein that increases the percentage of expression of non-adult globin such as embryonic or fetal globin (HbF), or the proliferation of embryonic, fetal or adult globin expressing cells.

Administration

In some embodiments, compositions comprising at least one or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide, desloratadine or MS-275 can be directly or indirectly administered to the patient. Indirect administration can also be performed, for example, by administering the composition to cells *ex vivo* and subsequently introducing the treated cells to the patient. The cells may be obtained from the subject to be treated or from a genetically related or unrelated patient. Related patients offer some advantage by lowering the immunogenic response to the cells to be introduced. For example, using techniques of antigen matching, immunologically compatible donors can be identified and utilized.

Direct administration of compositions comprising at least one or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide, desloratadine or MS-275 can also be by oral, parenteral, sublingual, rectal such as suppository or enteral administration, or by pulmonary absorption or topical application. Parenteral administration may be by intravenous injection, subcutaneous injection. In some embodiments, a composition comprising at least one or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, or MS-275, in particular benserazide, desloratadine or MS-275 can be administered by transdermal transfusion such as with a dermal or cutaneous patch, by direct contact with, for example, bone marrow through an incision or some other artificial opening into the body. Compositions may also be administered to the nasal passages as a spray. Arteries of the nasal area provide a rapid and efficient access to the bloodstream and immediate access to the pulmonary system. Access to the gastrointestinal tract, which can also rapidly introduce substances to the blood stream, can be gained using oral, enema, or injectable forms of administration. Compositions may be administered as a bolus injection or spray, or administered sequentially over time (episodically) such as every two, four, six or eight hours, every day (QD) or every other day (QOD), or over longer periods of time such as weeks to months. Compositions may also be administered in a timed-release fashion such as

by using slow-release resins and other timed or delayed release materials and devices.

Orally active compositions comprising at least one or any combination of ambroxol, benserazide, desloratadine, resveratrol, NSC-95397, idarubicin or MS-275, in particular benserazide, desloratadine or MS-275 are more preferred as oral administration is usually the safest, most convenient and economical mode of drug delivery. Oral administration is usually disadvantageous because compositions are poorly absorbed through the gastrointestinal lining. Compounds which are poorly absorbed tend to be highly polar. Consequently, compounds which are effective, as described herein, may be made orally bioavailable by reducing or eliminating their polarity. This can often be accomplished by formulating a composition with a complimentary reagent which neutralizes its polarity, or by modifying the compound with a neutralizing chemical group. Oral bioavailability is also a problem because drugs are exposed to the extremes of gastric pH and gastric enzymes. These problems can be overcome in a similar manner by modifying the molecular structure to withstand very low pH conditions and resist the enzymes of the gastric mucosa such as by neutralizing an ionic group, by covalently bonding an ionic interaction, or by stabilizing or removing a disulfide bond or other relatively labile bond.

Prophylactic treatments involve administration of a composition of the invention to a subject having a confirmed or suspected blood disorder without having any overt symptoms. For example, otherwise healthy patients who have been genetically screened and determined to be at high risk for the future development of a blood disorder may be administered compositions of the invention prophylactically. Administration can begin at birth and continue, if necessary, for life. Both prophylactic and therapeutic uses are readily acceptable because these compounds are generally safe and non-toxic.

Individual pulses of an HbF-inducing agent as disclosed herein can be delivered to the patient continuously over a period of several hours, such as about 2, 4, 6, 8, 10, 12, 14 or 16 hours, or several days, such as 2, 3, 4, 5, 6, or 7 days, preferably from about 1 hour to about 24 hours and more preferably from about 3 hours to about 9 hours. Alternatively, periodic doses can be administered in a single bolus or a small number of injections of the composition over a short period of time, typically less than 1 or 2 hours. For example, arginine butyrate has been administered over a period of 4 days with infusions for about 8 hours per day or overnight, followed by a period of 7 days of no treatment. This has been shown to be an effective regimen for many thalassemic disorders. Fetal hemoglobin levels rise substantially and there is a

significant rise in the number of both adult and fetal hemoglobin expressing cells. In certain instances, a substantial rise in HbF means that there are positive consequences that raise the patient's standard of living such as, for example, increased activity or mobility, fewer side-effects, fewer hospital stays or visits to the physician, or fewer transfusions. For instance, HbF levels above 20% are generally considered to be sufficient to eliminate symptoms associated with sickle cell disease.

With pulse therapy, *in vivo* levels of an HbF-inducing agent thereof can drop below that level required for effective continuous treatment. Pulsed administration can reduce the amount of a composition comprising an HbF-inducing agent thereof administered to the patient per dose, and/or per total treatment regimen with an increased effectiveness. Pulsed administration can provide a saving in time, effort and expense and a lower effective dose can lessen the number and severity of complications that can be experienced by a subject. As such, pulsing can be more effective than continuous administration of the same composition.

In some embodiments, individual pulses can be delivered to a subject continuously over a period of several hours, such as about 2, 4, 6, 8, 10, 12, 14 or 16 hours, or several days, such as 2, 3, 4, 5, 6, or 7 days, or from about 1 hour to about 24 hours or from about 3 hours to about 9 hours. Alternatively, periodic doses can be administered in a single bolus or a small number of injections of the composition comprising an HbF-inducing agent thereof over a short period of time, for example, less than 1 or 2 hours. For example, arginine butyrate can be administered over a period of 4 days with infusions for about 8 hours per day or overnight, followed by a period of 7 days of no treatment.

The interval between pulses or the interval of no delivery can be greater than 24 hours or can be greater than 48 hours, and can be for even longer such as for 3, 4, 5, 6, 7, 8, 9 or 10 days, two, three or four weeks or even longer. The interval between pulses can be determined by one of ordinary skill in the art, for example, as demonstrated herein in the Examples, by measuring the gamma-globin expression level in the blood in the subject after administration of the pulse dose, and administering a pulse when the mRNA gamma-globin level reaches a certain pre-defined low threshold limit. Such pre-defined low threshold limits can be determined by one of ordinary skill in the art, and can be, for example, about baseline level, or about 100% or about 200% above baseline level mRNA gamma-globin expression (e.g., mRNA gamma-globin expression without administration of an HbF-inducing agent). Alternatively, in some

embodiments, the interval between pulses can be calculated by administering another dose of a composition comprising an HbF-inducing agent, and when the active component of the composition is no longer detectable in the patient prior to delivery of the next pulse. Alternatively, intervals can also be calculated from the *in vivo* half-life of the composition.

The interval between pulses can also be determined by one of ordinary skill in the art, for example, as demonstrated herein in the Examples, by measuring the percent increase in absolute hemoglobin (see **Fig 3**), percent F-reticulocytes (see **Figure 5),** percent increase in F-cells (see **Figure 6**), in the blood in the subject after administration of the pulse dose, and administering a pulse when the mRNA gamma-globin or total hemoglobin (Hb) level increases by, for example about a 1.0 or about 0.5 g/dL increase in total hemoglobin or by 0.3-fold increase in fetal globin mRNA above levels prior to treatment.

In some embodiments, the number of pulses in a single therapeutic regimen can be as little as two, but can be from about 5 to 10, 10 to 20, 15 to 30 or more.

In some embodiments, a subject can receive one or more compositions comprising an HbF-inducing agent for life according to the methods of this invention, for example, where the subject has a permanent or incurable blood disorder, e.g., an inherited blood disorder. Compositions can be administered by most any means, and can be delivered to the subject as an oral formulation, or injection (e.g. intravenous, subcutaneous, intra-arterial), infusion or instillation. Various methods and apparatus for pulsing compositions by infusion or other forms of delivery to the patient are disclosed in U.S. Pat. Nos. 4,747,825; 4,723,958; 4,948,592; 4,965,251 and 5,403,590, which are incorporated herein in their entirety by reference.

In one embodiment, a composition comprising an HbF-inducing agent thereof can be administered to a subject for about 2, or about 3, or about 4, or about five days, or more than five days, and then a subsequently administered after an appropriate interval for an additional period of time, for example, for about 2, or about 3, or about 4, or about five days, or more than five days. Cycles of treatment may occur in immediate succession or with an interval of no treatment between cycles.

In some embodiments, a composition comprising an HbF-inducing agent can be administered to a subject before a chemotherapeutic treatment, or radiation treatment is administered to the subject. In alternative embodiments, a composition comprising an HbF-inducing agent can be co-administered to a subject In some embodiments, a composition

comprising an HbF-inducing agent can be co-administered with a pharmaceutical composition comprising an comprising one or more addition agents. The pharmaceutical compositions can be provided by pulsed administration. For example, a composition comprising an HbF-inducing agent can be administered to a subject, followed by a chemotherapeutic treatment, or radiation treatment after an interval of time has passed, and this order of administration the same or similar time interval can be repeated, for example, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more times.

Method for Increasing Percent HbF and/or Total Hemoglobin

Provided herein are methods and compositions for increasing the percentage of fetal hemoglobin in the blood of a subject comprising administering to a subject a composition comprising an HbF-inducing agent as disclosed herein as the free acid, a pharmaceutically acceptable salt, or ester thereof. Further provided herein are methods and compositions for increasing total hemoglobin in the blood of a subject, comprising administering to the subject a composition comprising an HbF-inducing agent as disclosed herein as the free acid, a pharmaceutically acceptable salt, or ester thereof. Also provided herein are methods and compositions for increasing total hemoglobin, hematocrit, and red blood cells in a subject, comprising administering to said subject an HbF-inducing agent as disclosed herein as the free acid, a pharmaceutically acceptable salt, or ester thereof. Further provided herein are methods and compositions for increasing total hemoglobin, hematocrit, or red blood cells, or a combination thereof, comprising administering to said subject an HbF-inducing agent as disclosed herein.

In some embodiments, administering an HbF-inducing agent as disclosed herein does not suppress erythropoiesis at concentrations associated with biologic activity. In certain embodiments, administering an HbF-inducing agent as disclosed herein stimulates cell proliferation. In some embodiments, administering an HbF-inducing agent as disclosed herein inhibits apoptosis of erythroid progenitors. In further embodiments, administering an HbF-inducing agent as disclosed herein stimulates erythroid cell proliferation and survival. In some embodiments, administering an HbF-inducing agent as disclosed herein stimulates erythroid cell proliferation. In certain embodiments, administering an HbF-inducing agent as disclosed herein stimulates erythroid cell survival. In some embodiments, administering an HbF-inducing agent as disclosed herein stimulates red blood cell production. In certain embodiments, administering an HbF-inducing agent as disclosed herein leads to a longer survival of sickled blood cells.

In some embodiments, administering an HbF-inducing agent as disclosed herein stimulates erythropoiesis. In certain embodiments, administering an HbF-inducing agent as disclosed herein induces expression of the fetal globin gene promoter. In some embodiments, administering an HbF-inducing agent as disclosed herein increases fetal globin levels. In certain embodiments, administering an HbF-inducing agent as disclosed herein increases RBC production. In some instances, increased RBC production is assayed by reticulocytes, total hemoglobin (Hgb), and hematocrit (Hct).

In certain embodiments, administering an HbF-inducing agent as disclosed herein increases the amount of fetal globin in the blood of the subject. In some embodiments, administering an HbF-inducing agent as disclosed herein increases the amount of fetal hemoglobin in the blood of the subject. In certain embodiments, administering an HbF-inducing agent as disclosed herein increases the amount of total hemoglobin in the blood of the subject. In some embodiments, administering an HbF-inducing agent as disclosed herein increases the percentage of reticulocytes in the blood of the subject. In certain embodiments, administering an HbF-inducing agent as disclosed herein increases the number of reticulocytes in the blood of the subject. In some embodiments, administering an HbF-inducing agent as disclosed herein increases hematocrit.

In contrast to ST20 or hydroxyurea, an HbF-inducing agent as disclosed herein is effective at increasing percent HbF at a total daily dose which is below the maximum tolerated dose. In some instances, administering an HbF-inducing agent as disclosed herein does not necessitate the careful dose titration currently required for treatment with ST20.

In addition, the total daily dose of an HbF-inducing agent as disclosed herein which is effective in increasing the percentage of HbF is significantly lower than the dose required for other SCFAD like arginine butyrate. In some embodiments, a subject can be administered an HbF-inducing agent as disclosed herein with other agents, including but not limited to 2,2-dimethylbutyrate is administered as sodium 2,2-dimethylbutyrate. 2,2-Dimethylbutyrate includes, but is not limited to, 2,2-dimethylbutyric acid, sodium 2,2-dimethylbutyrate, potassium 2,2-dimethylbutyrate, magnesium 2,2-dimethylbutyrate, calcium 2,2-dimethylbutyrate, arginine 2,2-dimethylbutyrate, lysine 2,2-dimethylbutyrate, choline 2,2-dimethylbutyrate, methyl 2,2-dimethylbutyrate (2,2-dimethylbutyric acid methyl ester), ethyl 2,2- dimethylbutyrate, propyl 2,2-dimethylbutyrate, or isopropyl 2,2-dimethylbutyrate.

In certain embodiments, the subject has been diagnosed with a blood disorder. In some embodiments, the blood disorder is sickle cell disease. In other embodiments, the blood disorder is a beta thalassemia. In certain embodiments, the beta-thalassemia is beta thalassemia intermedia. In some embodiments, the beta thalassemia is beta-thalassemia major. In certain embodiments, the beta thalassemia is beta thalassemia minor (beta-thalassemia trait). In some embodiments, the beta thalassemia is HbE beta-thalassemia. In certain embodiments, the beta thalassemia is HbS beta-thalassemia.

In certain embodiments, a subject is administered a composition comprising an HbF-inducing agent as disclosed herein, daily. In further embodiments, administration is continuous. In some embodiments, the administration of a composition comprising an HbF-inducing agent as disclosed herein is by pulsed administration. In certain embodiments, pulsed administration comprises administering an HbF-inducing agent pulse for about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 10 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 9 months, about 12 months. In some embodiments, pulsed administration comprises intervals of not administering an HbF-inducing agent for about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 10 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 9 months, about 12 months. In certain embodiments, administration is for the lifetime of the subject.

In some embodiments, a composition comprising an HbF-inducing agent is administered every other day. In certain embodiments, the pulsed administration comprises administering a composition comprising an HbF-inducing agent for about 5 days per week. In some embodiments, the pulsed administration comprises administering a composition comprising an HbF-inducing agent for about 5 days, followed by not administering an HbF-inducing agent for about 2 days. In certain embodiments, the pulsed administration comprises administering an HbF-inducing agent for about 2 weeks, followed by not administering an HbF- inducing agent for about 1 week. In some embodiments, the pulsed administration comprises administering an HbF-inducing agent for about 2 weeks, followed by not administering an HbF-inducing agent for about 2 weeks. In certain embodiments, the pulsed administration comprises administering an

HbF-inducing agent for about 4 weeks, followed by not administering an HbF-inducing agent for about 1 week. In some embodiments, the pulsed administration comprises administering an HbF-inducing agent for about 4 weeks, followed by not administering an HbF-inducing agent for about 2 weeks. In further embodiments, the pulsed administration comprises administering an HbF-inducing agent for about 6 weeks, followed by not administering an HbF- inducing agent for about 2 weeks. In certain embodiments, the pulsed administration comprises administering an HbF-inducing agent for about 8 weeks, followed by not administering an HbF-inducing agent for about 2 weeks. In some embodiments, the pulsed administration comprises administering an HbF-inducing agent for about 8 weeks, followed by not administering an HbF-inducing agent for about 4 weeks.

In some instances, administering a composition comprising an HbF-inducing agent to a subject with one genotype of beta thalassemia is more effective in raising percent HbF than administering DMB (ST20) to a subject with a different genotype of beta-thalassemia. Further provided herein are methods and compositions comprising diagnosing a beta-thalassemia genotype of a patient, determining a treatment plan considering the beta-thalassemia genotype, and optionally increasing the percentage of fetal hemoglobin in the blood of the patient, comprising administering to the patient an HbF-inducing agent as the free acid, a pharmaceutically acceptable salt, or ester thereof.

Pharmaceutical Compositions

In some embodiments, a pharmaceutical composition comprising an HbF-inducing agent administered according to a method of the invention are administered orally in effective dosages, depending upon the weight, body surface area, and condition of the subject being treated. In some instances, variations occur depending upon the species of the subject being treated and its individual response to said medicament, as well as on the type of pharmaceutical formulation chosen and the time period and interval at which such administration is carried out. In some embodiments, the administration of the pharmaceutical composition comprising an HbF-inducing agent according to a method of the invention is carried out in single or multiple doses. For example, the composition can be administered in a wide variety of different dosage forms, i.e., it may be combined with various pharmaceutically acceptable inert carriers in the form of tablets, dragees, capsules, lozenges, troches, hard candies, aqueous suspensions, elixirs, syrups, and the like. Such carriers include solid diluents or fillers, sterile aqueous media and

various non-toxic organic solvents, etc. Moreover, oral pharmaceutical compositions can be suitably sweetened and/or flavored.

In certain embodiments, pharmaceutical compositions comprising an HbF-inducing agent are suitable for oral administration. Suitable pharmaceutical compositions for oral administration can be in the form of capsules, tablets, pills, lozenges, cachets, dragees, powders, granules; or as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil liquid emulsion; or as an elixir or syrup; and the like; each containing a predetermined amount of a compound of the present invention as an active ingredient. When intended for oral administration in a solid dosage form (i.e., as capsules, tablets, pills and the like), the pharmaceutical compositions of the invention will typically comprise a compound of the present invention as the active ingredient and one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate. Optionally or alternatively, such solid dosage forms may also comprise: filters or extenders, such as starches, microcrystalline cellulose, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and/or sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as cetyl alcohol and/or glycerol monostearate; absorbents, such as kaolin and/or bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and/or mixtures thereof; coloring agents; and buffering agents.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

Release agents, wetting agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the pharmaceutical compositions of

the invention. Examples of pharmaceutically-acceptable antioxidants include: water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfate sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like. Coating agents for tablets, capsules, pills and like, include those used for enteric coatings, such as cellulose acetate phthalate (CAP), polyvinyl acetate phthalate (PVAP), hydroxypropyl methylcellulose phthalate, methacrylic acid, methacrylic acid ester copolymers, cellulose acetate trimellitate (CAT), carboxymethyl ethyl cellulose (CMEC), hydroxypropyl methyl cellulose acetate succinate (HPMCAS), and the like.

In addition, the pharmaceutical compositions of the present invention may optionally contain opacifying agents and may be formulated so that they release the active ingredient only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

If desired, pharmaceutical compositions of the present invention may also be formulated to provide slow or controlled release of the active ingredient using, by way of example, hydroxypropyl methyl cellulose in varying proportions; or other polymer matrices, liposomes and/or microspheres. Sustained release compositions can be formulated including those wherein the active component is derivatized with differentially degradable coatings, e.g., by microencapsulation, multiple coatings, etc.

It will be appreciated that the actual preferred amounts of active compounds used in a given therapy will vary according to the particular compositions formulated. Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art using conventional dosage determination tests conducted with regard to the foregoing guidelines.

It will also be understood that normal, conventionally known precautions will be taken regarding the administration of the compounds of the invention generally to ensure their efficacy under normal use circumstances. Especially when employed for treatment of humans and

animals in vivo, the practitioner should take all sensible precautions to avoid conventionally known contradictions and toxic effects.

The composition, shape, and type of dosage forms of the invention will typically vary depending on their use. This aspect of the invention will be readily apparent to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences (1990) 18th ed., Mack Publishing, Eastern Pa.

In certain embodiments, the pharmaceutical compositions of the invention are packaged in a unit dosage form. The term "unit dosage form" or "unit dose" refers to a physically discrete unit suitable for dosing a patient, i.e., each unit containing a predetermined quantity of active agent calculated to produce the desired therapeutic effect either alone or in combination with one or more additional units. For example, such unit dosage forms may be capsules, tablets, pills, and the like. Unit doses can also be prepared to contain any useful amount of an active ingredient (e.g., an HbF-inducing agent). For example, a unit dose can comprise 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 310 mg, 320 mg, 330 mg, 340 mg, 350 mg, 360 mg, 370 mg, 380 mg, 390 mg, 400 mg, 410 mg, 420 mg, 430 mg, 440 mg, 450 mg, 460 mg, 470 mg, 480 mg, 490 mg, 500 mg, 510 mg, 520 mg, 530 mg, 540 mg, 550 mg, 560 mg, 570 mg, 580 mg, 590 mg, 600 mg, 625 mg, 650 mg, 675 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, or more of an HbF-inducing agent per unit dose. Milligrams per dose can refer to either the free acid form of an HbF-inducing agent, or an HbF-inducing agent in a salt or ester form.

Administrations can be repeated on consecutive or non-consecutive days. Thus, daily administrations can be performed for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, or more consecutive days. For example, administration of 10 mg/kg of an HbF-inducing agent is performed twice a day (at a total daily dose of 20 mg/kg) for 14 consecutive days. Alternatively, administration may occur for multiple days, but on non-consecutive days separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more days. For example, administration of 15 mg/kg of an HbF-inducing agent is performed on every other day following therapy onset. In another instance, administration of an HbF-inducing agent is performed for 5 days per week. Such dosing regimens can be tailored to an individual patient, based on any number of clinically relevant parameters including, but not limited to toxicity, tolerance, side-

effects, effectiveness, etc.

Combination Therapy

In certain embodiments, the pharmaceutical composition is administered alone or in combination with other known compositions for treating blood disorders in a subject, e.g., a mammal. In some embodiments, mammals include cats, dogs, pigs, horses, cows, rats, mice, monkeys, chimpanzees, baboons, and humans. In specific embodiments, the mammal is a human. In some embodiments, the human is a child. In certain embodiments the human is under the age of 18. In some embodiments, the human is under the age of 10. In some embodiments, the human is under the age of 2. In one embodiment, the subject is suffering from a blood disorder. In another embodiment, the subject is at risk of suffering from a blood disorder.

The language "in combination with" a known composition is intended to include simultaneous administration of the composition of the invention and the known composition, administration of the composition of the invention first, followed by the known composition and administration of the known composition first, followed by the composition of the invention. Any of the compositions known in the art for treating blood disorders can be used in the methods of the invention.

In some embodiments, in addition to the use of an HbF-inducing agent for the treatment of blood disorders, concomitant administration of other pharmaceutical and nutraceutical compounds occurs. For example, persons suffering from sickle cell disease are given an HbF-inducing agent other agents as disclosed herein), folic acid supplements (for blood cell production), opioids or analgesics (for pain management), and/or antibiotics (for treating secondary infections). In further embodiments, administration of an HbF-inducing agent for the treatment of blood disorders is combined with the administration of natural or synthetic erythropoietin. In certain instances, concomitant treatment with an HbF-inducing agent and a second agent occurs at the same time, or on different regimen schedules. In some embodiments an HbF-inducing agent is an orally bio-available compound that is active at well tolerated doses.

Administration of the compositions comprising HbF-inducing agents as described herein may be by oral, parenteral, sublingual, rectal, or enteral administration, or pulmonary absorption or topical application. Compositions can be directly or indirectly administered to the patient. Indirect administration is performed, for example, by administering the composition to cells *ex vivo* and subsequently introducing the treated cells to the subject, e.g., human patient.

Alternatively, the cells may be obtained from the patient to be treated or from a genetically related or unrelated patient. Related patients offer some advantage by lowering the immunogenic response to the cells to be introduced. For example, using techniques of antigen matching, immunologically compatible donors can be identified and utilized.

The compositions comprising HbF-inducing agents can be purchased commercially and prepared as a mixed composition using techniques well-known to those of ordinary skill in the art. Direct administration of a composition comprising HbF-inducing agents to a subject can be by oral, parenteral, sublingual, rectal such as suppository or enteral administration, or by pulmonary absorption or topical application. Parenteral administration may be by intravenous (IV) injection, subcutaneous (s.c.) injection, intramuscular (i.m.) injection, intra-arterial injection, intrathecal (i.t.) injection, intra-peritoneal (i.p.) injection, or direct injection or other administration to the subject.

Alternatively, pharmaceutical compositions comprising HbF-inducing agents and/or salts thereof can be added to the culture medium of cells *ex vivo.* In addition to the active compound, such compositions comprising HbF-inducing agents can contain pharmaceutically-acceptable carriers and other ingredients known to facilitate administration and/or enhance uptake (e.g., saline, dimethyl sulfoxide, lipid, polymer, affinity-based cell specific-targeting systems). In some embodiments, a composition comprising HbF-inducing agents and/or salts thereof can be incorporated in a gel, sponge, or other permeable matrix (e.g., formed as pellets or a disk) and placed in proximity to the endothelium for sustained, local release. In some embodiments, a composition comprising HbF-inducing agents and/or salts thereof can be administered in a single dose or in multiple doses which are administered at different times.

Pharmaceutical compositions comprising HbF-inducing agents and/or salts thereof can be administered by any known route. By way of example, a composition c comprising HbF-inducing agents and/or salts thereof can be administered by a mucosal, pulmonary, topical, or other localized or systemic route (e.g., enteral and parenteral). The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal

injection, infusion and other injection or infusion techniques, without limitation. The phrases "systemic administration," "administered systemically", "peripheral administration" and "administered peripherally" as used herein mean the administration of the agents as disclosed herein such that it enters the animal's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agents from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, for example the carrier does not decrease the impact of the agent on the treatment. In other words, a carrier is pharmaceutically inert.

Suitable choices in amounts and timing of doses, formulation, and routes of administration of a composition comprising HbF-inducing agents and/or salts thereof can be made with the goals of achieving a favorable response in the subject with a blood disorder, e.g., thalassemia, aplastic anemia and hemoglobinopathy and avoiding undue toxicity or other harm thereto (i.e., safety). Therefore, "effective" refers to such choices that involve routine manipulation of conditions to achieve a desired effect.

A bolus of the formulation of a composition comprising HbF-inducing agents and/or salts thereof administered to an individual over a short time once a day is a convenient dosing schedule. Alternatively, the effective daily dose can be divided into multiple doses for purposes of administration, for example, two to twelve doses per day. Dosage levels of active ingredients in a pharmaceutical composition comprising HbF-inducing agents and/or salts thereof can also be varied so as to achieve a transient or sustained concentration of the compound or derivative thereof in an individual, especially in and around the blood circulation and to result in the desired therapeutic response or protection. But it is also within the skill of the art to start doses at levels

lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

In some embodiments, the amount of a composition comprising HbF-inducing agents and/or salts thereof can be administered is dependent upon factors known to a person skilled in the art such as bioactivity and bioavailability of the compound (e.g., half-life in the body, stability, and metabolism); chemical properties of the compound (e.g., molecular weight, hydrophobicity, and solubility); route and scheduling of administration, and the like. It will also be understood that the specific dose level to be achieved for any particular individual can depend on a variety of factors, including age, gender, health, medical history, weight, combination with one or more other drugs, and severity of disease.

Production of compounds comprising HbF-inducing agents and/or salts thereof according to present regulations are regulated for good laboratory practices (GLP) and good manufacturing practices (GMP) by governmental regulatory agencies for pharmaceuticals (e.g., U.S. Food and Drug Administration). This requires accurate and complete record keeping, as well as monitoring of QA/QC. Oversight of patient protocols by agencies and institutional panels is also envisioned to ensure that informed consent is obtained; safety, bioactivity, appropriate dosage, and efficacy of products are studied in phases; results are statistically significant; and ethical guidelines are followed. Similar oversight of protocols using animal models, as well as the use of toxic chemicals, and compliance with regulations is required.

Dosages, formulations, dosage volumes, regimens, and methods for analyzing results aimed at increasing the proliferation of blood cells, and increasing absolute neutrophil count (ANC) can vary. Thus, minimum and maximum effective dosages vary depending on the method of administration. Increase in ANC in a subject can occur within a specific dosage range, which varies depending on, for example, the race, sex, gender, age, and overall health of the subject receiving the dosage, the route of administration, whether a composition comprising HbF-inducing agents and/or salts thereof is administered in conjunction with other molecules, and the specific regimen of administration of the composition comprising HbF-inducing agents and/or salts thereof. For example, in general, nasal administration requires a smaller dosage than oral, enteral, rectal, or vaginal administration.

In an alternative embodiment, for oral and/or enteral formulations of a composition comprising HbF-inducing agents and/or salts thereof, tablets can be formulated in accordance

with conventional procedures employing solid carriers well-known in the art. Capsules employed for oral formulations to be used with the methods of the present invention can be made from any pharmaceutically acceptable material, such as gelatin or cellulose derivatives. Sustained release oral delivery systems and/or enteric coatings for orally administered dosage forms are also contemplated, such as those described in U.S. Pat. No. 4,704,295, "Enteric Film-Coating Compositions," issued Nov. 3, 1987; U.S. Pat. No.4, 556,552, "Enteric Film-Coating Compositions," issued Dec. 3, 1985; U.S. Pat. No. 4,309,404, "Sustained Release Pharmaceutical Compositions," issued Jan. 5, 1982; and U.S. Pat. No. 4,309,406, "Sustained Release Pharmaceutical Compositions," issued Jan. 5, 1982, which are all incorporated herein in their entirety by reference. Examples of solid carriers include starch, sugar, bentonite, silica, and other commonly used carriers. Further non-limiting examples of carriers and diluents which can be used in the formulations of the present invention include saline, syrup, dextrose, and water.

Enteric Coated Formulation

In some embodiments, oral formulations of a composition comprising HbF-inducing agents and/or salts thereof can be in the form of a tablet formulation, for example, comprising HbF-inducing agents and/or salts thereof with an enteric polymer casing. An example of such a preparation can be found in W02005/021002, which is incorporated herein in its entirety by reference. The active material in the core can be present in a micronized or solubilized form. In addition to active materials the core can contain additives conventional to the art of compressed tablets. Appropriate additives in such a tablet can comprise diluents such as anhydrous lactose, lactose monohydrate, calcium carbonate, magnesium carbonate, dicalcium phosphate or mixtures thereof; binders such as microcrystalline cellulose, hydroxypropylmethylcellulose, hydroxypropyl-cellulose, polyvinylpyrrolidone, pre-gelatinised starch or gum acacia or mixtures thereof; disintegrants such as microcrystalline cellulose (fulfilling both binder and disintegrant functions) cross-linked polyvinylpyrrolidone, sodium starch glycollate, croscarmellose sodium or mixtures thereof; lubricants, such as magnesium stearate or stearic acid, glidants or flow aids, such as colloidal silica, talc or starch, and stabilizers such as desiccating amorphous silica, coloring agents, flavors etc. In some embodiments, a tablet comprises lactose as diluent. When a binder is present, it is preferably hydroxypropylmethyl cellulose. In some embodiments, a tablet comprises magnesium stearate as lubricant. In some embodiments, a tablet comprises croscarmellose sodium as disintegrant, or can comprise a microcrystalline cellulose.

In some embodiments, a diluent can be present in a range of 10 - 80% by weight of the core. The lubricant can be present in a range of 0.25- 2% by weight of the core. The disintegrant can be present in a range of 1 - 10% by weight of the core. Microcrystalline cellulose, if present, can be present in a range of 10- 80% by weight of the core.

In some embodiments, the active ingredient, e.g., HbF-inducing agents and/or a salt thereof comprises between 10 and 50% of the weight of the core, more preferably between 15 and 35% of the weight of the core (calculated as free base equivalent). The core can contain any therapeutically suitable dosage level of the active ingredient e.g., HbF-inducing agents and/or a salts thereof, but preferably contains up to 150 mg as free base of the active ingredient. In some embodiments, the core contains 20, 30, 40, 50, 60, 80 or 100 mg as free base of the active ingredient. The active ingredient e.g., HbF-inducing agents and/or a salts thereof can be present as the free base, or as any pharmaceutically acceptable salt. If the active ingredient e.g., HbF-inducing agents is present as a salt, the weight is adjusted such that the tablet contains the desired amount of active ingredient, calculated as free base of the salt. In some embodiments, the active ingredient e.g., HbF-inducing agents is present as a hydrochloride salt.

In some embodiments, the core can be made from a compacted mixture of its components. The components can be directly compressed, or can be granulated before compression. Such granules can be formed by a conventional granulating process as known in the art. In an alternative embodiment, the granules can be individually coated with an enteric casing, and then enclosed in a standard capsule casing.

In some embodiments, the core can be surrounded by a casing which comprises an enteric polymer. Examples of enteric polymers are cellulose acetate phthalate, cellulose acetate succinate, methylcellulose phthalate, ethylhydroxycellulose phthalate, polyvinylacetate pthalate, polyvinylbutyrate acetate, vinyl acetate-maleic anhydride copolymer, styrene-maleic mono-ester copolymer, methyl acrylate- methacrylic acid copolymer or methacrylate-methacrylic acid-octyl acrylate copolymer. These can be used either alone or in combination, or together with other polymers than those mentioned above. The casing can also include insoluble substances which are neither decomposed nor solubilized in living bodies, such as alkyl cellulose derivatives such as ethyl cellulose, cross-linked polymers such as styrene-divinylbenzene copolymer, polysaccharides having hydroxyl groups such as dextran, cellulose derivatives which are treated with bifunctional crosslinking agents such as epichlorohydrin, dichlorohydrin or 1, 2-, 3, 4-

diepoxybutane. The casing can also include starch and/or dextrin.

In some embodiments, an enteric coating materials are the commercially available EUDRAGIT® enteric polymers such as EUDRAGIT® L, EUDRAGIT® Sand EUDRAGIT® NE, used alone or with a plasticizer. Such coatings are normally applied using a liquid medium, and the nature of the plasticizer depends upon whether the medium is aqueous or non-aqueous. Plasticizers for use with aqueous medium include propylene glycol, triethyl citrate, acetyl triethyl citrate or CITROFLEX® or CITROFLEX® A2. Non- aqueous plasticizers include these, and also diethyl and dibutyl phthalate and dibutyl sebacate. A preferred plasticizer is Triethyl citrate. The quantity of plasticizer included will be apparent to those skilled in the art.

In some embodiments, a casing can also include an anti-tack agent such as talc, silica or glyceryl monostearate. In some embodiments, an anti-tack agent is glyceryl monostearate. Typically, the casing can include around 5 - 25 wt% plasticizer and up to around 50 wt % of anti-tack agent, preferably 1-10 wt% of anti-tack agent.

If desired, a surfactant can be included to aid with forming an aqueous suspension of the polymer. Many examples of possible surfactants are known to the person skilled in the art. Preferred examples of surfactants are polysorbate 80, polysorbate 20, or sodium lauryl sulphate. If present, a surfactant can form 0.1- 10% of the casing, preferably 0.2- 5% and particularly preferably 0.5- 2%.

In one embodiment, there is a seal coat included between the core and the enteric coating. A seal coat is a coating material which can be used to protect the enteric casing from possible chemical attack by any alkaline ingredients in the core. The seal coat can also provide a smoother surface, thereby allowing easier attachment of the enteric casing. A person skilled in the art would be aware of suitable coatings. Preferably the seal coat is made of an OPADRY® coating, and particularly preferably it is OPADRY® White OY-S-28876.

In one embodiment, the pharmaceutically active ingredient is HbF-inducing agents or a salt thereof. In some embodiments, an example of an enteric-coated formulation as described in W02005/021002, comprises varying amounts of HbF-inducing agents. In that example, lactose monohydrate, microcrystalline cellulose, the active ingredient, the hydroxypropyl methyl cellulose and half of the croscarmellose sodium were screened into a 10 Litre Fielder high-shear blender (any suitable high shear blender could be used) and blended for 5 minutes at 300 rpm with the chopper off. The mixture was then granulated by the addition of about 750 ml water

whilst continuing to blend. The granules were dried in a Glatt 3/5 fluid bed drier, screened by Comil into a Pharmatec 5 Liter bin blender and then blended with any lactose anhydrous given in the formula plus the remainder of the croscarmellose sodium over 5 minutes at 20 rpm. Magnesium stearate was screened into the blender and the mixing process continued for a further 1 minute at 10 rpm. The lubricated mix was compressed using a Riva Piccolla rotary tablet press fitted with 9.5mm round normal convex punches (any suitable tablet press could be used). The sealcoat, and subsequently the enteric coat, are applied by spraying of an aqueous suspension of the coat ingredients in a Manesty 10 coater using parameters for the coating process as recommended by the manufacturers of the coating polymers (again, any suitable coater could be used). Other enteric-coated preparations of this sort can be prepared by one skilled in the art, using these materials or their equivalents.

Other formulations and routes of administration

In alternative embodiments, the compositions as disclosed herein is by an infusion pump (to infuse, for example, the compositions as disclosed herein into the subject's circulatory system) is generally used intravenously, although subcutaneous, arterial, and epidural infusions are occasionally used. Injectable forms of administration are sometimes preferred for maximal effect. When long-term administration by injection is necessary, medi-ports, in-dwelling catheters, or automatic pumping mechanisms are also preferred, wherein direct and immediate access is provided to the arteries in and around the heart and other major organs and organ systems.

In some embodiments, compositions as disclosed herein comprising HbF-inducing agents and/or salts thereof can be administered to a specific site may be by transdermal transfusion, such as with a transdermal patch, by direct contact to the cells or tissue, if accessible, such as a skin tumor, or by administration to an internal site through an incision or some other artificial opening into the body. Alternatively, in some embodiments, compositions as disclosed herein comprising HbF-inducing agents and/or salts thereof can also be administered to the nasal passages as a spray. Diseases localized to the head and brain area are treatable in this fashion, as arteries of the nasal area provide a rapid and efficient access to the upper areas of the head. Sprays also provide immediate access to the pulmonary system and are the preferable methods for administering compositions to these areas. Access to the gastrointestinal tract is gained using oral, enema, or injectable forms of administration. For example, administration of the

compositions as disclosed herein comprising HbF-inducing agents and/or salts thereof to a subject is preferably oral. As a result, the subject can undergo administration of a composition comprising HbF- inducing agents and/or salts at home.

As indicated above, orally active compositions comprising HbF-inducing agents and/or salts thereof are preferred for at least a portion of the cycle of therapy, as oral administration is usually the safest, most convenient, and economical mode of drug delivery. Consequently, compositions as disclosed herein comprising HbF-inducing agents and/or salts thereof can be modified to increase their oral bioavailable by reducing or eliminating their polarity. This can often be accomplished by formulating a composition with a complimentary reagent that neutralizes its polarity, or by modifying the compound with a neutralizing chemical group. Oral bioavailability is also a problem, because drugs are exposed to the extremes of gastric pH and gastric enzymes. Accordingly, problems associated with oral bioavailability can be overcome by modifying the molecular structure to be able to withstand very low pH conditions and resist the enzymes of the gastric mucosa such as by neutralizing an ionic group, by covalently bonding an ionic interaction, or by stabilizing or removing a disulfide bond or other relatively labile bond.

In some embodiments, the compositions as disclosed herein comprising HbF-inducing agents and/or salts thereof can be used in combination with other agents to maximize the effect of the compositions administered in an additive or synergistic manner. Accordingly, compositions as disclosed herein comprising HbF-inducing agents and/or salts thereof can also comprise proteinaceous agents such as growth factors and/or cytokines. Such proteinaceous agents may also be aminated, glycosylated, acylated, neutralized, phosphorylated, or otherwise derivatized to form compositions that are more suitable for the method of administration to the patient or for increased stability during shipping or storage. Cytokines that are useful to be included in the compositions comprising HbF-inducing agents and/or salts thereof include, but are not limited to, granulocyte/macrophage colony stimulating factor (GM-CSF), stem cell factor (SCF erythropoietin (EPO), steel factor, activin, inhibin, the bone morphogenic proteins (BMPs), retinoic acid or retinoic acid derivatives such as retinol.

Compositions as disclosed herein comprising HbF-inducing agents and/or salts thereof can be physiologically stable at therapeutically effective concentrations. Physiological stable compounds of HbF-inducing agents or salts thereof not break down or otherwise become ineffective upon administration to a subject or prior to having a desired effect. Compounds of

HbF-inducing agents that are structurally resistant to catabolism, and, thus, physiologically stable, or coupled by electrostatic or covalent bonds to specific reagents to increase physiological stability. Such reagents include amino acids such as arginine, glycine, alanine, asparagine, glutamine, histidine, or lysine, nucleic acids including nucleosides or nucleotides, or substituents such as carbohydrates, saccharides and polysaccharides, lipids, fatty acids, proteins, or protein fragments. Useful coupling partners include, for example, glycol, such as polyethylene glycol, glucose, glycerol, glycerin, and other related substances.

Physiological stability of a composition comprising an HbF-inducing agent and/or salts thereof can be measured from a number of parameters such as the half-life of the an HbF-inducing agent compound or salts thereof, or the half-life of active metabolic products derived from the an HbF-inducing agent compound or salts thereof. In some embodiments, compositions comprising an HbF-inducing agent and/or salts thereof have in vivo half-lives of greater than about fifteen minutes, greater than about one hour, greater than about two hours, and greater than about four hours, eight hours, twelve hours, or longer. A compound of an HbF-inducing agent or its salts is stable using this criteria, however, physiological stability can also be measured by observing the duration of biological effects on the patient. Clinical symptoms that are important from the patient's perspective include a reduced frequency or duration, or elimination of the need for transfusions or chelation therapy. Preferably, a stable composition comprising an HbF-inducing agent and/or salts thereof has an in vivo half-life of greater than about 15 minutes, a serum half-life of greater than about 15 minutes, or a biological effect which continues for greater than 15 minutes after treatment has been terminated or the serum level of the compound has decreased by more than half. Preferably, compositions as disclosed herein comprising an HbF-inducing agent and/or salts thereof are also not significantly biotransformed, degraded, or excreted by catabolic processes associated with metabolism. Although there may be some biotransformation, degradation, or excretion, these functions are not significant, if the composition is able to exert its desired effect.

In some embodiments, compositions as disclosed herein comprising an HbF-inducing agent and/or salts thereof are also safe at effective dosages. Safe compositions are compositions that are not substantially toxic (e.g. cytotoxic or myelotoxic), or mutagenic at required dosages, do not cause adverse reactions or side effects, and are well-tolerated. Although side effects may occur, compositions are substantially safe if the benefits achieved from their use outweigh

disadvantages that may be attributable to side effects. Unwanted side effects include nausea, vomiting, hepatic or renal damage or failure, hypersensitivity, allergic reactions, cardiovascular problems, gastrointestinal disturbances, seizures, and other central nervous system difficulties, fever, bleeding or hemorrhaging, serum abnormalities, and respiratory difficulties. Preferably, compositions useful for treating blood disorders preferably do not substantially affect the viability of a blood cell such as a normal mammalian blood cell. Normal cell viability or the viability of blood cell, e.g., hematopoietic cell can be determined from analyzing the effects of the composition on one or more biological processes of the blood or hematopoietic cell. Useful combination therapies will be understood and appreciated by those of skill in the art. Potential advantages of such combination therapies include the ability to use less of each of the individual active ingredients to minimize toxic side effects, synergistic improvements in efficacy, improved ease of administration or use, and/or reduced overall expense of compound preparation or formulation.

In some embodiments, the composition comprising an HbF-inducing agent and/or salts thereof can be administered to an adult, an adolescent, a child, a neonate, an infant or in utero. In some embodiments, a composition comprising an HbF-inducing agent and/or salts thereof can be administered to a subject via a continuous infusion throughout the cycle of therapy. Alternatively, a composition comprising an HbF-inducing agent and/or salts thereof can be administered to a the subject over a single span of a few to several hours per day every day throughout the first period of the cycle of therapy. Alternatively, in some embodiments a composition comprising an HbF-inducing agent and/or salts thereof can be administered to a subject in a single parenteral bolus, or orally, daily for several days throughout the treatment regimen or cycle, or weekly.

In some embodiments, a composition comprising an HbF-inducing agent and/or salts thereof can be prepared in solution as a dispersion, mixture, liquid, spray, capsule, or as a dry solid such as a powder or pill, as appropriate or desired. Solid forms may be processed into tablets or capsules or mixed or dissolved with a liquid such as water, alcohol, saline or other salt solutions, glycerol, saccharides or polysaccharide, oil, or a relatively inert solid or liquid. Liquids, pills, capsules or tablets administered orally may also include flavoring agents to increase palatability. Additionally, in some embodiments, a composition comprising an HbF-inducing agent and/or salts thereof can further comprise agents to increase shelf-life, such as

preservatives, anti-oxidants, and other components necessary and suitable for manufacture and distribution of the composition. Compositions comprising an HbF-inducing agent and/or salts thereof can further comprise a pharmaceutically acceptable carrier or excipient. Carriers are chemical or multi-chemical compounds that do not significantly alter or affect the active ingredients of the compositions. Examples include water, alcohols such as glycerol and polyethylene glycol, glycerin, oils, salts such as sodium, potassium, magnesium, and ammonium, fatty acids, saccharides, or polysaccharides. Carriers may be single substances or chemical or physical combinations of these substances.

Administration Therapy

In some embodiments, a composition comprising an HbF-inducing agent and/or salts thereof can contain chemicals that are substantially non-toxic. Substantially non-toxic means that the composition, although possibly possessing some degree of toxicity, is not harmful to the long-term health of the patient. Although the active component of the composition may not be toxic at the required levels, there may also be problems associated with administering the necessary volume or amount of the final form of the composition to the patient. For example, if composition comprising an HbF-inducing agent contains a salt, although the active ingredient may be at a concentration that is safe and effective, there can be a harmful build-up of sodium, potassium, or another ion. With a reduced requirement for the composition or at least the active component of that composition, the likelihood of such problems can be reduced or even eliminated. Consequently, although patients may suffer minor or short term detrimental side-effects, the advantages of taking the composition outweigh the negative consequences.

Doses of administration

The amount of an HbF-inducing agent that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound that produces a therapeutic effect. Generally out of one hundred percent, this amount will range from about 0.01% to 99% of the compound, preferably from about 5% to about 70%, most preferably from 10% to about 30%. The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 (the dose therapeutically effective in 50% of the population) with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration

utilized.

The therapeutically effective dose of an HbF-inducing agent can be estimated initially from cell culture assays, for example, one can measure the percent increase in mRNA gamma-globin in the blood on administration, as disclosed herein. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the therapeutic which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Levels in plasma may be measured, for example, by high performance liquid chromatography. The effects of any particular dosage can be monitored by a suitable bioassay.

The dosage of an HbF-inducing agent may be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment. Generally, the compositions are administered so that an HbF-inducing agent or a prodrug thereof is given at a dose from 1 μg/kg to 150 mg/kg, 1 μg /kg to 100 mg/kg, 1 μg /kg to 50 mg/kg, 1 μg /kg to 20 mg/kg, 1 μg /kg to 10 mg/kg, 1 μg /kg to 1 mg/kg, 100 μg /kg to 100 mg/kg, 100 μg /kg to 50 mg/kg, 100 μg /kg to 20 mg/kg, 100 μg /kg to 10 mg/kg, 100 μg/kg to 1 mg/kg, 1 mg/kg to 100 mg/kg, 1 mg/kg to 50 mg/kg, 1 mg/kg to 20 mg/kg, 1 mg/kg to 10 mg/kg, 10 mg/kg to 100 mg/kg, 10 mg/kg to 50 mg/kg, or 10 mg/kg to 20 mg/kg. It is to be understood that ranges given here include all intermediate ranges, for example, the range 1 mg/kg to 10 mg/kg includes 1 mg/kg to 2 mg/kg, 1 mg/kg to 3 mg/kg, 1 mg/kg to 4 mg/kg, 1 mg/kg to 5 mg/kg, 1 mg/kg to 6 mg/kg, 1 mg/kg to 7 mg/kg, 1 mg/kg to 8 mg/kg, 1 mg/kg to 9 mg/kg, 2 mg/kg to 10 mg/kg, 3 mg/kg to 10 mg/kg, 4 mg/kg to 10 mg/kg, 5 mg/kg to 10 mg/kg, 6 mg/kg to 10 mg/kg, 7 mg/kg to 10 mg/kg, 8 mg/kg to 10 mg/kg, 9 mg/kg to 10 mg/kg, and the like. It is to be further understood that the ranges intermediate to the given above are also within the scope of this invention, for example, in the range 1 mg/kg to 10 mg/kg, dose ranges such as 2 mg/kg to 8 mg/kg, 3 mg/kg to 7 mg/kg, 4 mg/kg to 6 mg/kg, and the like.

In some embodiments, the compositions comprising an HbF-inducing agent are administered at a dosage so that an HbF-inducing agent or a metabolite thereof has an *in vivo,* e.g., serum or blood, concentration of less than 500 nM, less than 400 nM, less than 300 nM, less than 250 nM, less than 200 nM, less than 150 nM, less than 100 nM, less than 50 nM, less than 25 nM, less than 20, nM, less than 10 nM, less than 5 nM, less than 1 nM, less than 0.5 nM, less than 0.1 nM, less than 0.05 nM, less than 0.01 nM, less than 0.005 nM, or less than 0.001 nM

after 15 mins, 30 mins, 1 hr, 1.5 hrs, 2 hrs, 2.5 hrs, 3 hrs, 4 hrs, 5 hrs, 6 hrs, 7 hrs, 8 hrs, 9 hrs, 10 hrs, 11 hrs, 12 hrs or more of time of administration.

With respect to duration and frequency of treatment, it is typical for skilled clinicians to monitor subjects in order to determine when the treatment is providing therapeutic benefit, and to determine whether to increase or decrease dosage, increase or decrease administration frequency, discontinue treatment, resume treatment or make other alteration to treatment regimen. The dosing schedule can vary from once a week to daily depending on a number of clinical factors, such as the subject's sensitivity to an HbF-inducing agent acid. The desired dose can be administered every day or every third, fourth, fifth, or sixth day. The desired dose can be administered at one time or divided into subdoses, e.g., 2-4 subdoses and administered over a period of time, e.g., at appropriate intervals through the day or other appropriate schedule. Such sub-doses can be administered as unit dosage forms. In some embodiments of the aspects described herein, administration is chronic, e.g., one or more doses daily over a period of weeks or months. Examples of dosing schedules are administration daily, twice daily, three times daily or four or more times daily over a period of 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, or 6 months or more.

An HbF-inducing agent or a prodrug thereof can be administrated to a subject in combination with one or more pharmaceutically active agents. Exemplary pharmaceutically active compound include, but are not limited to, those found in *Harrison's Principles of Internal Medicine,* 13th Edition, Eds. T.R. Harrison *et al.* McGraw-Hill N.Y., NY; Physician's Desk Reference, 50th Edition, 1997, Oradell New Jersey, Medical Economics Co.; Pharmacological Basis of Therapeutics, 8th Edition, Goodman and Gilman, 1990; United States Pharmacopeia, The National Formulary, USP XII NF XVII, 1990; current edition of Goodman and Gilman's *The Pharmacological Basis of Therapeutics;* and current edition of *The Merck Index,* the complete content of all of which are herein incorporated in its entirety.

The following examples illustrate embodiments of the invention, but should not be viewed as limiting the scope of the invention.

Examples

Dual luciferase reporter assay

A counter-screening assay to determine the gamma-globin-specificity of hits, using an assay which measures gamma-globin gene promoter induction relative to beta-globin gene

promoter induction. This assay is fluorescence-based, and consists of a dual-luciferase reporter construct containing the LCR and beta-globin promoter linked to renilla luciferase and the A-gamma-globin promoter linked to firefly luciferase (μLCR-beta-pr-Rluc-Agamma-pr-Fluc cassette), stably-transfected into GM979 cells.

The orientation of the promoters and μLCR allowed detection only of strong, specific inducers of the gamma-globin gene promoter.

Erythroid progenitor cultures

Fetal globin-inducing activity in human erythroid progenitors was assessed. Human erythroid progenitors were cultured from peripheral blood samples, exposed to the test compounds at varying concentrations and for varying durations and analyzed for globin chain mRNA ratios. Erythroid progenitors were cultured from cord blood CD34+ cells. Briefly, CD34+ cells in the human cord blood were separated using a Ficoll-paque density gradient. CD34+ cells were cultured in H4230 medium containing 2mM L-glutamine, 1% Methylcellulose in Iscove's Medium, 30% Fetal Bovine Serum, 1% Bovine Serum Albumin and 10-4 M beta-mercaptoethanol. Methylcellulose H4230 medium was supplemented with EPO (0.5 U/ml) and IL-3 (20 ng/ml) to support BFU-e growth. Cells were cultured in 35 x 100mm mini-dishes and incubated in a humidified atmosphere containing 5% CO2, at 37°C. Different concentrations of the test compounds were added at the time the cultures were established. Each compound was tested in three different cultures. BFU-e colonies grown in mini-dishes were counted on day 14 and harvested for mRNA analysis.

mRNA analysis by real-time PCR

On day 14, RNA was extracted from cultured erythroid cells, and relative quantification PCR was performed. Briefly, cDNA was generated from equal amounts of total RNA extracted using The PerfectPure RNA Purification Kit (5 Prime Inc Gaithersburg, MD). Real-time PCR was performed using an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA). Levels of globin mRNA were calculated by the delta-delta Ct method. Isolated total RNA was used as a template for cDNA synthesis and real-time PCR was performed using appropriate primer sets. GAPDH levels were used for standardization. Western blotting nuclear extract of K562 cells and 14-day-old BFU-e were analyzed by electrophoresis using 5-24% gradient SDS-polyacrylamide mini-gels (BIORAD Laboratories. Hercules CA). Proteins in the gels were transferred to Immobilon-P membranes. Blots were then incubated with BCL-11A polyclonal

(Novus Biologicals, Littleton Co) or mouse monoclonal (Santa Cruz Biotechnology, Santa Cruz CA) antibodies, after washing blots were incubated with anti-rabbit IgG-horseradish peroxidase secondary antibody (ECL, Little Chalfont Buckinghamshire UK). BCL-11A bands were detected on the X-ray film using Western Lightning Reagents (Perking Elmer Inc., Waltham, MA).

Studies in non-human primates

Studies to evaluate pharmacokinetic properties and gamma-globin induction were performed in juvenile baboons (Papio hamadryas anubis). Briefly, animals were chronically phlebotomized on a daily basis to achieve stable anemia, maintaining a total hemoglobin level of 7.0 to 7.5 g/dl. Candidate compounds were administered intravenously or orally once daily in single doses for pharmacokinetic studies, or once daily in single doses, 4-5 days per week for 4-5 weeks, for pharmacodynamic studies. The compound Desloratadine was administered intravenously to a baboon at doses of 50 or 200 mg/kg once daily, 5 days per week for 4 weeks, to evaluate gamma-globin gene expression. MS-275 was administered intravenously at a dose of 10 mg/kg once daily, 4-5 days per week for four weeks, to assess gamma-globin gene expression in baboon 5002. Levels of gamma-globin mRNA expression and globin chain synthesis were assessed in baboons before and during treatment with test compounds. A washout period between administrations of different compounds in the same baboon was provided. MS-275 and desloratidine both induced fetal globin mRNA and total hemoglobin levels increased following MS-275 administration.

EXAMPLE 1: Benserazide is identified as a novel HbF inducing agent using a High throughput Screening Assay (HTS). Benserazide was discovered in a novel high-throughput screening (HTS) program which interrogated 3 chemical libraries of compounds and included a library of medicinal products which are already approved in USA or in the European Union (by the European Medicines Agency) for other medical indications. Prior to the HTS, the potential of Benserazide to induce HbF was unknown. Using a gamma-globin gene promoter linked to GFP (**Figure 1**), the HTS assay was adapted from low throughput to a robotic high-throughput screening system, and screening was performed over a 3–log concentration range, on the diverse chemical libraries. The screening assay was developed from a cell-based reporter, stably transfected with a construct containing the 1.4-kilobase (kb) KpnI-BglII fragment of the human locus control region (LCR) linked to the gamma-globin gene promoter and the enhanced green fluorescent protein (EGFP) reporter gene (**Figure 1B**). Because EGFP messenger RNA (mRNA)

is very stable, positive changes averaged 1.2- to 2-fold, and weak inducers are not detectable in this system. Two-fold or higher induction over control indicates strong inducers of gamma-globin gene activity. The HTS assay was developed in 96-well format on a Tecan SpectraFluor Plus, incorporating multiple positive and negative control wells in each plate. Optimal fluorescence measurements were identified. Analysis of two sets of 10,000 compounds was performed, testing over a 3-log range of concentrations. From the library of compounds and US and EMA approved medicinal products tested, a small panel of approved therapeutics were found to induce γ-globin expression (**Figure 1B**). The activity of the potential candidate compounds identified using the HTS was validated in a secondary assay using dual luciferases linked to the fetal and adult globin promoters. The secondary assay confirmed that Benserazide induces only the gamma globin promoter and not general globin promoters. The racemate form of Benserazide was selected for further analysis and was referred to as PB-04.

EXAMPLE 2: Benserazide induces a mean 42-fold induction of Fetal globin (gamma-globin) mRNA in erythroid progenitors from sickle cell and beta thalassemia patients. Erythroid progenitors were isolated from cord blood and from human patients with beta thalassaemia or sickle cell disease and CD34+ cells were enriched. CD34+ erythroid progenitors were cultured for a first phase to eliminate white cells and then in a second phase to expand and differentiate the erythroid cells in the presence of erythropoietin. This method produces 95% erythroid cells. mRNA was extracted after 12 days and real-time RT-PCR was performed for expression levels of gamma-globin mRNA. Briefly, cDNA was generated from equal amounts of total RNA extracted using the PerfectPure RNA purification kit (5 Prime Inc. Gaithersburg, MD) or RNA STAT-60 isolation reagent (Teltest, Friendswood TX). Real-time PCR was performed using an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA). Levels of gamma-globin mRNA were calculated by the ΔΔCt method. GAPDH and 18S levels were used for standardization. Benserazide caused a mean 42-fold increase in fetal globin (gamma-globin) mRNA in human erythroid progenitors (**Figure 2**), which is significantly higher than the magnitude of induction by other known fetal globin inducing agents, which only caused a 2-6.5 fold increase in gamma-globin mRNA.

EXAMPLE 3: In vivo results: Anemic Baboon Study. Benserazide and Desloratidine (DLT) induce a 33-fold and 11-fold increase in fetal Globin Induction respectively, and increase erythroid cell production in anemic baboons. The effect of Benserazide to induce fetal globin

was assessed in vivo in a baboon model of anemia. This baboon model (Pace et al., 2002) requires that anemia be present for induction of gamma-globin to be detected. One juvenile baboon (Papio hamadryas anubis) was chronically phlebotomized (by 3.7 to 5 mL/kg/day) to achieve and maintain stable anemia with total hemoglobin of 7.0 to 7.5 g/dl, a level necessary for the modulation of globin genes in this species. The animals were supplemented with iron dextran and folic acid to support active erythropoiesis. The phlebotomy regimen effectively exchanged the animals' total blood volume every 10 to 20 days. Assays of gamma-globin mRNA expression were performed with the approval of the corresponding animal research institutions. The drug candidate was administered based on doses adjusted for more rapid metabolism in the baboon, but projected as equivalent to doses previously used or studied in humans as follows: Benserazide was administered orally, at 1 mg/kg for 4 days/week and 2 mg/kg for two weeks. A washout period was provided between administrations of different compounds in the same baboon. Levels of gamma-globin mRNA expression, total hemoglobin, and % F-cells were assessed before and during treatment with test compounds. Benserazide was compared to 2 other candidates (i) desloratadine (DLT), which was administered orally (0.5 mg/kg/dose), three times a week over two weeks, and (ii) MS-275, which was administered orally three times a week for two weeks at 0.2 mg/kg/dose. Doses of Benserazide are shown in the bars above the graph, with Benserazide administered at 1 mg/kg (Open squares) and 2 mg/kg (dark squares). The time course is consistent with the time required for new red blood cells to develop and enter the circulation. A lag follows each time-off the drug. Gamma globin mRNA was analyzed by RT-PCR prior to drug administration, 3 times/week during and following drug administration, and change from baseline was assessed. Changes in gamma-globin mRNA is shown in **Figure 3A**. Benserazide resulted in up to 33-fold induction of gamma-globin mRNA in vivo. Benserazide also caused an increase in total hemoglobin (Hb) (by 1.5g/dL) (**Figure 3B**) despite the daily phlebotomy, indicating that Benserazide has an independent effect on erythropoiesis, which is also beneficial in the treatment of patients with beta-thalassemia and sickle cell disease. Furthermore, both Benserazide (BEN) and Desloratidine (DLT) resulted in a mean increase in fetal globin mRNA in vivo in the Anaemic Baboon model, with Benserazide inducing a mean 27-fold increase, and Desloratidine (DLT) inducing a 11-fold increase above baseline (**Figure 4**), both of which are significantly higher than other known inducers of fetal globin (Hydroxyurea (HU) and ST20 (dimethylbutyrate)), which induced only a 1.5-3.5-fold increase of fetal globin mRNA above the

baseline.

EXAMPLE 4: In vivo results: Anemic Baboon Study. Benserazide results in a 15% increase the number of F-reticulocytes in Anemic Baboons. F-reticulocytes of Benserazide treated anemic baboons were analyzed using flow cytometry. The peripheral blood of the baboons was analyzed using Flow cytometry to detect HbF protein and proportions of cells expressing fetal globin protein as previously described (Pace, 2002). Briefly, cells were washed with PBS containing 0.1% BSA, and fixed. After washing twice, the cells were permeabilized in PBS with 0.1% Triton-X100 and incubated. Cells were then washed, resuspended in PBS containing 0.1 % BSA. PerCP isotype labeled and unstained cells were used as controls. Thiazole orange was used to determine proportions and populations of reticulocytes. A custom-synthesized PerCP mouse anti-human antibody that detects baboon F-cells was used to label fetal globin-containing cells. Samples were incubated in the dark at room temperature for 30 min, washed several times with PBS containing 0.1 % BSA and analyzed by flow cytometry on a FACScalibur (Becton Dickinson, Franklin Lakes, NJ). **Figure 5** shows that Benserazide increases F-reticulocytes by 15% following the 2 mg/kg dose. The time course of response is consistent with the time required for new red blood cells to mature and enter the circulation.

EXAMPLE 5: In vivo results: Transgenic Murine Study. Benserazide induces human γ-globin gene expression in a mouse model. The effect of Benserazide was compared to a known Hb inducer (Hydoxyurea (HU) in a transgenic mice model comprising the entire human non-alpha (gamma delta beta-globin) gene locus. Mice transgenic for the human beta-globin gene locus including the locus control region (LCR) were previously described (Pace 2002). Mice were treated with either (i) hydroxyurea (HU) - administered at 100 mg/kg/dose once daily for 5 days/week, or (ii) Benserazide - administered at 2 mg/kg/dose, 3 times per week for 5 weeks or (iii) water in the same volume (100 microliters) as the drug candidates as a negative control. Dosing was performed by intraperitoneal injection (i.p) to ensure consistent drug delivery. Blood was sampled for globin mRNA and for F-cell quantitation and fluorescent intensity of F-cells by flow cytometry. Total hemoglobin (Hb) was assayed on a Horiba ABX60 at baseline, 2 and 5 weeks (sampling was limited due to constraints on amount of blood that can be withdrawn safely from mice). Benserazide induced a mean increase in total hemoglobin (Hb) at 5 weeks, which was significantly better as compared to mice administered hydroxyurea (HU), (the only FDA approved drug for fetal globin induction) (**Figure 6A**). Importantly, Benserazide induced an

absolute 13% mean increase in the number of F-cells (from 0.1% to 9% (mice #1), 0.4% to 18% (mice #2), and 0.13% to 12% (mice #3); and 10 to 33-fold increase in mean fluorescence intensity (MFI) (**Figure 6B**). Responses were observed within one week, and continually increased in the Benserazide treated mice, whereas the percentage of F-cells declined in the Hydroxyurea-treated mice.

Figure 9 depicts a comparison of the magnitude of fetal globin mRNA induction over baseline levels in anemic baboons by new drugs compared to hydroxyurea (HU) (**Figure 9A**), and proportions of new red blood cells with fetal globin protein, F-reticulocytes, (**Figure 9B**). Reponses are significantly higher than those induced by the only US and EMA-approved drug, hydroxyurea. F-reticulocytes are 3 to 5 times higher with the new drugs than induced by hydroxyurea.

Figures 10A-F depict Benserazide reduction of binding of two repressors, LSD1 and HDAC3, to the fetal globin gene promoter in erythroid progenitors from sources as indicated.

Figure 11 depicts the suppression of repressor proteins BCL11A and KLF1 in different erythroid progenitors treated with new drugs as compared with untreated control levels by Western blot analysis.

Prior Experience in Development of Fetal Globin-Inducers and Selection of Higher Potency Candidates

Three lead drug candidates are expected to have higher clinical activity than prior-generation HbF-inducers. This expectation is based on their activity in multiple assays shown to be clinically predictive for prior-generation drugs. The first-generation inducer, Arginine Butyrate, induced beta-globin expression 3-fold in sickle cell patients from a mean baseline HbF of 7% to mean 21% and significantly increased HbF and total Hb levels (by a mean 2.8 g/dl) in subjects with beta-thalassemia and rendered some beta-thalassemia patients transfusion-independent for greater than 7 years. However, arginine butyrate (AB) is rapidly-metabolized, requires intravenous (IV) infusions which are difficult long-term, and is also a broad HDAC inhibitor, suppressing cell proliferation. Experience with this therapy demonstrated the importance of preserving erythroid cell proliferation for optimal HbF induction. This fundamental principle is important for successful application of any fetal globin induction therapy. Next-generations of oral derivatives have rapidly induced HbF in short Phase I trials,

(compared to the 6 months required for HU effects); SDMB increased HbF by a mean of 9% (range 5-21%) in beta-thalassemia within 8 weeks. Yet, higher potency inducers are likely needed for the most severe patients. Inducers of fetal globin structurally-unrelated compounds which actively induce the gamma-globin genes *in vitro* and *in vivo*. From a library of 13,000 structurally unrelated compounds tested against a putative receptor site, 2 candidates, RB16 and RB7 have 5- to 7-fold higher potency and favorable PK profiles in primates. RB7 is particularly novel, in that it displaces a repressor complex containing HDAC3 and recruits EKLF to the promoter. Yet all such new chemical entities must pass daunting, lengthy, and costly toxicology and manufacturing hurdles, and may have unknown safety issues.

The system utilizes a stably-transfected construct containing the human LCR linked to the gamma-globin gene promoter and the enhanced green fluorescent protein (EGFP) reporter gene, and was adapted from low-throughput to high-throughput application. The HTS assay also allowed an estimate of the cytotoxicity of the tested compounds. Because EGFP mRNA has long stability, positive changes average 1.2- to 2-fold; weak inducers are not detectable in this system; and 2-fold or higher induction indicates very strong inducers of beta-globin promoter activity. Two campaigns screening 10,000 compounds were performed. From the "FDA-approved compound" library, a number of hits were identified, of which a few showed higher potency than butyrates in secondary, confirmatory assays. Several were eliminated as hemoglobinopathy therapeutics because of cytotoxicity *(e.g.,* Idarubicin) or the need for parenteral administration, (but these were nonetheless validated in confirmatory assays as potent HbF-inducers). Follow-on confirmatory assays included a dual-luciferase reporter assay that analyzes specificity by comparative induction of the gamma- *vs* beta-*g*lobin promoters, globin mRNA analyses by RT-PCR, and initial erythroid cultures from normal subjects with low baseline HbF Bfu-e; these published assays are validated for their correlation with subsequent clinical activity in humans, with induction in Bfu-e having greater correlation than reporter assays.

Significantly, the HTS assay identified 10 drugs, already FDA-approved by the U.S. or Canadian drug authorities for other indications (and confirmed in secondary assays) as potent inducers of gamma-globin gene expression (active at 2-3 logs lower concentration than butyrate, and generating higher induction of gamma-globin mRNA in human erythroid progenitors). These drugs hits are 3-8 times more potent than an oral SCFAD HbF inducer currently in clinical trials (SDMB). Three drugs were selected with high potency in specifically inducing the gamma-

globin promoter. One (MS-275) also suppresses a repressor of gamma-globin transcription, *BCL-11A*. Two of the 3 are approved in young children, which is important as organ damage in these diseases begins in childhood.

One issue which confounds prediction of clinical efficacy in the beta-globin disease population, however, is patient variability in HbF genetic modifier profiles and resulting baseline HbF levels. Such variables can only be studied in erythroid cells cultured from beta-hemoglobinopathy patients. Analysis of the effects of the 3 lead drug candidates in genotyped hemoglobinopathy patient erythroid progenitor cultures are used for final selection of the lead compound for clinical development. This selected therapeutic can then immediately be evaluated in Phase 2 clinical trials in patients with b-globin diseases, without need for the costly (and "high risk") preclinical toxicology, mutagenicity, and manufacturing development necessary for a new chemical entity.

The targeted therapeutic action is induction of high-level expression of fetal globin, without concomitant erythroid cell cytostasis or cytotoxicity. Two clinically-approved therapeutics (desloratidine and benserazide) and a third extensively clinically-studied therapeutic, MS-275, were selected for evaluation in patients' erythroid progenitors. The agents have shown 5-fold gamma-globin induction in erythroid cells cultured from normal subjects, compared to untreated controls from the same subjects, and each had greater effects than induced by HU or butyrate. Because hemoglobinopathy patients (as opposed to cultures from normal subjects) have a wide range of basal HbF levels which may affect the magnitude of responses to therapeutics. These agents augment HbF expression to the greatest degree in erythroid progenitors cultured from patients with lower baseline HbF levels.

The 3 preferred oral therapeutics include:

- **Desloratidine (DLT):** A drug requiring prescription for treatment of allergic symptoms, with a benign safety profile in extended use. In baboon studies, this agent induced HbF within 4 days of administration to a higher degree than almost all other test agents within the same animal..
- **Benserazide (BEN):** Approved in a combination formulation to enhance the PK profile of L-dopa, and reported to have no clinical effects itself. No adverse effects of this therapeutic or drug interactions have been identified. A proprietary extended-release formulation is developed. This drug has never been approved for any use except in combination with other

drugs.

- **MS-275**, an oral, class I-specific HDAC inhibitor. Its primary adverse effect is fatigue, but it is administered infrequently, once every 2 weeks, and is therefore worthwhile to evaluate. It does not have adverse cardiac side effects of many pan-HDAC inhibitors. MS-275 suppresses expression of *BCL-11A,* a transcriptional repressor which down-regulates beta-globin transcriptional activity. As (pan-)HDAC inhibitors inhibit erythroid cell proliferation, MS-275 would be given in an intermittent or pulsed regimen in patients. Of the 3 leading candidates, BEN is a preferred pharmaceutical agent as it has slight pro- erythropoietic activity. DLT is another preferred agent as stable GMP formulations for extended release are available.

Erythroid progenitor cultures

Three drugs/compounds are investigated for their efficacy in up-regulating HbF synthesis in erythroid progenitor culture experiments (**Figure 2**). Progenitor cultures in patients were predictive of subsequent HbF response rates in patients to prior-generation inducers which have been less effective in the baboon than the newly discovered agents. Peripheral blood is collected from (de-identified) sickle cell patients given a unique patient identifier number (UPIN), and in whom alpha- and beta-globin genotypes, and the status of the 3 genetic modifiers (QTL) are documented. Erythroid progenitors are cultured +/- the 3 candidate drugs and vehicle controls, and +/- HU, at the optimal concentrations established. Two drugs which have shown activity in clinical trials (Arginine butyrate and hydroxyurea) are used as positive controls for comparison with the candidate drugs. The gamma-globin induction assays are performed on erythroid progenitors cultured from 3 groups of at least 15 genotyped sickle cell patients, stratified for baseline HbF in 3 ranges: low (less than 2%), average HbF (3-8%), and the typical range in HU-responsive adults (8-11%). HbF levels are obtained on the submitted de-identified samples by HPLC. Approximately 8 samples with baseline HbF levels greater than 15% may also be analyzed. Analyses is performed as previously described on cultured progenitors (Boosalis, 2001; 2011) for: (i) gamma-globin mRNA, by quantitative RT-PCR; (ii) F-cells by flow cytometry; (iii) HbF by reverse-phase HPLC; and (iv) erythroid colony size (mean cells/colony) and Bfu-e number/ per CD34 or mononuclear cells cultured.

The magnitude of positive changes in these parameters, in absolute terms and as a percent of baseline, and the proportion of subjects' cultures in which a positive change occurs, are compared to the subjects' untreated control progenitors, and +/- addition of HU. Descriptive

statistics are used to assess differences between treated and untreated control progenitors from the same subjects. 1) highest magnitude of HbF induction above baseline in low and moderate basal HbF patient groups; and, 2) the highest proportion of positive responses, defined as at least a 5% absolute increase compared to untreated control. Paired-tests and Fisher's exact test (GraphPad Prism V software) are used to determine if significant differences are produced in% F-cells, HbF, and gamma-globin mRNA parameters, compared to levels in the same subjects' vehicle-treated control cultures. Whether addition of HU with test drugs results in higher induction compared to cultures treated with HU alone, or with test drugs alone, are statistically evaluated; t-tests and Wilcoxon tests for paired differences and other tests as indicated are used. Positive changes in greater than 35% of cultures are based on positive changes in 75% of progenitors cultured from normal subjects; however, the magnitude of change in hemoglobinopathy patients' progenitors is much more likely significant. All drug candidates should increase HbF expression in greater than or equal to 60% of subjects' colonies, based on response rates in cells cultured from normal subjects. As growth of erythroid cells is inhibited by HU, and by HDAC inhibitors to a lesser degree, HU (positive control) are utilized at low nanomolar concentrations. If colony growth is severely suppressed by HU, it may be added transiently. Selection is based on the agent that produces the greatest magnitude of HbF induction compared to untreated controls in cultures from the low and moderate baseline HbF levels. If there is no significant difference between the magnitude of HbF responses, or the response rates with the different drugs, selection of the clinical candidate are based on effects on erythroid proliferation and side-effect profile (favoring DLT or Benserazide), and the most rapid regulatory path (DLT).

Formulation of the drug Desloratidine may include higher dose requirements than required for allergy treatment. Benserazide may include extended-release preparations based on its published pharmacokinetics. Benserazide is currently formulated commercially in combination with L-dopa. Because of its solubility profile, Desloratidine is prepared in a proprietary medicinal formulation as a solution or tablet. Validated assays for the analytical work are established for the required formulation development for all 3. Formulation developments include pH solubility and pH stability profiles; selection of buffer and buffer strength; selection of formulation type-solution, tablet, immediate or extended release; selection of co-solvents and complexing agents, *(e.g.,* for extended release, Benserazide); selection of preservatives based on

pH; selection of sweeteners and flavoring agents (from a panel of 15 taste-masking agents); composition of final formulation (active pharmaceutical ingredient [API], excipients, preservatives); dissolution of any extended release formulation; establishment of specifications for future batches, including sterility testing; accelerated stability study.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. All references cited herein, including all publications, U.S. and foreign patents and patent applications, are specifically and entirely incorporated by reference. The term comprising, where ever used, is intended to include the terms consisting and consisting essentially of. Furthermore, the terms comprising, including, containing and the like are not intended to be limiting. It is intended that the specification and examples be considered exemplary only with the true scope and spirit of the invention indicated by the following claims.

Claims

1. Benserazide for use as a fetal hemoglobin inducing agent in the treatment of a blood disorder.

2. The agent of claim 1, wherein the blood disorder is one or more of a sickle-cell syndrome, sickle cell disease, HbSS, HbSC, HbS/beta-thalassemia, HbS-OArab, a beta-thalassemia syndrome, beta-zero-thalassemia, beta plus thalassemia, thalassemia major, thalassemia intermedia, HbE/beta-thalassemia, delta/beta-thalassemia, or a globin gene mutation disease.

3. The agent of claim 1, which is formulated to increase the percentage of fetal hemoglobin in the blood of a patient or proportion of fetal hemoglobin in relation to non-fetal hemoglobin.

4. The agent of claim 3, wherein the formulation comprises an amount of agent that, 10 minutes or more after administration to the patient, produces a blood concentration of at least 1 nM.

5. The agent of claim 3, wherein the formulation comprises an amount of agent that, 10 minutes or more after administration to the patient, produces a blood concentration in the patient of at least 0.2 μM.

6. The agent of claim 1, wherein the amount of fetal hemoglobin in the blood increases by two percent or more within 120 days.

7. The agent of claim 1, which is a salt, an acid, an ester, an amine, an aldehyde, a ketone, an amide, an alcohol, a carbonyl, an amine, an alkene, a polymorph or a combination thereof.

8. The agent of claim 1, which, at ambient temperature, is a liquid, a suspension, a powder, a gel or a combination thereof.

9. The agent of claim 1, which is formulated for administration to the patient orally, by injection, by suppository or by a transdermal patch.

10. The agent of claim 1, which is formulated to be administered as a single dose, continuously, in pulsed doses or by intermittent or non-continuous doses.

11. The agent of claim 1, which increases the total hemoglobin or the hematocrit value of the blood.

12. The agent of claim 1, further comprising a second fetal hemoglobin inducing agent or an erythropoietic agent.

13. A pharmaceutical composition comprising benserazide and a pharmaceutically acceptable carrier formulated for oral, injectable, transdermal or rectal administration to a patient for treatment of a blood disorder.

14. A method for increasing the percentage of fetal hemoglobin or proportion of fetal hemoglobin in relation to non-fetal hemoglobin in the blood of a patient for treating or preventing a blood disorder comprising administering a therapeutically effective dose of a composition containing benserazide.

15. The method of claim 14, wherein the blood disorder is one or more of a sickle-cell syndrome, sickle cell disease, HbSS, HbSC, HbS/beta-thalassemia, HbS-OArab, a beta-thalassemia syndrome, beta-zero-thalassemia, beta plus thalassemia, thalassemia major, thalassemia intermedia, HbE/beta-thalassemia, delta/beta-thalassemia, or a globin gene mutation disease.

16. The method of claim 14, wherein the composition is administered as a liquid, a suspension, a powder, a gel or a combination thereof.

17. The method of claim 14, wherein the composition is administered as a single dose, continuously, in pulsed doses or by intermittent or non-continuous doses.

18. A benzamide histone deacetylase inhibitor for use as a fetal hemoglobin inducing agent in the treatment of a blood disorder.

19. The agent of claim 18, which is entinostat (MS-275).

20. The agent of claim 18 or 19, wherein the blood disorder is one or more of a sickle-cell syndrome, sickle cell disease, HbSS, HbSC, HbS/beta-thalassemia, HbS-OArab, a beta-thalassemia syndrome, beta-zero-thalassemia, beta plus thalassemia, thalassemia major, thalassemia intermedia, HbE/beta-thalassemia, delta/beta-thalassemia or a globin gene mutation disease.

21. The agent of claim 18 or 19, which is formulated to increase the percentage of fetal hemoglobin in the blood of a patient or proportion of fetal hemoglobin in relation to non-fetal hemoglobin.

22. The agent of claim 21, wherein the formulation comprises an amount of agent that, 10 minutes or more after administration to the patient, produces a blood concentration of at least 1 nM.

23. The agent of claim 21, wherein the formulation comprises an amount of agent that, 10

minutes or more after administration to the patient, produces a blood concentration in the patient of at least 0.2 μM.

24. The agent of claim 18 or 19, wherein the amount of fetal hemoglobin in the blood increases by two percent or more within 120 days.

25. The agent of claim 18 or 19, which is a salt, an acid, an ester, an amine, an aldehyde, a ketone, an amide, an alcohol, a carbonyl, an amine, an alkene, a polymorph or a combination thereof.

26. The agent of claim 18 or 19, which, at ambient temperature, is a liquid, a suspension, a powder, a gel or a combination thereof.

27. The agent of claim 18 or 19, which is formulated for administration to the patient orally, by injection, by suppository or by a transdermal patch.

28. The agent of claim 18 or 19, which is formulated to be administered as a single dose, continuously, in pulsed doses or by intermittent or non-continuous doses.

29. The agent of claim 18 or 19, which increases the total hemoglobin or the hematocrit value of the blood.

30. The agent of claim 18 or 19, further comprising a second fetal hemoglobin inducing agent or an erythropoietic agent.

31. A pharmaceutical composition comprising a benzamide histone deacetylase inhibitor and a pharmaceutically acceptable carrier formulated for oral, injectable, transdermal or rectal administration to a patient for treatment of a blood disorder.

32. The pharmaceutical composition of claim 31, wherein the benzamide histone deacetylase inhibitor is entinostat (MS-275).

33. A method for increasing the percentage of fetal hemoglobin or proportion of fetal hemoglobin in relation to non-fetal hemoglobin in the blood of a patient for treating or preventing a blood disorder comprising administering a therapeutically effective dose of a composition containing benzamide histone deacetylase inhibitor.

34. The method of claim 33, wherein the benzamide histone deacetylase inhibitor is entinostat.

35. The method of claim 33 or 34, wherein the blood disorder is one or more of a sickle-cell syndrome, sickle cell disease, HbSS, HbSC, HbS/beta-thalassemia, HbS-OArab, a beta-thalassemia syndrome, beta-zero-thalassemia, beta plus thalassemia, thalassemia major,

thalassemia intermedia, HbE/beta-thalassemia, delta/beta-thalassemia, or a globin gene mutation disease.

36. The method of claim 33 or 34, wherein the composition is administered as a liquid, a suspension, a powder, a gel or a combination thereof.

37. The method of claim 33 or 34, wherein the composition is administered as a single dose, continuously, in pulsed doses or by intermittent or non-continuous doses.

38. A pharmaceutical composition comprising one or more of ambroxol, desloratadine, resveratrol or NSC-95397, and a pharmaceutically acceptable carrier formulated for oral, injectable, transdermal or rectal administration to a patient for treatment of a blood disorder.

39. The pharmaceutical composition of claim 29, wherein the blood disorder is one or more of a sickle-cell syndrome, sickle cell disease, HbSS, HbSC, HbS/beta-thalassemia, HbS-OArab, a beta-thalassemia syndrome, beta-zero-thalassemia, beta plus thalassemia, thalassemia major, thalassemia intermedia, HbE/beta-thalassemia, delta/beta-thalassemia or a globin gene mutation disease.

40. A method for inducing fetal hemoglobin in a patient to treat or prevent a blood disorder comprising administering a therapeutically effective dose of a composition containing one or more of ambroxol, desloratadine, resveratrol or NSC-95397, and a pharmaceutically acceptable carrier formulated for oral, injectable, transdermal or rectal administration to a patient for treatment of a blood disorder.

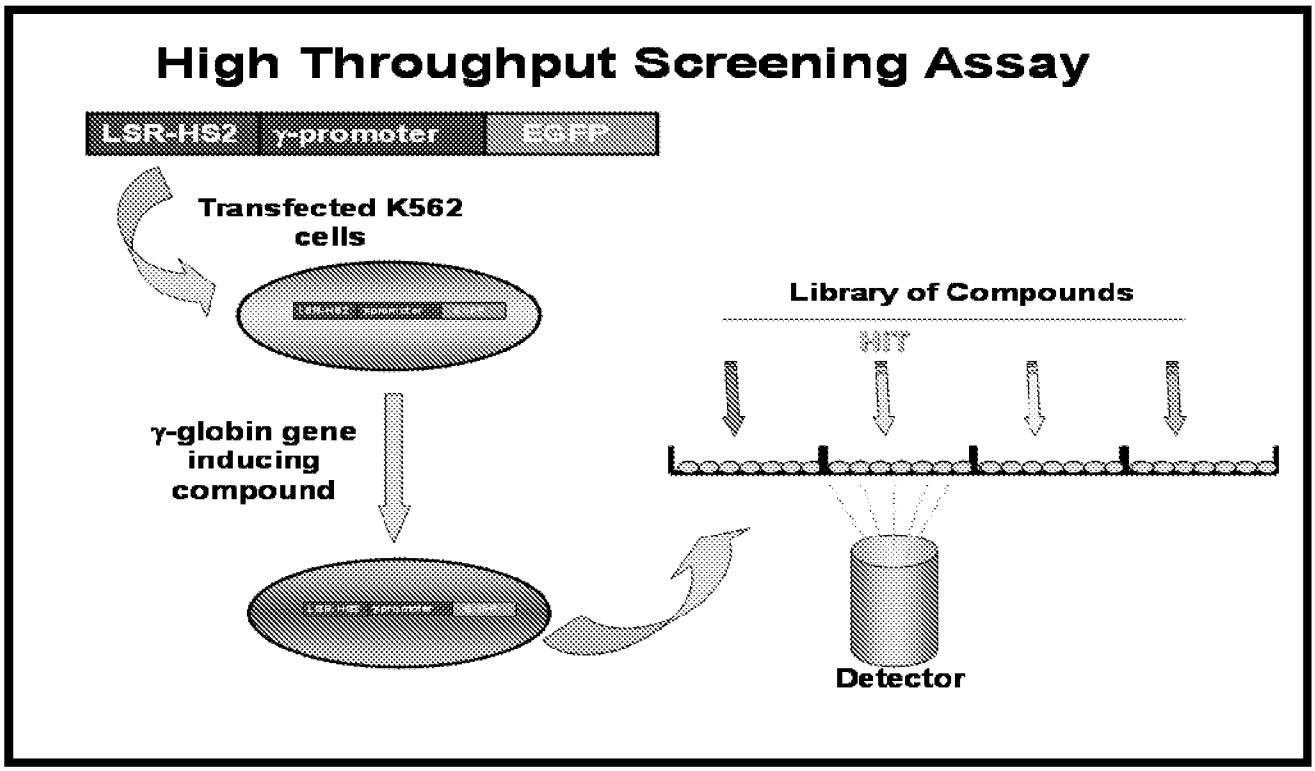


FIGURE 1A

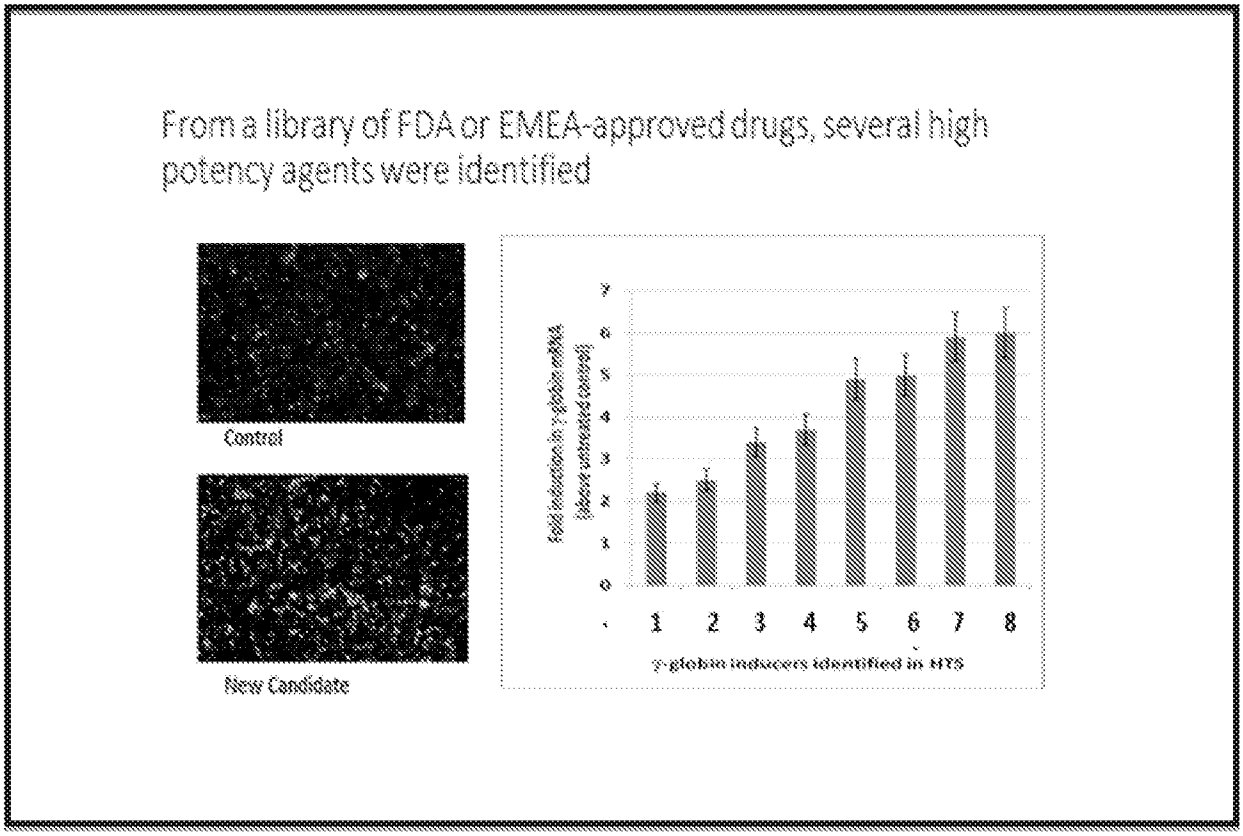


FIGURE 1B

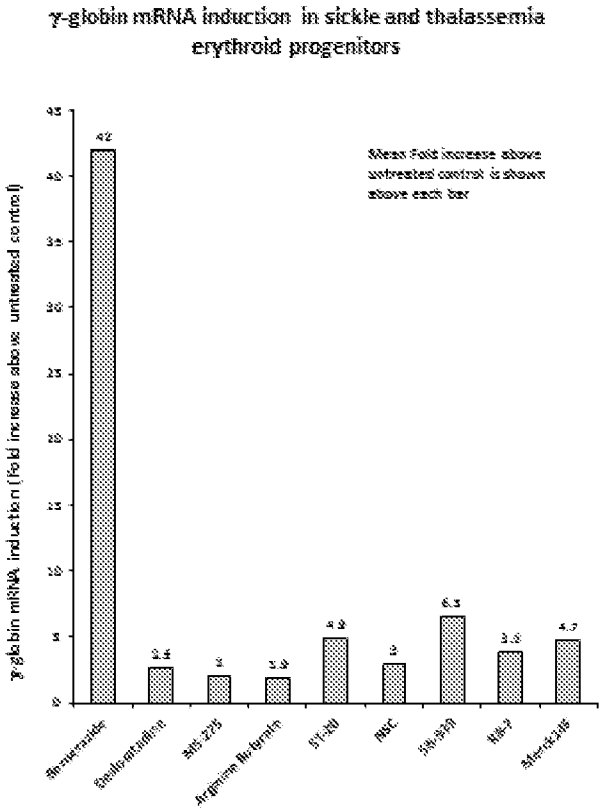


FIGURE 2

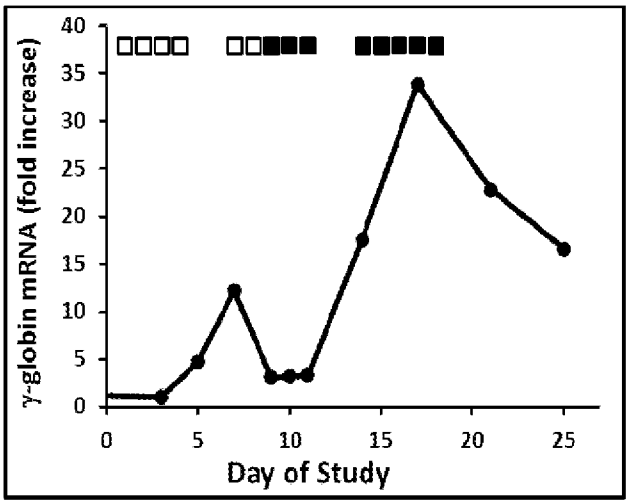


FIGURE 3A

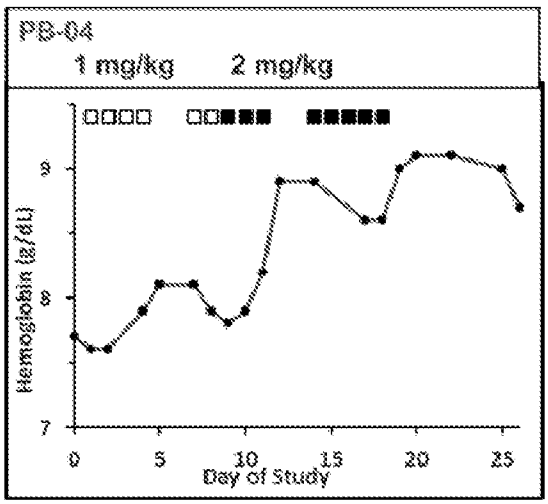


FIGURE 3B

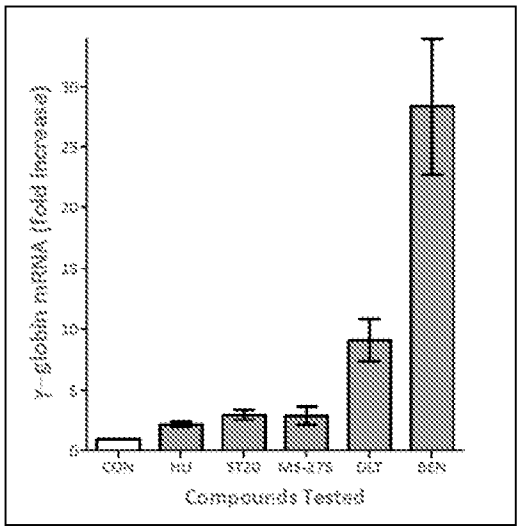


FIGURE 4

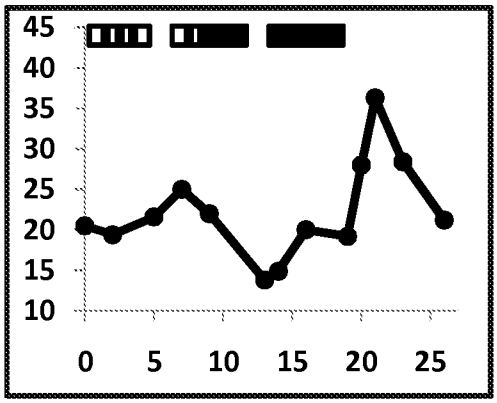


FIGURE 5

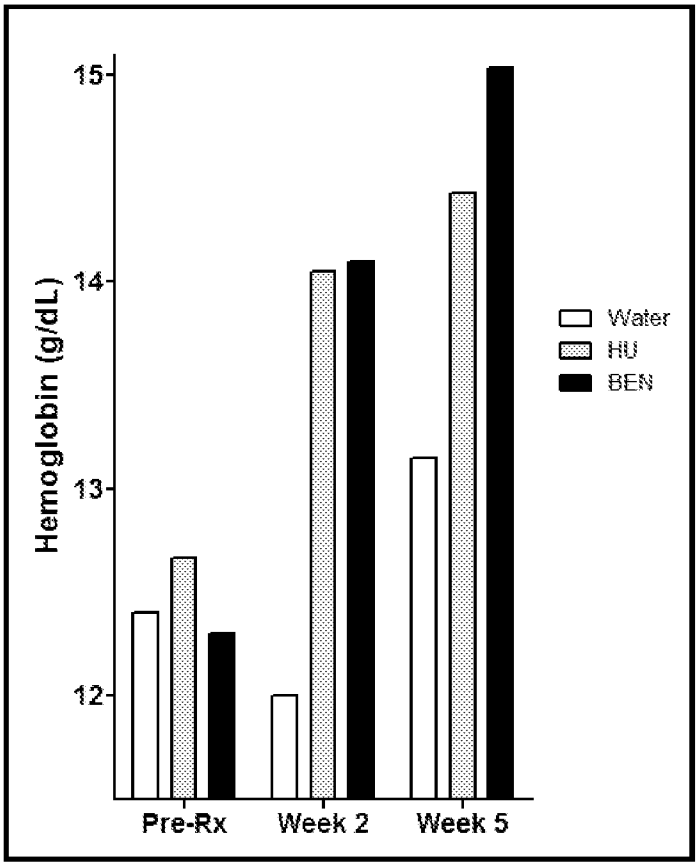
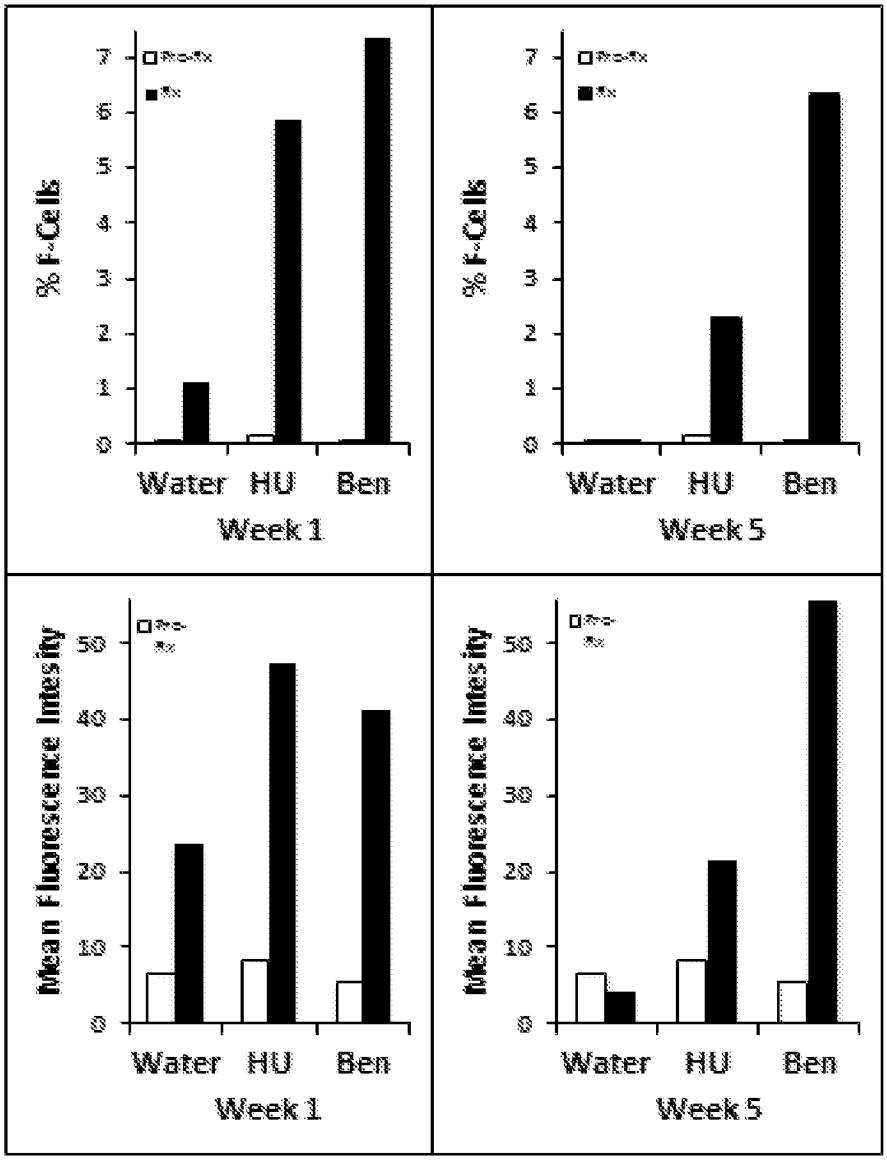


FIGURE 6A


% F-Cells
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Mean Fluorescence Intesity
Water
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Ben
Week 5

FIGURE 6B

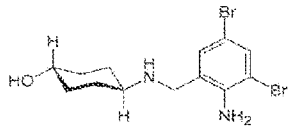
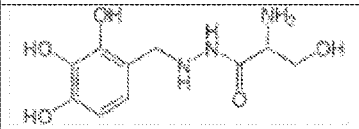
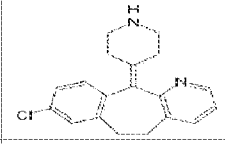
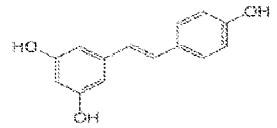
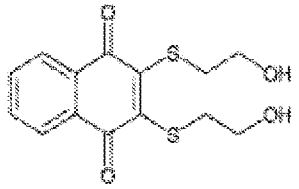
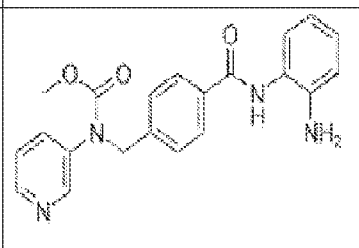
Ambroxol	A mucolytic agent which has extensive clinical experience and a benign safety profile.	
Benserazide	Used in a combination to enhance the PK profile of L-dopa, and is reported to have no clinical effects itself.	
Desloratadine	This therapeutic is approved in children and adults as an allergy medication. Its safety profile is benign in extensive clinical use	
Resveratrol	This is a natural substance identified from red wine and has been studied clinically in cancer trials and for anti-oxidant actions	
NSC-95397	This phosphatase inhibitor is not currently a clinical stage agent and may have cytotoxicity,but is a known bioactive. It will undergo *in vitro* evaluation, although it may not advance to *in vivo* studies	
MS-275	An oral pan-HDAC inhibitor, was initially developed by the NCI and has been studied in many oncology trials. The major adverse effect is fatigue, but it can be administered infrequently, eg, once every 2 weeks and is therefore worthwhile to evaluate. It does not have the cardiac side effects of many HDAC inhibitors.	

FIGURE 7: Structures of the compounds named in this application

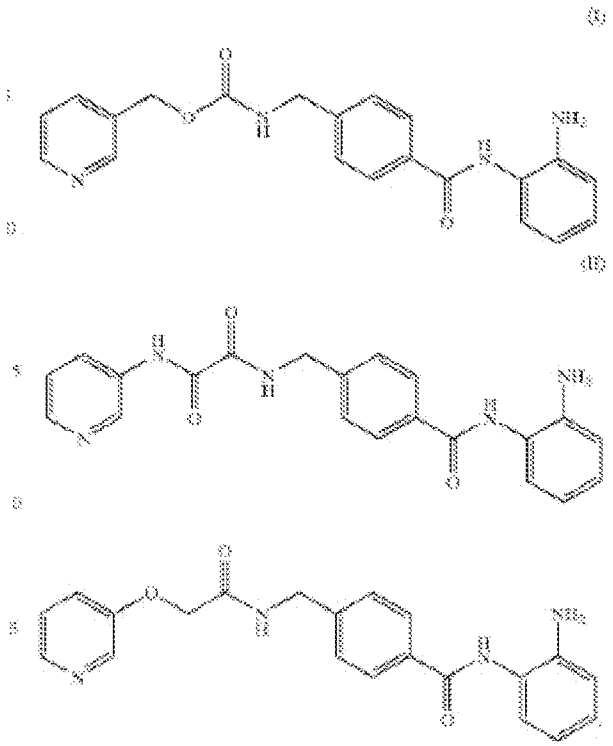


FIGURE 8: Forms of MS-275

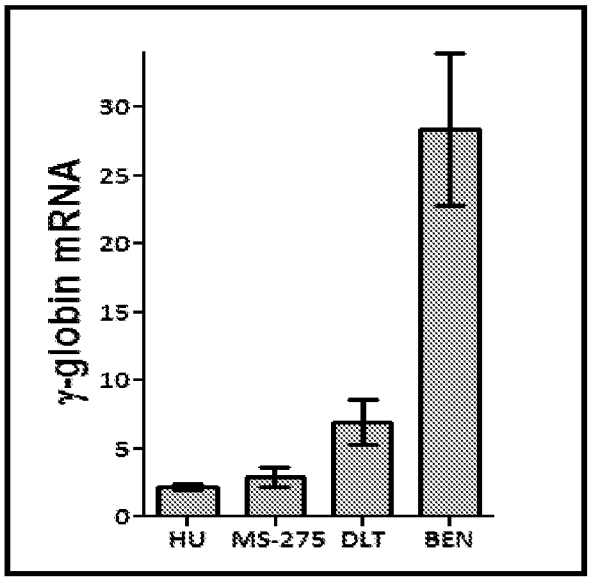


FIGURE 9A

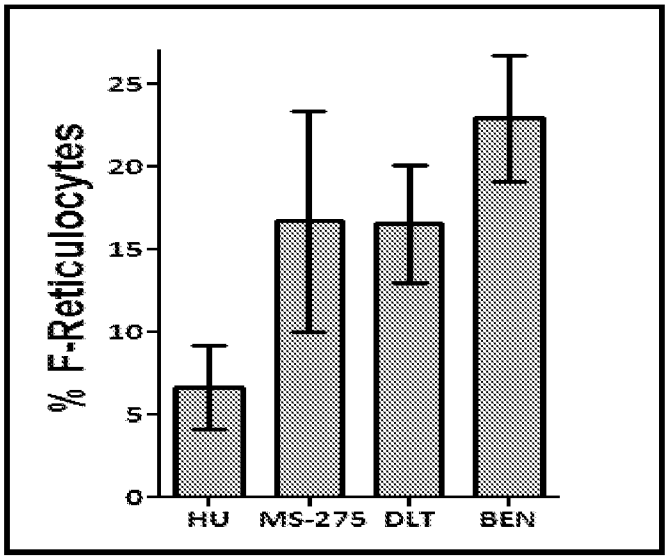


FIGURE 9B

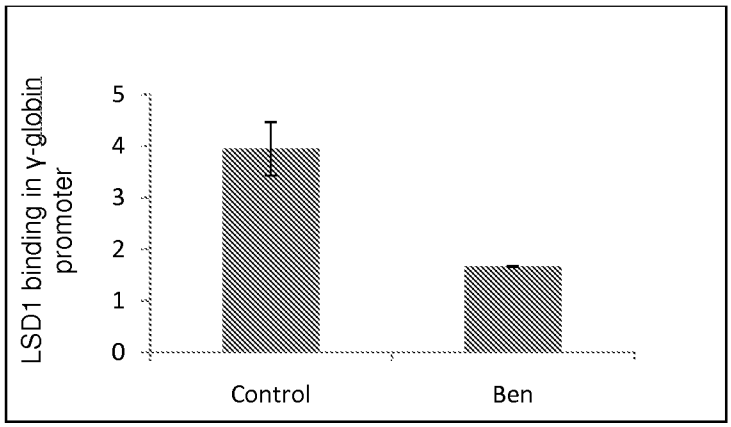


FIGURE 10A

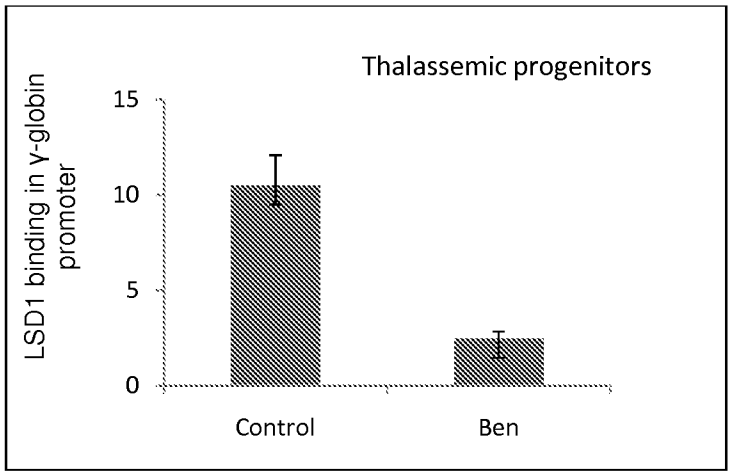


FIGURE 10B

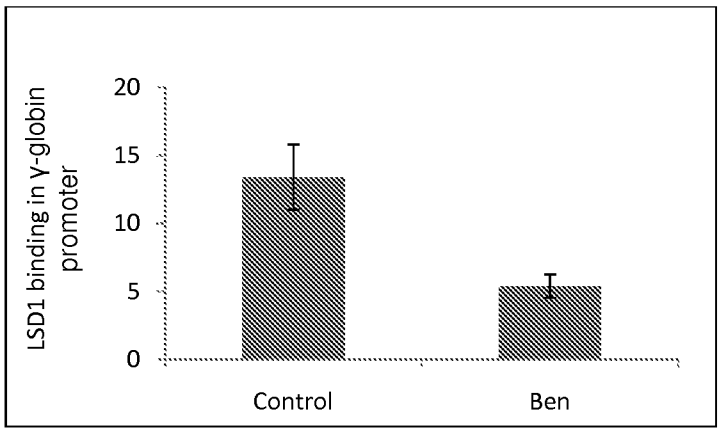


FIGURE 10C

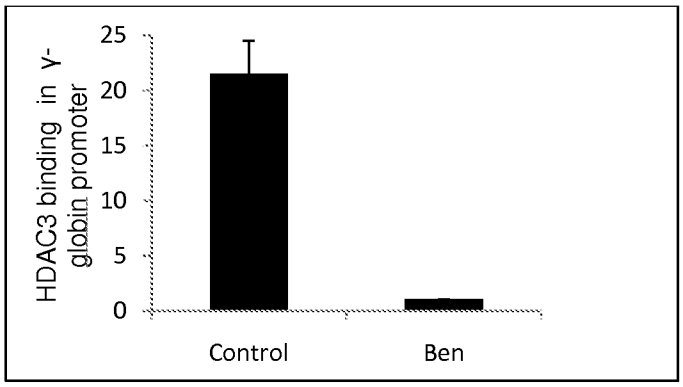


FIGURE 10D

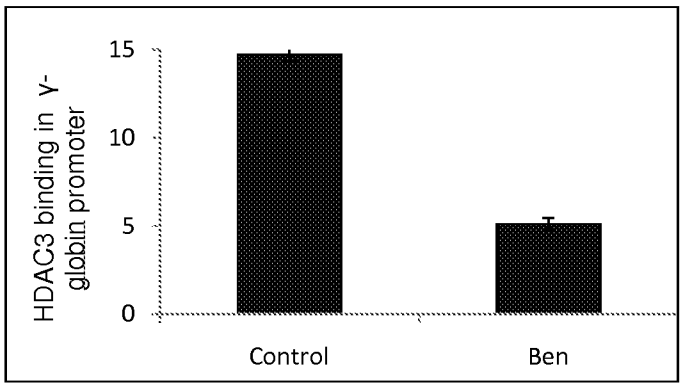


FIGURE 10E

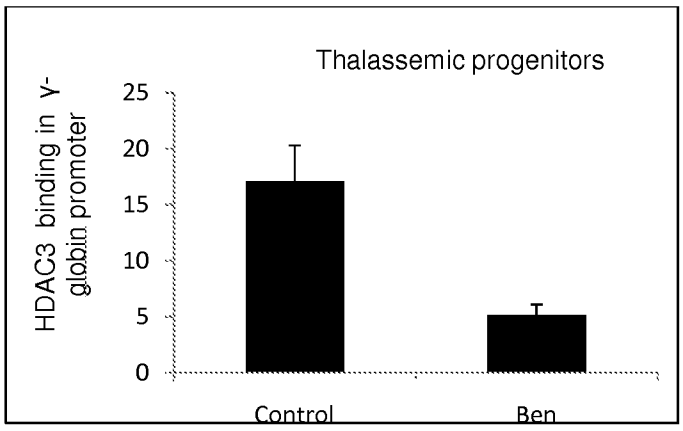


FIGURE 10F

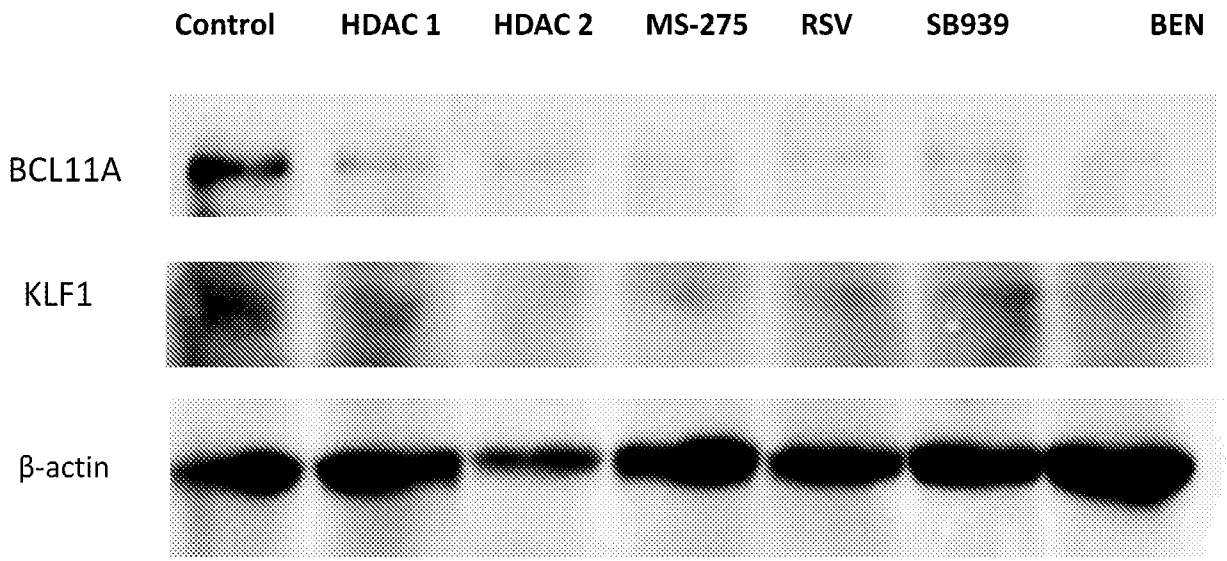


FIGURE 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/051887

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/42 (2014.01)
CPC - A61K 38/42 (2014.09)
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 31/4427, 35/18, 38/42 ; C07C 215/28, 229/34
USPC - 435/7.25, 372, 375 ; 514/13.5, 357, 565, 646, 649

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 31/4427, 35/18, 38/42, 2035/124 ; C12N 5/0641 ; C07C 215/28, 229/34 (keyword delimited)

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

Orbit, Google Patents, Google Scholar, Google; Search terms used: fetal hemoglobin, inducer, Benserazide, entinostat, MS-275, ambroxol, desloratadine, resveratrol, NSC-95397, histone deacetylase inhibitor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	SANGERMAN et al. "Identification of New and Diverse Inducers of Fetal Hemoglobin with High Throughput Screening (HTS)," Amer. Soc. Hematology, 56th Annual Meeting and Exposition, San Francisco, CA (06 December 2010); Thalassemia and Globin Gene Regulation, Poster 4277; entire document	1-3, 4-6 ---------- 7-17, 38-40
X -- Y	US 2011/0015168 A1 (KEEGAN et al.) 20 January 2011 (20.01.2011) entire document	18-21, 22-24 ---------- 7-17, 38-40
A	US 2009/0137567 A1 (PERRINE et al.) 28 May 2009 (28.05.2009) entire document	1-40
P, A	PERRINE et al. "Targeted fetal hemoglobin induction for treatment of beta hemoglobinopathies," Hematol Oncol Clin North Am. 01 April 2014 (01.04.2014), Vol. 28, No. 2, Pgs. 233-248. entire document	1-40

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

* 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	Date of mailing of the international search report
24 October 2014	21 NOV 2014

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)