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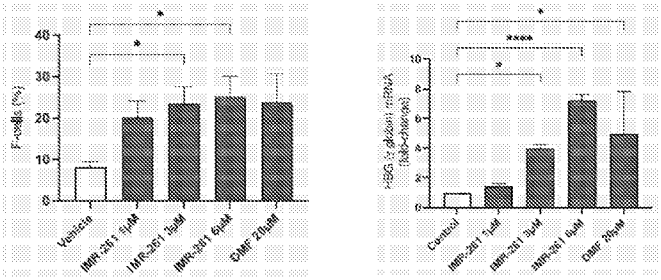
(54) **Title:** NITRATED FATTY ACIDS FOR THE TREATMENT OF SICKLE CELL DISORDERS

Fig. 3

IMR-261 Induces Fetal Hemoglobin in CD34+ Cells from Healthy Donors



*p<0.05, **p<0.01, ***p<0.005, ****p<0.001, IMR-261 6 µM : N=4 (N=2 health subjects + N=2 SCD sample) due to decreased cell viability

(57) **Abstract:** The present disclosure relates to methods of using nitrated fatty acids such as 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof, in the treatment in sickle cell disease.

[Continued on next page]

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NITRATED FATTY ACIDS FOR THE TREATMENT OF SICKLE CELL DISORDERS

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/229,001, filed August 3, 2021, the content of each of which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

[0002] Sickle cell disease (SCD) is an autosomal recessive disorder where mutated hemoglobin (HbS) polymerizes and can lead to irreversible red blood cell (RBC) sickling and painful vaso-occlusive crisis (VOC). The RBC sickling is amplified by inflammation, resulting in tissue and organ damage. The transcription factor Nuclear factor erythroid 2-related factor 2 (Nrf2) coordinates the expression of antioxidant genes in response to oxidative stress, regulates inflammation, inhibits the NFκB pathway, and induces fetal hemoglobin (HbF), making it an attractive target in SCD and β-thalassemia.

SUMMARRY

[0003] In some embodiments, the method involves the method of treating a condition or disease in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof, and increasing HbF gene expression, increasing gamma-globin gene expression, increasing F-cell ratio, increasing Ery.C levels, increasing hemoglobin count, increasing hematocrit levels, increasing HbF positive cell count, decreasing reticulocyte levels, decreasing spleen cellularity, decreasing P-selectin levels, decreasing Ery.B levels, decreasing L-selectin levels, decreasing bilirubin levels, decreasing free heme levels, or any combination thereof. In some embodiments, the method involves a method of increasing Nrf2 gene expression level in a cell or in plasma of a subject, comprising administering Compound 1. In some embodiments, the method involves increasing the Nrf2 gene expression level wherein the increase is by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a method of increasing HbF gene expression level in a cell or in plasma of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of increasing HbF gene expression level by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a

method of increasing F-cell ratio in plasma or cell of a subject, comprising administering Compound 1.In some embodiments, the method involves a method of increasing the F-cell ratio in plasma or cell of a subject, wherein the increase is by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a method of increasing Ery.C level in a cell or in plasma of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of increasing the Ery.C level in plasma or cell of a subject by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a method of increasing red blood cell counts of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of increasing Ery.C level in plasma or cell of a subject by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a method of increasing hemoglobin counts of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of increasing the hemoglobin count of a subject by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a method of increasing gamma-globin gene expression in a cell or plasma of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of increasing gamma-globin gene expression level in plasma or cell of a subject is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a method of increasing hematocrit levels of a subject, comprising administering Compound 1. In some embodiments, the method involves increasing hematocrit level in plasma or cell of a subject by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves increasing fetal hemoglobin (HbF) positive cell number in a subject, comprising administering Compound 1. In some embodiments, the method involves a method of increasing HbF positive red blood cell number by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times. In some embodiments, the method involves a method of decreasing reticulocyte level in a cell or plasma of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing reticulocyte

level in a cell or plasma of a subject by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves a method of decreasing spleen cellularity of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing spleen cellularity of a subject by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves decreasing P-selectin protein levels of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing P-selectin level in a cell or plasma of a subject by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves a method of decreasing Ery.B levels of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing Ery.B level in a cell or plasma of a subject by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves a method of decreasing L-selectin protein levels of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing L-selectin level in a cell or plasma of a subject by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves a method of decreasing bilirubin levels of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing bilirubin levels in a cell or plasma of a subject by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves a method of decreasing free heme levels of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing free heme level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves a method of decreasing L-selectin levels of a subject, comprising administering Compound 1. In some embodiments, the method involves a method of decreasing L-selectin level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%. In some embodiments, the method involves any of the preceding methods, wherein the degree of change is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70 %. In some embodiments, the method involves a method of treating a patient wherein the patient is suffering from Vaso-occlusive crisis (VOC), beta-thalassemia, sickle cell disease, sickle cell crisis, hypertension, cardiovascular disease, cardiac hypertrophy, myocardial infarction, angina, heart failure, hypercholesterolemia, , chronic kidney disease, chronic liver disease, chronic renal disease, chronic kidney injury, inflammatory disease, obesity associated chronic kidney disease,

type II diabetes, diabetic nephropathy, kidney fibrosis, neuropathy, glomerulonephritis, kidney failure, ischemic kidney injury, acute kidney injury, focal segmental glomerulosclerosis, neurodegenerative disease, inflammatory bowel disease, colitis, idiopathic pulmonary fibrosis, fatty liver disease, heart disease, obesity, pulmonary arterial hypertension, postoperative atrial fibrillation, fatal arrhythmia, catecholaminergic polymorphic ventricular tachycardia, thrombosis, chronic obstructive pulmonary disease, valvular heart disease, anti-adipogenic disease, mitochondrial related disease, Charcot-Marie-Tooth disease, Alzheimer's disease, prostatic hyperplasia, urolithiasis, allergic airway disease, non-alcoholic steatohepatitis, solid organ fibrosis, inflammatory gastrointestinal disease, metabolic syndrome, or amyotrophic lateral sclerosis. In some embodiments, the patient is suffering from catecholaminergic polymorphic ventricular tachycardia. In some embodiments, the method involves the patient is suffering from post-operative atrial fibrillation. In some embodiments, the method involves a patient suffering from fatal arrhythmia. In some embodiments, the method involves a patient suffering from VOC. In some embodiments, the method involves a patient that is suffering from beta thalassemia disease. In some embodiments, the method involves a patient that is suffering from sickle cell disease. In some embodiments, the method involves a patient that is suffering from hypertension. In some embodiments, the method involves a patient that is suffering from cardiovascular disease. In some embodiments, the method involves a patient that is suffering from hypercholesterolemia. In some embodiments, the method involves a patient that is suffering from chronic kidney disease. In some embodiments, the method involves a patient that is suffering from chronic liver disease. In some embodiments, the method involves a patient that is suffering from renal disease. In some embodiments, the method involves a patient that is suffering from chronic kidney injury. In some embodiments, the method involves a patient that is suffering from inflammatory disease. In some embodiments, the method involves a patient that is suffering from obesity associated chronic kidney disease. In some embodiments, the method involves a patient that is suffering from diabetic nephropathy. In some embodiments, the method involves a patient that is suffering from kidney fibrosis. In some embodiments, the method involves a patient that is suffering from glomerulonephritis. In some embodiments, the method involves a patient that is suffering from kidney failure. In some embodiments, the method involves a patient that is suffering from ischemic kidney injury. In some embodiments, the method involves a patient that is suffering from neurodegenerative disease. In some embodiments, the method involves a patient that is suffering from inflammatory bowel disease. In some embodiments, the method involves a patient that is suffering from idiopathic pulmonary fibrosis. In some embodiments, the method involves a patient that is suffering from fatty liver disease. In some embodiments, the method involves a patient that is suffering from heart

disease. In some embodiments, the method involves a patient that is suffering from obesity. In some embodiments, the method involves a patient that is suffering from pulmonary arterial hypertension. In some embodiments, the method involves a patient that is suffering from type II diabetes. In some embodiments, the method involves a patient that is suffering from anti-adipogenic disease. In some embodiments, the method involves a patient that is suffering from mitochondrial related disease. In some embodiments, the method involves a patient that is suffering from Charcot-Marie-Tooth disease. In some embodiments, the method involves a patient that is suffering from allergic airway disease. In some embodiments, the method involves a patient that is suffering from non-alcoholic steatohepatitis. In some embodiments, the method involves a patient that is suffering from amyotrophic lateral sclerosis. In some embodiments, the method involves a patient that is suffering from sickle cell crisis. In some embodiments, the method involves a patient that is suffering from solid organ fibrosis. In some embodiments, the method involves a patient that is suffering from chronic obstructive pulmonary disease. In some embodiments, the method involves a patient that is suffering from cardiac hypertrophy. In some embodiments, the method involves a patient that is suffering from myocardial infarction. In some embodiments, the method involves a patient that is suffering from angina. In some embodiments, the method involves a patient that is suffering from sickle cell nephropathy. In some embodiments, the method involves a patient that is suffering from acute kidney injury. In some embodiments, the method involves a patient that is suffering from focal segmental glomerulosclerosis. In some embodiments, the method involves a patient that is suffering from inflammatory gastrointestinal disease. In some embodiments, the method involves a patient that is suffering from colitis. In some embodiments, the method involves a patient that is suffering from metabolic syndrome. In some embodiments, the method involves a patient that is suffering from neuropathy. In some embodiments, the method involves administering Compound 1 orally. In some embodiments, the method involves administering Compound 1 daily. In some embodiments, the method involves administering Compound 1 between about 0.3 mg/kg – about 500 mg/kg. In some embodiments, the method involves administering Compound 1 at about 0.3 mg/kg, about 1 mg/kg, about 3 mg/kg, about 10 mg/kg, about 30 mg/kg, about 50 mg/kg, about 100 mg/kg, about 150 mg/kg, about 200 mg/kg, or about 250 mg/kg. In some embodiments, the method involves administering Compound 1 for between 1 to 7 days.

INCORPORATION BY REFERENCE

[0004] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] FIG. 1 shows the effect of IMR-261 on human monocyte Nrf2-related gene expression.

[0006] FIG. 2 shows the protective effect of IMR-261 on LPS-induced NFκB-related gene expression.

[0007] FIG. 3 shows IMR-261 induction of fetal hemoglobin in CD34+ cells.

[0008] FIG. 4 shows IMR-261 experimental model of sickle cell disease in mice.

[0009] FIG. 5 shows the increase in fetal hemoglobin at 37.5 mg/kg in the mouse model of sickle cell disease upon administration of IMR-261.

[0010] FIG. 6 shows the effect of IMR-261 on biomarkers within the mouse model of sickle cell disease.

[0011] FIG. 7 shows the effect of IMR-261 on splenomegaly in the mouse model of sickle cell disease.

[0012] FIG. 8 shows the experimental plan of the VOC model in mice.

[0013] FIG. 9 shows stained images of Liver in Spleen tissue in the mouse VOC model with and without IMR-261.

[0014] FIG. 10 shows adhesion and hemolysis biomarkers in the mouse VOC model with and without IMR-261.

[0015] FIG. 11 shows the experimental plan of the mouse model of beta-thalassemia.

[0016] FIG. 12 shows the effect of IMR-261 in the mouse model of beta-thalassemia.

[0017] FIG. 13 shows the effect of IMR-261 on erythropoiesis in the mouse model of beta-thalassemia.

[0018] FIG. 14 shows the effect of IMR-261 on the mRNA of Nrf2 target genes in human monocytes.

[0019] FIG. 15 shows the effect of IMR-261 on fetal hemoglobin levels pre- and post-treatment.

[0020] FIG. 16 shows the effect of IMR-261 on bone marrow.

[0021] FIG. 17 shows the effect of IMR-261 on stress erythropoiesis

[0022] FIG. 18 shows the effect of IMR-261 on additional adhesion biomarkers

[0023] FIG. 19 shows the effect of IMR-261 on hemoglobin

[0024] FIG. 20 shows the effect of IMR-261 on ineffective erythropoiesis

DETAILED DESCRIPTION OF THE INVENTION

[0025] A nitrated fatty acid, 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), has been shown to be an oral Nrf2 activator and displays promise in treating sickle cell disorders.

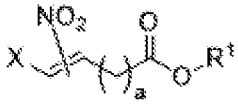
[0026] In the present disclosure, a nitrated fatty acid is used for the treatment of vaso-occlusive crisis (VOC), beta-thalassemia, sickle cell disease, sickle cell crisis, hypertension, cardiovascular disease, cardiac hypertrophy, myocardial infarction, angina, heart failure, hypercholesterolemia, , chronic kidney disease, chronic liver disease, chronic renal disease, chronic kidney injury, inflammatory disease, obesity associated chronic kidney disease, type II diabetes, diabetic nephropathy, kidney fibrosis, neuropathy, glomerulonephritis, kidney failure, ischemic kidney injury, acute kidney injury, focal segmental glomerulosclerosis, neurodegenerative disease, inflammatory bowel disease, colitis, idiopathic pulmonary fibrosis, fatty liver disease, heart disease, obesity, pulmonary arterial hypertension, postoperative atrial fibrillation, fatal arrhythmia, catecholaminergic polymorphic ventricular tachycardia, thrombosis, chronic obstructive pulmonary disease, valvular heart disease, anti-adipogenic disease, mitochondrial related disease, Charcot-Marie-Tooth disease, Alzheimer's disease, prostatic hyperplasia, urolithiasis, allergic airway disease, non-alcoholic steatohepatitis, solid organ fibrosis, inflammatory gastrointestinal disease, metabolic syndrome, or amyotrophic lateral sclerosis.

I. Methods of Treatment

[0001] In an aspect, provided herein are methods of treating vaso-occlusive crisis (VOC), beta-thalassemia, sickle cell disease, sickle cell crisis, hypertension, cardiovascular disease, cardiac hypertrophy, myocardial infarction, angina, heart failure, hypercholesterolemia, , chronic kidney disease, chronic liver disease, chronic renal disease, chronic kidney injury, inflammatory disease, obesity associated chronic kidney disease, type II diabetes, diabetic nephropathy, kidney fibrosis, neuropathy, glomerulonephritis, kidney failure, ischemic kidney injury, acute kidney injury, focal segmental glomerulosclerosis, neurodegenerative disease, inflammatory bowel disease, colitis, idiopathic pulmonary fibrosis, fatty liver disease, heart disease, obesity, pulmonary arterial hypertension, postoperative atrial fibrillation, fatal arrhythmia, catecholaminergic polymorphic ventricular tachycardia, thrombosis, chronic obstructive pulmonary disease, valvular heart disease, anti-adipogenic disease, mitochondrial related disease, Charcot-Marie-Tooth disease, Alzheimer's disease, prostatic hyperplasia, urolithiasis, allergic airway disease, non-alcoholic steatohepatitis, solid organ fibrosis, inflammatory gastrointestinal disease, metabolic syndrome, or amyotrophic lateral sclerosis in a patient in need thereof, comprising administering to the patient a pharmaceutically efficient amount of a nitrated fatty acid.

Compounds

[0002] In some embodiments, the nitrated fatty acid is a Compound having the structure of Formula (I), or a pharmaceutically acceptable salt or solvate thereof:


Formula (I),

wherein:

X is selected from H and  ;

R^1 is selected from H, alkyl, haloalkyl, cycloalkyl, aryl, and heteroaryl;

a is an integer from 0-30; and

b is an integer from 0-30.

[0003] In some embodiments, R^1 is H, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, or pentyl. In some embodiments, R^1 is methyl. In some embodiments, R^1 is ethyl. In some embodiments, R^1 is H.

[0004] In some embodiments, X is  . In some embodiments, X is H.

[0005] In some embodiments, b is an integer from 0-30, 0-25, 0-20, 0-15, 0-10, or 0-5. In some embodiments, b is an integer selected from 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, b is an integer selected from 5, 6, 7, 8, 9, or 10. In some embodiments, b is 6. In some embodiments, b is 7. In some embodiments, b is 8. In some embodiments, b is 9. In some embodiments, b is 10.

[0006] In some embodiments, a is an integer from 0-30, 0-25, 0-20, 0-15, 0-10, or 0-5. In some embodiments, a is an integer selected from 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, a is an integer selected from 5, 6, 7, 8, 9, or 10. In some embodiments, b is 6. In some embodiments, a is 7. In some embodiments, a is 8. In some embodiments, a is 9. In some embodiments, a is 10.

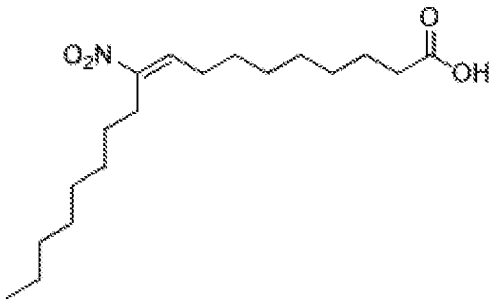
[0007] In some embodiments, the Compound of Formula (I) is a nitro-oleic acid.

[0008] Compounds encompassed by the formula described above include, but are not limited to, (E)-9-nitro-octadec-9-enoic acid, (E)-10-nitro-octadec-9-enoic acid, (E)-8-nitro-octadec-9-enoic acid, (E)-11-nitro-octadec-9-enoic acid, (E)-10-acetyltetradec-9-enoic acid, (E)-9-acetyltetradec-9-enoic acid, (E)-11-acetyltetradec-9-enoic acid, (E)-8-acetyltetradec-9-enoic acid, (E)-13-chloro-docosen-13-enoic acid, (E)-14-chloro-docosen-13-enoic acid, (E)-12-chloro-docosen-13-enoic acid, (E)-15-chloro-docosen-13-enoic acid, (E)-10-methylsulfonylhexadec-9-enoic acid, (E)-9-methylsulfonylhexadec-9-enoic acid, (E)-11-methylsulfonylhexadec-9-enoic acid, and (E)-8-methylsulfonylhexadec-9-enoic acid. Other embodiments include the Z-isomer of such

Compounds. Further embodiments include, for example, (E)-9-nitro-pentadec-9-enoic acid, (E)-10-nitro-pentadec-9-enoic acid, (E)-8-nitro-pentadec-9-enoic acid, (E)-11-nitro-pentadec-9-enoic acid, (E)-10-acetylheptadec-9-enoic acid, (E)-9-acetylheptadec-9-enoic acid, (E)-11-acetyloctahepta-9-enoic acid, (E)-8-acetylheptadec-9-enoic acid, (E)-10-chloro-pentadec-9-enoic acid, (E)-9-chloro-pentadec-9-enoic acid, (E)-11-chloro-pentadec-9-enoic acid, (E)-8-chloro-pentadec-9-enoic acid, (E)-10-methylsulfonylnonadec-9-enoic acid, (E)-9-methylsulfonylnonadec-9-enoic acid, (E)-11-methylsulfonylnonadec-9-enoic acid, (E)-8-methylsulfonylnonadec-9-enoic acid, and the (Z)-isomers thereof. Yet other embodiments include, for example, (E)-9-nitro-eicos-11, 14-ienoic acid, (E)-10-nitro-eicos-8, 13-ienoic acid, (E)-8-nitro-eicos-11, 14-ienoic acid, (E)-11-nitro-eicos-8, 13-ienoic acid, (E)-10-acetylnonadec-10,13-ienoic acid, (E)-9-acetylnonadec-9,12-enoic acid, (E)-11-acetylnonadec-10,13-ienoic acid, (E)-8-acetylnonadec-9, 12-enoic acid, (E)-10-chloro-heptadec-9, 11-ienoic acid, (E)-9-chloro-hetpadec-10,12-ienoic acid, (E)-11-chloro-heptadec-9,11-ienoic acid, (E)-8-chloro-heptadec-10,11-ienoic acid, (E)-10-methylsulfonylpentadec-9,11-ienoic acid, (E)-9-methylsulfonylpentadec-8,9-ienoic acid, (E)-11-methylsulfonylpentadec-9,10-ienoic acid, and (E)-8-methylsulfonylpentadec-8,9-ienoic acid, and (Z)-isomers thereof.

[0009] In some embodiments, the nitrated fatty acid is 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof.

[0010] In some embodiments, 10-nitro-9(E)-octadec-9-enoic acid (Compound 1) has the structure:



(Compound 1).

[0011] In some embodiments, 10-nitro-9(E)-octadec-9-enoic acid (Compound 1) is disclosed in WO2017059451A1, which is herein incorporated by reference in its entirety. In some embodiments, 10-nitro-9(E)-octadec-9-enoic acid is also known as CXA-10.

Pharmaceutical Compositions

[0027] The present disclosure further provides for a method of treating a sickle cell disorder by administering to the patient in need thereof with a pharmaceutical composition comprising a therapeutically effective amount of a nitrated fatty acid and a pharmaceutically acceptable carrier or diluent. In some embodiments, the pharmaceutical composition comprising a therapeutically effective amount of a Compound having the structure of Formula (I), a

pharmaceutically acceptable salt or solvate thereof; and a pharmaceutically acceptable carrier or diluent or excipient. In some embodiments, the pharmaceutical composition comprise a therapeutically acceptable amount of 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier or diluent or excipient.

Pharmaceutically Acceptable Salts

[0028] The present disclosure also comprises salts of the nitrated fatty acid, typically, pharmaceutically acceptable salts. Such salts include pharmaceutically acceptable acid addition salts. Acid addition salts include salts of inorganic acids as well as organic acids.

[0029] Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, sulfamic, nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, itaconic, lactic, methanesulfonic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methane sulfonic, ethanesulfonic, tartaric, ascorbic, pamoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, EDTA, glycolic, p-aminobenzoic, glutamic, benzenesulfonic, p-toluenesulfonic acids, theophylline acetic acids, as well as the 8-halotheophyllines, for example 8-bromotheophylline and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in Berge, S.M. et al., J. Pharm. Sci. 1977, 66, 2, the contents of which are hereby incorporated by reference.

[0030] Furthermore, the Compounds of this disclosure may exist in unsolvated as well as in solvated forms with pharmaceutically acceptable solvents such as water, ethanol and the like. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of this disclosure.

[0031] In some embodiments, the pharmaceutical composition comprises Compound 1 as the solvated, unsolvated, or crystalline/polymorph form. In some embodiments, Compound 1 is present as the unsolvated form. In some embodiments, Compound 1 is present as the solvated form. ***Formulations***

[0032] The Compounds of the disclosure may be administered alone or in combination with pharmaceutically acceptable carriers, diluents or excipients, in either single or multiple doses. The pharmaceutical compositions according to the disclosure may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington: The Science and Practice of Pharmacy, 22nd Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 2013.

[0033] The pharmaceutical compositions may be specifically formulated for administration by any suitable route such as oral, rectal, nasal, pulmonary, topical (including buccal and sublingual), transdermal, intracisternal, intraperitoneal, vaginal and parenteral (including subcutaneous, intramuscular, intrathecal, intravenous and intradermal) routes. It will be appreciated that the route will depend on the general health and age of the subject to be treated, the nature of the condition to be treated and the active ingredient.

[0034] Pharmaceutical compositions for oral administration include solid dosage forms such as capsules, tablets, dragees, pills, lozenges, powders and granules Where appropriate, the compositions may be prepared with coatings such as enteric coatings or they may be formulated so as to provide controlled release of the active ingredient such as sustained or prolonged release according to methods well known in the art. Liquid dosage forms for oral administration include solutions, emulsions, suspensions, syrups and elixirs.

[0035] Pharmaceutical compositions for parenteral administration include sterile aqueous and nonaqueous injectable solutions, dispersions, suspensions or emulsions as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use. Other suitable administration forms include, but are not limited to, suppositories, sprays, ointments, creams, gels, inhalants, dermal patches and implants.

[0036] For parenteral routes such as intravenous, intrathecal, intramuscular and similar administration, typical doses are on the order of half the dose employed for oral administration.

[0037] The present disclosure also provides a process for making a pharmaceutical composition comprising admixing a therapeutically effective amount of a Compound of the present disclosure and at least one pharmaceutically acceptable carrier or diluent.

[0038] The Compounds of this disclosure are generally utilized as the free substance or as a pharmaceutically acceptable salt thereof. Such salts are prepared in a conventional manner by treating a solution or suspension of a Compound of the present disclosure with a molar equivalent of a pharmaceutically acceptable acid. Representative examples of suitable organic and inorganic acids are described above.

[0039] For parenteral administration, solutions of the Compounds of the present disclosure in sterile aqueous solution, aqueous propylene glycol, aqueous vitamin E or sesame or peanut oil may be employed. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The Compounds of the present disclosure may be readily incorporated into known sterile aqueous media using standard techniques known to those skilled in the art.

[0040] Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solutions and various organic solvents. Examples of solid carriers include lactose, terra alba, sucrose, cyclodextrin, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and lower alkyl ethers of cellulose. Examples of liquid carriers include, but are not limited to, syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. The pharmaceutical compositions formed by combining the Compounds of the present disclosure and a pharmaceutically acceptable carrier are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

[0041] Formulations of the present disclosure suitable for oral administration may be presented as discrete units such as capsules or tablets, each containing a predetermined amount of the active ingredient, and optionally a suitable excipient. Furthermore, the orally available formulations may be in the form of a powder or granules, a solution or suspension in an aqueous or non-aqueous liquid, or an oil-in-water or water-in-oil liquid emulsion.

[0042] If a solid carrier is used for oral administration, the preparation may be tabletted, placed in a hard gelatin capsule in powder or pellet form or it may be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will range from about 25 mg to about 1 g per dosage unit. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

[0043] The pharmaceutical compositions of the disclosure may be prepared by conventional methods in the art. For example, tablets may be prepared by mixing the active ingredient with ordinary adjuvants and/or diluents and subsequently compressing the mixture in a conventional tableting machine prepare tablets. Examples of adjuvants or diluents comprise: corn starch, potato starch, talcum, magnesium stearate, gelatin, lactose, gums, and the like. Any other adjuvants or additives usually used for such purposes such as colorings, flavorings, preservatives etc. may be used provided that they are compatible with the active ingredients.

[0044] The pharmaceutical compositions may comprise at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% by weight of the nitrated fatty acid (e.g. Compound 1), or a pharmaceutically acceptable salt or solvate thereof. In some embodiments, the pharmaceutical composition may comprise at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% by weight of Compound 1, or a pharmaceutically acceptable salt or solvate thereof.

[0045] In some embodiments, Compound 1 or a pharmaceutically acceptable salt or solvate thereof is formulated as a composition for oral administration. For example, it may be in a solid tablet form. The composition for oral administration comprises at least a filler and/or a processing aid. The processing aid may be a glidant or a lubricant.

Dosing

[0046] Typical oral dosages range from about 0.001 to about 100 mg/kg body weight per day. Typical oral dosages also range from about 0.01 to about 50 mg/kg body weight per day. Typical oral dosages further range from about 0.05 to about 10 mg/kg body weight per day. Oral dosages are usually administered in one or more dosages, typically, one to three dosages per day. The exact dosage will depend upon the frequency and mode of administration, the gender, age, weight and general health of the subject treated, the nature and severity of the condition treated and any concomitant diseases to be treated and other factors evident to those skilled in the art.

[0047] In some embodiments, the nitrated fatty acid (e.g. Compound 1), or a pharmaceutically acceptable salt or solvate thereof is administered to a patient in need thereof at a dosing of less than 6.0 mg/kg or less than about 4.0 mg/kg. For example, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered at a dosing of between about 0.3 to about 3.0 mg/kg, or about 0.3 to about 1.0 mg/kg. The patient may have a sickle cell disorder, such as hemochromatosis. The patient may be an adult (≥18 years old) or a child (<18 years old).

[0048] In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 1 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 3 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 6 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 8.0 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 10 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 20 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 50 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 60 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 100 mg/kg. In some embodiments, the patient receives Compound 1, or a pharmaceutically acceptable salt or solvate thereof at about 200 mg/kg.

[0049] In some embodiments, the patient receives at least about 10 mg/kg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof per the weight of the patient. In some embodiments, the patient receives at least about 20 mg/kg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof per the weight of the patient. In some embodiments, the patient receives at least about 30 mg/kg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof per the weight of the patient. In some embodiments, the patient receives at least about 40 mg/kg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof per the weight of the patient. In some embodiments, the patient receives at least about 50 mg/kg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof per the weight of the patient. In some embodiments, the patient receives at least about 60 mg/kg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof per the weight of the patient. In some embodiments, the patient receives at least about 100 mg/kg of Compound 1, or a pharmaceutically acceptable salt or solvate thereof per the weight of the patient.

[0050] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount from about 500 mg to about 1,500 mg or from about 600 mg to about 1,000 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1,000 mg, 1,100 mg, 1,200 mg, 1,300 mg, 1,400 mg, or 1,500 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, or 1,000 mg.

[0051] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 50 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 100 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 200 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 300 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 400 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 500 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 600 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is

administered to the patient in an amount of about 700 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 800 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 900 mg. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient in an amount of about 1,000 mg.

[0052] In some embodiments, Compound 1 or a pharmaceutically acceptable salt or solvate thereof is administered to a patient once a day (QD). In some embodiments, Compound 1 or a pharmaceutically acceptable salt or solvate thereof is administered to a patient twice a day (BID). In some embodiments, Compound 1 or a pharmaceutically acceptable salt or solvate thereof is administered to a patient three times a day (TID).

[0053] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient orally.

[0054] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient orally once per day.

[0055] In some embodiments, Compound 1 or a pharmaceutically acceptable salt or solvate thereof, is administered with food. In other embodiments, Compound 1 or a pharmaceutically acceptable salt or solvate thereof, is administered without food.

[0056] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 4 weeks, 12 weeks, 16 weeks, or 24 weeks, 1 year, or 1.5 years.

[0057] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to a patient for at least 7 days, 10 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, a year, 1.5 years, or 2 years. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 4 weeks. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 12 weeks. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 16 weeks. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 24 weeks. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 6 months. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 1 year. In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered for at least 1.5 years.

[0058] In some embodiments, Compound 1, or a pharmaceutically acceptable salt or solvate thereof is administered to the patient until symptom resolution.

[0059] In some embodiments, Compound 1 or a pharmaceutically acceptable salt or solvate thereof is administered for life.

[0060] The formulations may also be presented in a unit dosage form by methods known to those skilled in the art. For illustrative purposes, a typical unit dosage form for oral administration may contain from about 0.01 to about 1000 mg, from about 0.05 to about 500 mg, or from about 0.5 mg to about 200 mg.

II. Biophysical Effects

[0061] In another aspect, 10-nitro-9-octadec-9-enoic acid (Compound 1), upregulates expression of the HbF gene and the gamma-globin gene. Hemoglobin F has a different structure than the predominant adult form of hemoglobin. Hemoglobin F tends to have better binding to oxygen and may alleviate circulatory deficiency in oxygen distribution. The gamma-globin gene encodes the gamma subunit of the hemoglobin tetramer of fetal hemoglobin.

[0062] In another aspect, provided herein is a method of modulating F-cell ratio, Ery.C levels, hemoglobin levels, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, L-selectin levels, bilirubin levels, free heme levels or any combination thereof over baseline levels prior to treatment in a patient suffering from an; the method comprising administering a therapeutically effective amount of 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof.

[0063] In some embodiments, the reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, L-selectin levels, bilirubin levels, or free heme levels are deceased over baseline levels in serum and/or one or more tissues selected from kidney, spleen, and liver.

[0064] In some embodiments, Compound 1 reduces reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, L-selectin levels, bilirubin levels, or free heme levels in the cell or plasma. In some embodiments, the reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, L-selectin levels, bilirubin levels, or free heme levels in the cell or plasma are reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, or more. In some embodiments, the reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, L-selectin levels, bilirubin levels, or free heme levels in the cell or plasma are reduced by about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, or 40%. In some embodiments, Compound 1 reduces reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, L-selectin levels, bilirubin levels, or free heme in the

cell or plasma tissue by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over the cell or plasma levels prior to treatment.

[0065]

[0066] Without being bound by theory, 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), upregulates expression of mRNA for certain protein including but not limited to the HbF gene and gamma-globin gene.

[0067] In another aspect, provided herein is a method of modulating one or more biomarkers over baseline levels prior to treatment in a patient suffering from a condition or disease, wherein the biomarkers are selected from HbF mRNA, gamma-globin mRNA, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, free heme levels; the method comprising administering a therapeutically effective amount of 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof.

[0068] In some embodiments, the biomarkers may include HbF mRNA, gamma-globin mRNA, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, free heme levels in the serum and/or the tissues, e.g. liver, kidney or spleen tissues. In some embodiments, the serum reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, free heme levels are reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, free heme levels are reduced by about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, or more.

[0069] In some embodiments, the HbF mRNA levels, gamma-globin mRNA levels, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, or HbF positive cell count levels are increased by about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 68%, 69%, or 70%, or more. In some embodiments, Compound 1 increases HbF mRNA levels, gamma-globin mRNA levels, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, or HbF positive cell count levels by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over protein levels prior to treatment. In some embodiments, the HbF, gamma-globin, F-cell ratio, Ery.C levels,

hemoglobin count, hematocrit levels, or HbF positive cell count are increased by about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 68%, 69%, 70%, or more. In some embodiments, Compound 1 increases HbF, gamma-globin, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, or HbF positive cell count by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over HbF mRNA, gamma-globin mRNA, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, or HbF positive cell count levels prior to treatment.

[0070] In some embodiments, the HbF mRNA levels, gamma-globin mRNA levels, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels are increased by about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 68%, 69%, or 70%, or more. In some embodiments, Compound 1 increases HbF mRNA levels, gamma-globin mRNA levels, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over protein levels prior to treatment. In some embodiments, the HbF mRNA levels, gamma-globin mRNA levels, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels are increased by about 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 68%, 69%, 70%, or more. In some embodiments, Compound 1 increases HbF mRNA levels, gamma-globin mRNA levels, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over HbF mRNA levels, gamma-globin mRNA levels, F-cell ratio, Ery.C levels, hemoglobin count, hematocrit levels, HbF positive cell count, reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels prior to treatment.

[0071] In some embodiments, the biomarkers are reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels. In some embodiments, the reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels are decreased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least

about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels are reduced by about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, or more. In some embodiments, Compound 1 decreases reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over reticulocyte levels, spleen cellularity, P-selectin levels, Ery.B levels, bilirubin levels, L-selectin levels, or free heme levels prior to treatment.

[0072] In some embodiments, the biomarker may be hematocrit. In some embodiments, Compound 1 increases the hematocrit in a subject. In some embodiments, the hematocrit is increased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the hematocrit is increased by about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, or more. In some embodiments, Compound 1 increases hematocrit by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over levels prior to treatment.

[0073] In some embodiments, the biomarker may be hemoglobin levels (Hb). In some embodiments, Compound 1 increases Hb levels in a subject. In some embodiments, the Hb levels increased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the Hb levels are increased by about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, or more. In some embodiments, Compound 1 increases Hb levels by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over levels prior to treatment. In some embodiments, the hemoglobin (Hb) levels of the subject are increased in the range of about 0.5 to about 3.0 g/dL of total Hb. In some embodiments, the hemoglobin (Hb) level of the subject is increased by about 0.5, about 1.0, about 1.5, about 2.0, about 2.5, or about 3.0 g/dL of total Hb.

[0074] In some embodiments, the biomarker may be red blood cell count (RBC). In some embodiments, Compound 1 increases RBC levels in a subject. In some embodiments, RBCs are

increased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the RBC levels are increased by about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, or more. In some embodiments, Compound 1 increases RBC levels by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over levels prior to treatment.

[0075] In some embodiments, the biomarker may be reticulocyte count. In some embodiments, Compound 1 may decrease reticulocyte levels in a subject. In some embodiments, reticulocytes are decreased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%. In some embodiments, the reticulocytes are decreased by about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, or more. In some embodiments, Compound 1 decreases reticulocyte levels by about 2-fold, 3-fold, 4-fold, 5-fold, or 10-fold over levels prior to treatment.

Combination Therapies

[0076] Another aspect of the present disclosure provides methods of using a nitrated fatty acid, e.g. 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof, in combination with at least one additional active agent or therapy. They may be administered simultaneously or sequentially. They may be present as a mixture for simultaneous administration, or may each be present in separate containers for sequential administration.

[0077] The term "simultaneous administration", as used herein, is not specifically restricted and means that the nitrated fatty acid of the present disclosure and the at least one other active agent are substantially administered at the same time, e.g. as a mixture or in immediate subsequent sequence.

[0078] The term "sequential administration", as used herein, is not specifically restricted and means that the nitrated fatty acid of the present disclosure and the at least one other active agent are not administered at the same time but one after the other, or in groups, with a specific time interval between administrations. The time interval may be the same or different between the respective administrations of the nitrated fatty acid of the present disclosure and the at least one other active agent and may be selected, for example, from the range of 2 minutes to 96 hours, 1

to 7 days or one, two or three weeks. Generally, the time interval between the administrations may be in the range of a few minutes to hours, such as in the range of 2 minutes to 72 hours, 30 minutes to 24 hours, or 1 to 12 hours. Further examples include time intervals in the range of 24 to 96 hours, 12 to 36 hours, 8 to 24 hours, and 6 to 12 hours.

[0079] The molar ratio of the nitrated fatty acid of the present disclosure and the at least one additional active agent is not particularly restricted. For example, when the nitrated fatty acid of the present disclosure and the one other additional active agent are combined in a composition, the molar ratio of them may be in the range of 1:500 to 500:1, or of 1:100 to 100:1, or of 1:50 to 50:1, or of 1:20 to 20:1, or of 1:5 to 5:1, or 1:1.

III. Kits and Devices

[0080] The disclosure provides a variety of kits and devices for conveniently and/or effectively carrying out methods of the present disclosure. Typically, kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[0081] In one embodiment, the present disclosure provides kits for treating a condition, disease, or disorder comprising a nitrated fatty acid, e.g. 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof.

[0082] The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent may comprise a saline, a buffered solution, or any delivery agent disclosed herein. The amount of each component may be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The components may also be varied in order to increase the stability of the nitrated fatty acid in the buffer solution over a period of time and/or under a variety of conditions.

[0083] The present disclosure provides for devices that may incorporate 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof of the present disclosure. These devices contain in a stable formulation available to be immediately delivered to a subject in need thereof, such as a human patient with an sickle cell disorder.

[0084] Non-limiting examples of the devices include a pump, a catheter, a needle, a transdermal patch, a pressurized olfactory delivery device, iontophoresis devices, multi-layered microfluidic devices. The devices may be employed to deliver the nitrated fatty acid of the present disclosure according to single, multi- or split-dosing regiments. The devices may be employed to deliver the nitrated fatty acid of the present disclosure across biological tissue, intradermal, subcutaneously, or intramuscularly. More examples of devices suitable for delivering the nitrated fatty acid include but not limited to a medical device for intravesical drug delivery

disclosed in International Publication WO 2014036555, a glass bottle made of type I glass disclosed in U.S. Publication No. 20080108697, a drug-eluting device comprising a film made of a degradable polymer and an active agent as disclosed in U.S. Publication No. 20140308336, an infusion device having an injection micropump, or a container containing a pharmaceutically stable preparation of an active agent as disclosed in US Patent No. 5716988, an implantable device comprising a reservoir and a channeled member in fluid communication with the reservoir as disclosed in International Publication WO 2015023557, a hollow-fibre-based biocompatible drug delivery device with one or more layers as disclosed in U.S. Publication No. 20090220612, an implantable device for drug delivery including an elongated, flexible device having a housing defining a reservoir that contains a drug in solid or semi-solid form as disclosed in International Publication WO 2013170069, a bioresorbable implant device disclosed in U.S. Patent No. 7326421, contents of each of which are incorporated herein by reference in their entirety.

Definitions

[0085] The articles "a" and "an," as used herein, should be understood to mean "at least one," unless clearly indicated to the contrary.

[0086] The phrase "and/or," as used herein, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to "A and/or B," when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements).

[0087] As used herein, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements.

[0088] In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as

"either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[0089] As used herein, the phrase "at least one" in reference to a list of one or more elements should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified.

[0090] Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0091] As used herein, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," and the like are to be understood to be open-ended, i.e., to mean including but not limited to.

[0092] Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures.

[0093] As used herein, a "subject" or a "patient" refers to any mammal (e.g., a human), such as a mammal that may be susceptible to a disease or disorder, for example, tumorigenesis or cancer. Examples include a human, a non-human primate, a cow, a horse, a pig, a sheep, a goat, a dog, a cat, or a rodent such as a mouse, a rat, a hamster, or a guinea pig. In various embodiments, a subject refers to one that has been or will be the object of treatment, observation, or experiment. For example, a subject can be a subject diagnosed with cancer or otherwise known to have cancer or one selected for treatment, observation, or experiment on the basis of a known cancer in the subject.

[0094] As used herein, "treatment" or "treating" refers to amelioration of a disease or disorder, or at least one sign or symptom thereof. "Treatment" or "treating" can refer to reducing the progression of a disease or disorder, as determined by, e.g., stabilization of at least one sign or symptom or a reduction in the rate of progression as determined by a reduction in the rate of

progression of at least one sign or symptom. In another embodiment, "treatment" or "treating" refers to delaying the onset of a disease or disorder.

[0095] As used herein, "prevention" or "preventing" refers to a reduction of the risk of acquiring or having a sign or symptom a given disease or disorder, i.e., prophylactic treatment.

[0096] The phrase "therapeutically effective amount" as used herein means that amount of a Compound, material, or composition comprising a Compound of the present teachings that is effective for producing a desired therapeutic effect. Accordingly, a therapeutically effective amount treats or prevents a disease or a disorder, e.g., ameliorates at least one sign or symptom of the disorder. In various embodiments, the disease or disorder is a cancer.

[0097] A dash ("-") that is not between two letters or symbols is used to indicate a point of attachment for a substituent. For example, $-CONH_2$ is attached through the carbon atom (C).

[0098] By "optional" or "optionally," it is meant that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" encompasses both "aryl" and "substituted aryl" as defined herein. It will be understood by those ordinarily skilled in the art, with respect to any group containing one or more substituents, that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical, synthetically non-feasible, and/or inherently unstable.

[0099] All numerical ranges herein include all numerical values and ranges of all numerical values within the recited range of numerical values. As a non-limiting example, (C_1-C_6) alkyls also include any one of C_1, C_2, C_3, C_4, C_5, C_6, (C_1-C_2), (C_1-C_3), (C_1-C_4), (C_1-C_5), (C_2-C_3), (C_2-C_4), (C_2-C_5), (C_2-C_6), (C_3-C_4), (C3-C_5), (C_3-C_6), (C_4-C_5), (C_4-C_6), and (C_5-C_6) alkyls.

[0100] Further, while the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations as discussed above, the numerical values set forth in the Examples section are reported as precisely as possible. It should be understood, however, that such numerical values inherently contain certain errors resulting from the measurement equipment and/or measurement technique.

LIST OF ABBREVIATIONS AND TERMS

^{1}H-NMR: Proton Nuclear Magnetic Resonance spectroscopy

ADME: Absorption, Distribution, Metabolism, and Excretion

AE: adverse event

AUC_{0-24}: area under the concentration-time curve from time 0 to 24 hours postdose

C_{max}: maximum plasma concentration

CV: coefficient of variation

CYP: cytochrome p450

DMC: Data Monitoring Committee

DMSO: dimethyl sulfoxide

ECG: electrocardiogram

EOT: end of treatment

IC: inhibitory concentration

IC_{50}: a half minimal inhibitory concentration

IV: intravenous

MAD: multiple-ascending dose

MTD: maximum tolerated dose

NOAEL: no-observed-adverse-effect level

PD: pharmacodynamic

PK: pharmacokinetic(s)

RBC: red blood cell

QD: once daily

QoL: quality of life

SAD: single ascending dose

SAE: serious adverse event

SD: standard deviation

$t_{½}$: half-life

TK: Toxicokinetic

T_{max}: time of maximum concentration

ULN: upper limit of normal

WBC: white blood cell

w/w%: weight/weight percent

EXAMPLES

[0101] It will be appreciated that the following examples are intended to illustrate but not to limit the present disclosure. Various other examples and modifications of the foregoing description and examples will be apparent to a person skilled in the art after reading the disclosure without departing from the spirit and scope of the disclosure, and it is intended that all such examples or modifications be included within the scope of the appended claims. All publications and patents referenced herein are hereby incorporated by reference in their entirety.

Example 1

[0102] CD14+ human monocytes were exposed to Compound 1 at 3μM and 10μM for 3 hours, to determine via quantitative PCR (qPCR) its ability to induce expression of antioxidant genes. Compound 1 at 10 μM significantly increased ($p<0.05$) the expression of Nrf2-dependent genes ($p<0.05$), including HMOX1, HSPA1A, HSP90, GCLM, SOD1 and TXNRD1 ($p<0.05$). Human monocytes were treated with lipopolysaccharide (LPS) to test the ability of Compound 1 to block inflammatory genes with a NFKB target dataset. Compound 1 significantly inhibited ($p<0.05$) LPS-induced expression of IL-1-beta, TNF-alpha and IL-6 in human monocytes. Data are shown in FIGs 1-10.

Example 2

[0103] To test the effects of Compound 1 on HbF induction, human erythroblasts were derived from CD34+ blood marrow progenitor cells sourced from healthy or SCD subjects. Compound 1 induced expression of the γ-globin gene (4.0-fold change at 3μM and 7.18-fold change at 6 μM). This was accompanied by an increased % of F-cells (2.8-fold change at 3μM and 3.0-fold change at 6 μM).

[0104] Compound 1 was also tested in the Townes HbSS mouse model of SCD to assess the potential for HbF induction. Mice were dosed with Compound 1 at 12.5 mg/kg or 37.5 mg/kg BID for 4 weeks (N=4-8/group). After 4 weeks of treatment, Compound 1 at 12.5 mg/kg and 37.5 mg/kg resulted in a significant increase in % of F-cells (1.44±0.33 % on vehicle-treated vs. 4.58±0.67 % on Compound 1 HbF relative to control, and 37.5 mg/kg resulted in a significant increase in %F-cells relative to control (*see* Table 1, $p<0.05$) and fetal hemoglobin (3.74±0.32 ng/ml on vehicle-treated vs. 8.26±0.35 ng/ml on Compound 1 37.5 mg/kg, $p<0.05$). In addition, both doses of Compound 1 led to significant increases in RBC counts ($8.01\pm0.36\times10^6$ cells/mm^3 in vehicle group vs $8.86\pm0.48\times10^6$ cells/mm^3 in Compound 1 12.5 mg/kg and $8.94\pm0.34\times10^6$ cells/mm^3 in Compound 1 37.5 mg/kg, $p<0.01$) and total hemoglobin (Hb) (7.55±0.20 g/dL in vehicle group vs 8.47±0.48 g/dL in Compound 1 12.5 mg/kg and 8.71±0.21 g/dL in Compound 1 37.5 mg/kg, $p<0.01$). (*see* Table 1, $p<0.05$). Compound 1 at 37.5 mg/kg also significantly decreased ($p<0.05$) both reticulocyte counts and spleen cellularity. Data are shown in FIGs 1-10.

Example 3

[0105] The ability of Compound 1 to reduce VOCs was assessed in separate HbSS-Townes mice after the administration of TNF-alpha (0.5 μg/mice i.p.). Compound 1 was dosed at 37.5 mg/kg BID for 5 days before triggering VOCs. RBCs were stained with Ter-119 antibodies on spleen and liver of mice. Compared to controls, Compound 1 significantly reduced the presence of RBC on occluded vessels. This was coupled with a reduction of P-selectin (3109±97 Mean Fluorescence Units [MFI] in vehicle-treated vs. 1974±379 MFI in Compound 1 group, $p<0.05$)

and L-selectin (375±20 MFI in vehicle-treated vs. 242±60 MFI in Compound 1 group, $p<0.05$). Compound 1 also reduced select hemolysis biomarkers: bilirubin (11.2±0.3 mg/dL in vehicle-treated vs. 8.4±0.7 mg/dL in Compound 1 group, ($p<0.05$) and free-heme (325±52 μM in vehicle-treated vs. 203±51 μM in Compound 1 group, $p<0.05$).

[0106] A beta-thalassemia experimental model $Hbb^{th1/th1}$ was tested to evaluate whether Compound 1 could reduce ineffective erythropoiesis seen in beta-thalassemia. Compound 1 treatment at 37.5 mg/kg BID significantly increased hemoglobin levels, RBC counts and hematocrit ($p<0.05$), with significant reductions observed in reticulocytes ($p<0.05$). flow cytometry analysis (CD71/Ter119) showed that Compound 1 significantly decreased late basophilic and polychromatic erythroblasts (Ery.B) and increased orthochromatic erythroblasts and reticulocytes (Ery.C) cell numbers in the spleen ($p<0.05$). Data are shown in FIGs 11-20.

Example 4

[0107] A beta-thalassemia experimental model $Hbb^{th1/th1}$ was tested to evaluate whether Compound 1 could reduce ineffective erythropoiesis seen in beta-thalassemia. Compound 1 treatment at 37.5 mg/kg BID significantly increased hemoglobin levels, RBC counts and hematocrit ($p<0.05$), with significant reductions observed in reticulocytes ($p<0.05$). flow cytometry analysis (CD71/Ter119) showed that Compound 1 significantly decreased late basophilic and polychromatic erythroblasts (Ery.B) and increased orthochromatic erythroblasts and reticulocytes (Ery.C) cell numbers in the spleen ($p<0.05$). Data are shown in Figs 11-20.

[0108] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating a condition or disease in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof, and

i. increasing HbF gene expression;
ii. increasing gamma-globin gene expression
iii. increasing F-cell ratio
iv. increasing Ery.C levels
v. increasing hemoglobin count
vi. increasing hematocrit levels
vii. increasing HbF positive cell count
viii. decreasing reticulocyte levels
ix. decreasing spleen cellularity
x. decreasing P-selectin levels
xi. decreasing Ery.B levels
xii. decreasing L-selectin levels
xiii. decreasing bilirubin levels
xiv. decreasing free heme levels

or any combination thereof.

2. A method of increasing Nrf2 gene expression level in a cell or in plasma of a subject, comprising administering Compound 1.

3. The method of claim 2, wherein the Nrf2 gene expression level is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

4. A method of increasing HbF gene expression level in a cell or in plasma of a subject, comprising administering Compound 1.

5. The method of claim 4, where the HbF gene expression level is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

6. A method of increasing F-cell ratio in plasma or cell of a subject, comprising administering Compound 1.

7. The method of claim 6, wherein the F-cell ratio in plasma or cell of a subject is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

8. A method of increasing Ery.C level in a cell or in plasma of a subject, comprising administering Compound 1.

9. The method of claim 8, wherein the Ery.C level in plasma or cell of a subject is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

10. A method of increasing red blood cell counts of a subject, comprising administering Compound 1.

11. The method of claim 10, wherein the Ery.C level in plasma or cell of a subject is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

12. A method of increasing hemoglobin counts of a subject, comprising administering Compound 1.

13. The method of claim 12, wherein the hemoglobin count of a subject is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

14. A method of increasing gamma-globin gene expression in a cell or plasma of a subject, comprising administering Compound 1.

15. The method of claim 14, wherein the gamma-globin gene expression level in plasma or cell of a subject is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

16. A method of increasing hematocrit levels of a subject, comprising administering Compound 1.

17. The method of claim 16, wherein the hematocrit level in plasma or cell of a subject is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

18. A method of increasing fetal hemoglobin (HbF) positive cell number in a subject, comprising administering Compound 1.

19. The method of claim 18, wherein the HbF positive red blood cell number is increased by at least about 50%, about 100%, about 150%, about 2 times, about 3 times, about 4 times, about 5 times, about 10 times, about 15 times, about 20 times, or about 25 times.

20. A method of decreasing reticulocyte level in a cell or plasma of a subject, comprising administering Compound 1.

21. The method of claim 20, wherein the reticulocyte level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

22. A method of decreasing spleen cellularity of a subject, comprising administering Compound 1.

23. The method of claim 22, wherein the spleen cellularity of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

24. A method of decreasing P-selectin protein levels of a subject, comprising administering Compound 1.

25. The method of claim 24, wherein the P-selectin level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

26. A method of decreasing Ery.B levels of a subject, comprising administering Compound 1.

27. The method of claim 26, wherein the Ery.B level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

28. A method of decreasing L-selectin protein levels of a subject, comprising administering Compound 1.

29. The method of claim 28, wherein the L-selectin level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

30. A method of decreasing bilirubin levels of a subject, comprising administering Compound 1.

31. The method of claim 30, wherein the bilirubin levels in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

32. A method of decreasing free heme levels of a subject, comprising administering Compound 1.

33. The method of claim 32, wherein the free heme level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

34. A method of decreasing L-selectin levels of a subject, comprising administering Compound 1.

35. The method of claim 34, wherein the L-selectin level in a cell or plasma of a subject is reduced by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70%.

36. The method of any of the preceding claims, wherein the degree of change is at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, or about 70 %.

37. The method of claim 1, wherein the patient is suffering from Vaso-occlusive crisis (VOC), beta-thalassemia, sickle cell disease, sickle cell crisis, hypertension, cardiovascular disease, cardiac hypertrophy, myocardial infarction, angina, heart failure, hypercholesterolemia, , chronic kidney disease, chronic liver disease, chronic renal disease, chronic kidney injury, inflammatory disease, obesity associated chronic kidney disease, type II diabetes, diabetic nephropathy, kidney fibrosis, neuropathy, glomerulonephritis, kidney failure, ischemic kidney injury, acute kidney injury, focal segmental glomerulosclerosis, neurodegenerative disease, inflammatory bowel disease, colitis, idiopathic pulmonary fibrosis, fatty liver disease, heart disease, obesity, pulmonary arterial hypertension, postoperative atrial fibrillation, fatal arrhythmia, catecholaminergic polymorphic ventricular tachycardia, thrombosis, chronic obstructive pulmonary disease, valvular heart disease, anti-adipogenic disease, mitochondrial related disease, Charcot-Marie-Tooth disease, Alzheimer's disease, prostatic hyperplasia, urolithiasis, allergic airway disease, non-alcoholic steatohepatitis, solid organ fibrosis, inflammatory gastrointestinal disease, metabolic syndrome, or amyotrophic lateral sclerosis.

38. The method of claim 1, wherein the patient is suffering from catecholaminergic polymorphic ventricular tachycardia.

39. The method of claim 1, wherein the patient is suffering from post-operative atrial fibrillation.

40. The method of claim 1, wherein the patient is suffering from fatal arrhythmia.

41. The method of claim 1, wherein the patient is suffering from VOC.

42. The method of claim 1, wherein the patient is suffering from beta thalassemia disease.

43. The method of claim 1, wherein the patient is suffering from sickle cell disease.

44. The method of claim 1, wherein the patient is suffering from hypertension.

45. The method of claim 1, wherein the patient is suffering from cardiovascular disease.

46. The method of claim 1, wherein the patient is suffering from hypercholesterolemia.

47. The method of claim 1, wherein the patient is suffering from chronic kidney disease.

48. The method of claim 1, wherein the patient is suffering from chronic liver disease.

49. The method of claim 1, wherein the patient is suffering from renal disease.

50. The method of claim 1, wherein the patient is suffering from chronic kidney injury.

51. The method of claim 1, wherein the patient is suffering from inflammatory disease.

52. The method of claim 1, wherein the patient is suffering from obesity associated chronic kidney disease.

53. The method of claim 1, wherein the patient is suffering from diabetic nephropathy.

54. The method of claim 1, wherein the patient is suffering from kidney fibrosis.

55. The method of claim 1, wherein the patient is suffering from glomerulonephritis.

56. The method of claim 1, wherein the patient is suffering from kidney failure.

57. The method of claim 1, wherein the patient is suffering from ischemic kidney injury.

58. The method of claim 1, wherein the patient is suffering from neurodegenerative disease.

59. The method of claim 1, wherein the patient is suffering from inflammatory bowel disease.

60. The method of claim 1, wherein the patient is suffering from idiopathic pulmonary fibrosis.

61. The method of claim 1, wherein the patient is suffering from fatty liver disease.

62. The method of claim 1, wherein the patient is suffering from heart disease.

63. The method of claim 1, wherein the patient is suffering from obesity.

64. The method of claim 1, wherein the patient is suffering from pulmonary arterial hypertension.

65. The method of claim 1, wherein the patient is suffering from type II diabetes.

66. The method of claim 1, wherein the patient is suffering from anti-adipogenic disease.

67. The method of claim 1, wherein the patient is suffering from mitochondrial related disease.

68. The method of claim 1, wherein the patient is suffering from Charcot-Marie-Tooth disease.

69. The method of claim 1, wherein the patient is suffering from allergic airway disease.

70. The method of claim 1, wherein the patient is suffering from non-alcoholic steatohepatitis.

71. The method of claim 1, wherein the patient is suffering from amyotrophic lateral sclerosis.

72. The method of claim 1, wherein the patient is suffering from sickle cell crisis.

73. The method of claim 1, wherein the patient is suffering from solid organ fibrosis.

74. The method of claim 1, wherein the patient is suffering from chronic obstructive pulmonary disease.

75. The method of claim 1, wherein the patient is suffering from cardiac hypertrophy.

76. The method of claim 1, wherein the patient is suffering from myocardial infarction.

77. The method of claim 1, wherein the patient is suffering from angina.

78. The method of claim 1, wherein the patient is suffering from sickle cell nephropathy.

79. The method of claim 1, wherein the patient is suffering from acute kidney injury.

80. The method of claim 1, wherein the patient is suffering from focal segmental glomerulosclerosis.

81. The method of claim 1, wherein the patient is suffering from inflammatory gastrointestinal disease.

82. The method of claim 1, wherein the patient is suffering from colitis.

83. The method of claim 1, wherein the patient is suffering from metabolic syndrome.

84. The method of claim 1, wherein the patient is suffering from neuropathy.

85. The method of any of the previous claims, wherein the Compound is administered orally.

86. The method of any of the previous claims, wherein Compound 1 is administered daily.

87. The method of any of the previous claims, wherein Compound 1 is administred at between about 0.3 mg/kg – about 500 mg/kg.

88. The method of claim 87, wherein Compound 1 is administered at about 0.3 mg/kg, about 1 mg/kg, about 3 mg/kg, about 10 mg/kg, about 30 mg/kg, about 50 mg/kg, about 100 mg/kg, about 150 mg/kg, about 200 mg/kg, or about 250 mg/kg.

89. The method of any of the previous claims, wherein Compound 1 is administered for between 1 to 7 days.

90. The method of any of the previous claims, wherein Compound 1 is administered for at least 7 days.

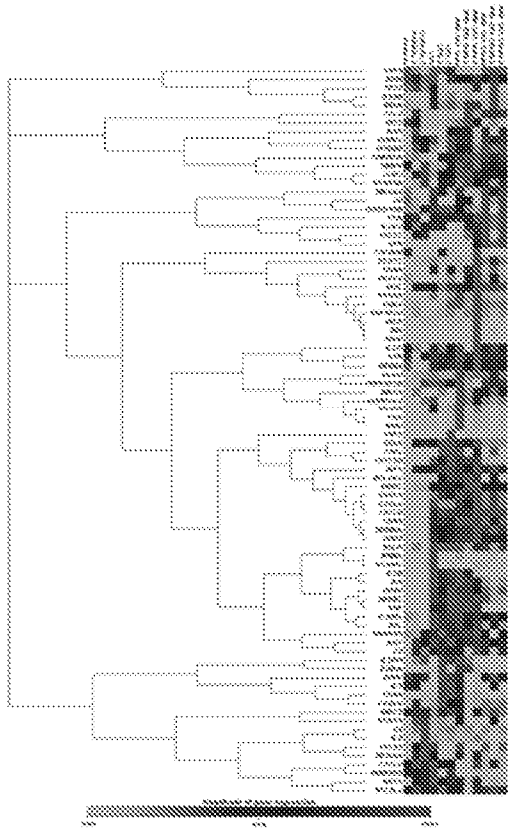
Fig. 1

IMR-261 activates NRF2 target genes in Human Monocytes from Healthy Donors

Oxidative Stress Gene Array

	IMR261 3uM	IMR261 10uM	p (IMR261 10μM vs. Vehicle	DMF 20uM
HMOX1	5.85	21.21	0.000803	1.32
HSPA1A	3.08	20.49	0.000025	1.21
HSP90AA1	3.23	15.58	0.000094	1.07
GCLM	2.93	5.04	0.000041	1.17
SOD1	1.61	3.83	0.000912	1.07
AKR1C2	4.17	3.56	0.000801	1.31
BAG2	1.44	3.55	0.002277	0.9
SRXN1	1.64	2.08	0.00012	0.56
PTGR1	1.82	2.01	0.001295	1.18
SPINK1	1.09	1.93	0.000352	0.88
SQSTM1	1.43	1.89	0.000513	1.05
TXNRD1	1.48	1.81	0.000736	1.18
DUSP1	1.23	1.75	0.008364	1.15
GCLC	2.06	1.73	0.011043	1.3
PRNP	1.1	1.52	0.005663	0.82

Fig. 2



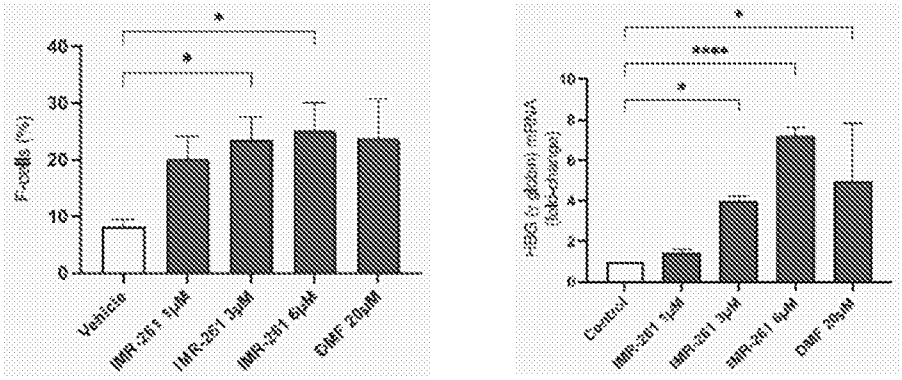
IMR-261 blocks LPS-induced NFkB target genes in Human Monocytes from Healthy Donors

NFkB target Gene Array

				Fold Change (comparing to LPS)	
	LPS	IMR-261 3μM	IMR-261 10μM	IMR-261 3μM	IMR-261 10μM
IL1B	392,01	101,58	9,61	0,28	0,02
TNF	129,31	166,43	9,54	1,26	0,07
BCL2A1	112,08	253,51	25,12	2,26	0,22
NFKBIA	106,75	177,61	73,9	1,66	0,69
CD83	81,84	108,58	37,99	1,33	0,46
NFKB1	93,45	47,92	10,3	1,10	0,24
CXCL10	23,38	20,36	5,84	0,90	0,25
REL	21,43	19,11	7,54	0,89	0,35
CD40	14,6	13,46	5,22	0,92	0,36
IL6	8,52	4,81	2,35	0,51	0,25
CSF2	7,74	3,78	1,08	0,49	0,17
ICAM1	7,23	5,84	4,25	0,81	0,59
CSF1	4,58	4,65	1,89	1,06	0,41
CD80	3,69	6,2	2,13	1,68	0,58
CD86	3,33	1,84	1,04	0,58	0,31
IFNB1	3,21	1,95	1,6	0,61	0,50
IL2RA	2,18	3,46	1,64	1,59	0,46
VCAM1	2,07	1,22	1,04	0,59	0,50

Fig. 3

IMR-261 Induces Fetal Hemoglobin in CD34+ Cells from Healthy Donors



*p<0.05, **p<0.01, ***p<0.005, ****p<0.001, IMR-261 6 μM : N=4 (N=2 health subjects + N=2 SCD sample) due to decreased cell viability

Fig. 4

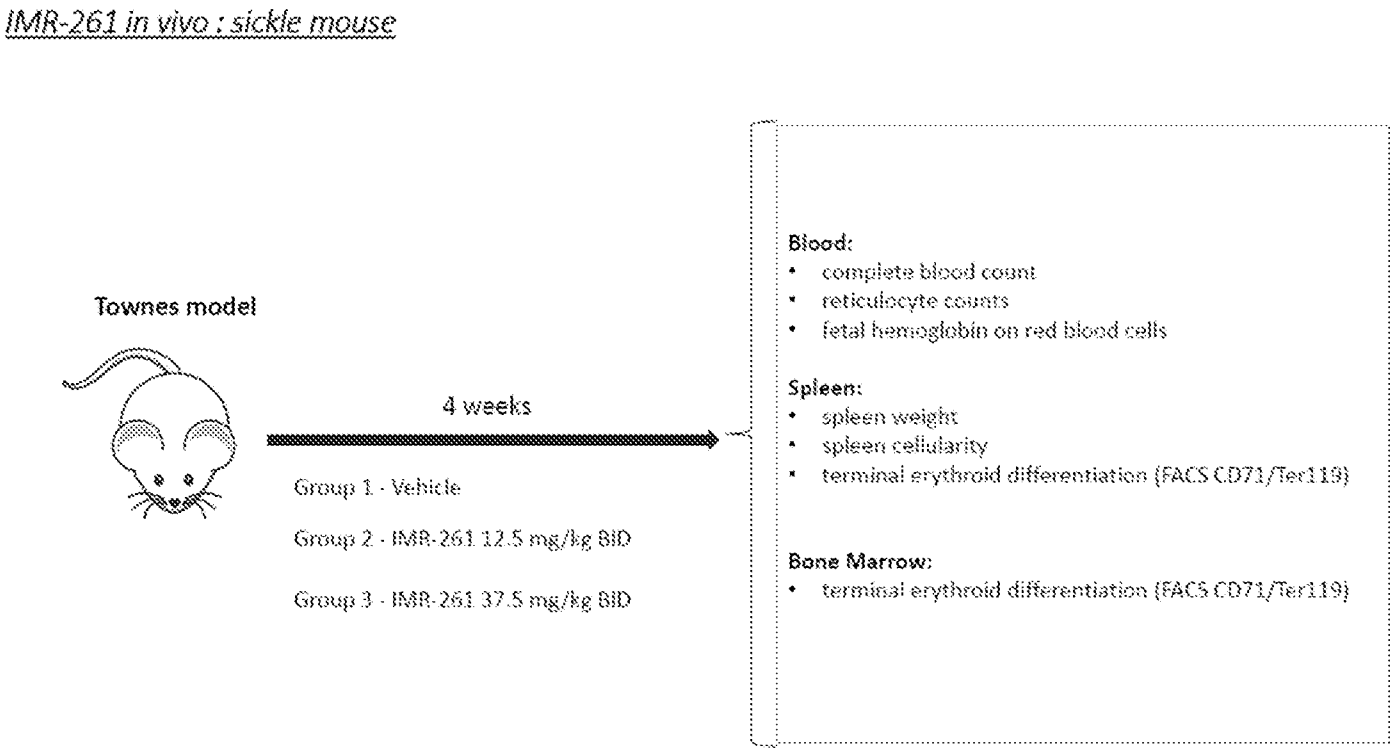
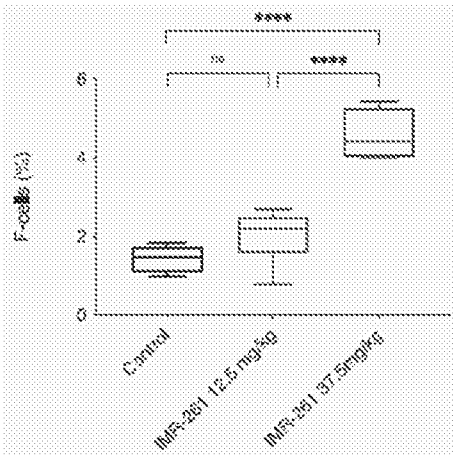

IMR-261 in vivo : sickle mouse
Townes model
4 weeks
Group 1 - Vehicle
Group 2 - IMR-261 12.5 mg/kg BID
Group 3 - IMR-261 37.5 mg/kg BID
Blood:
• complete blood count
• reticulocyte counts
• fetal hemoglobin on red blood cells
Spleen:
• spleen weight
• spleen cellularity
• terminal erythroid differentiation (FACS CD71/Ter119)
Bone Marrow:
• terminal erythroid differentiation (FACS CD71/Ter119)

Fig. 5

IMR-261 in vivo : increase fetal hemoglobin at 37.5 mg/kg



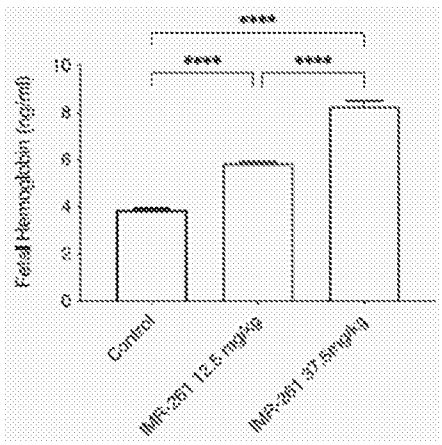
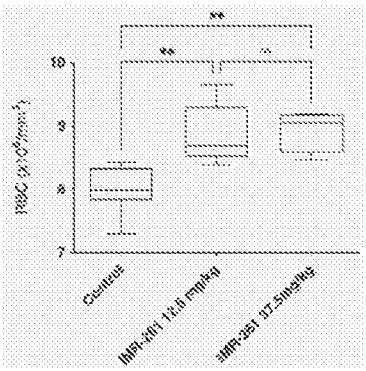
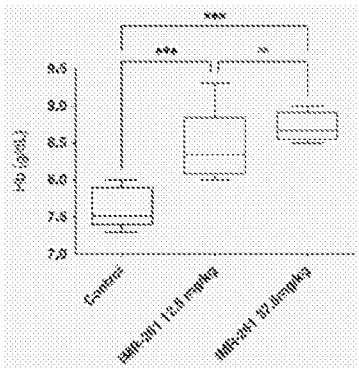


Fig. 6

IMR-261 in vivo: other biomarkers





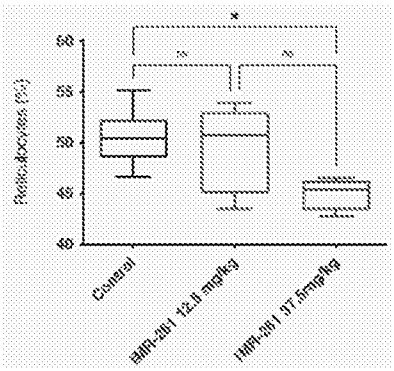


Fig. 7

IMR-261 in vivo : effect on splenomegaly

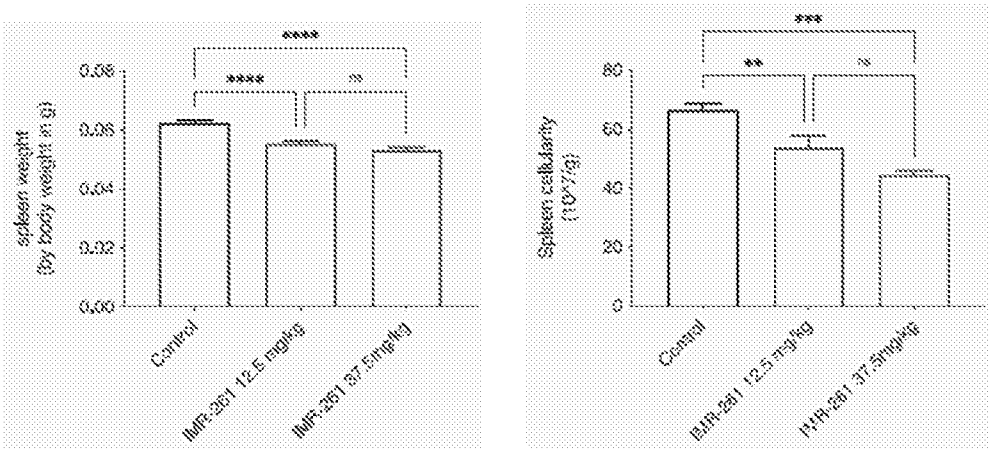


Fig. 8

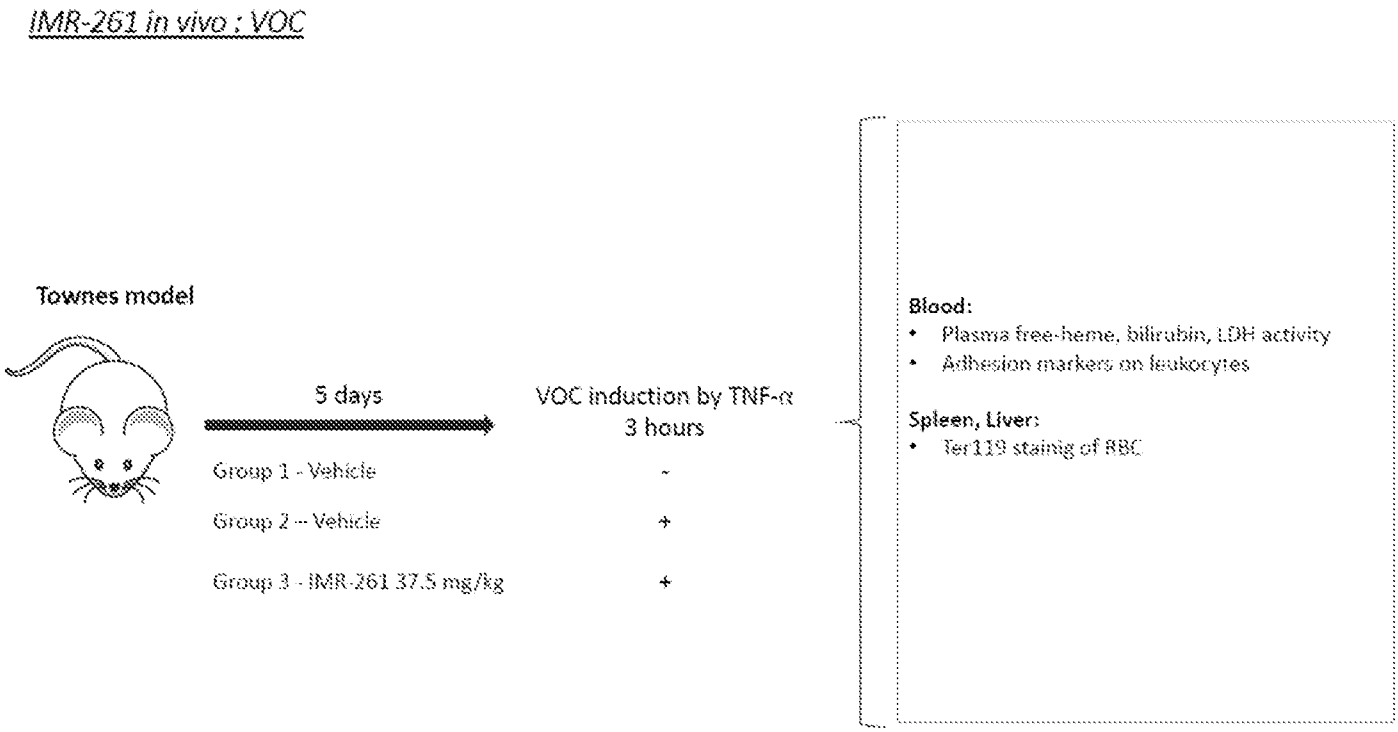

IMR-261 in vivo : VOC
Townes model
5 days
VOC induction by TNF-α
3 hours
Group 1 - Vehicle
-
Group 2 – Vehicle
+
Group 3 - IMR-261 37.5 mg/kg
+
Blood:
• Plasma free-heme, bilirubin, LDH activity
• Adhesion markers on leukocytes
Spleen, Liver:
• Ter119 staining of RBC

Fig. 9

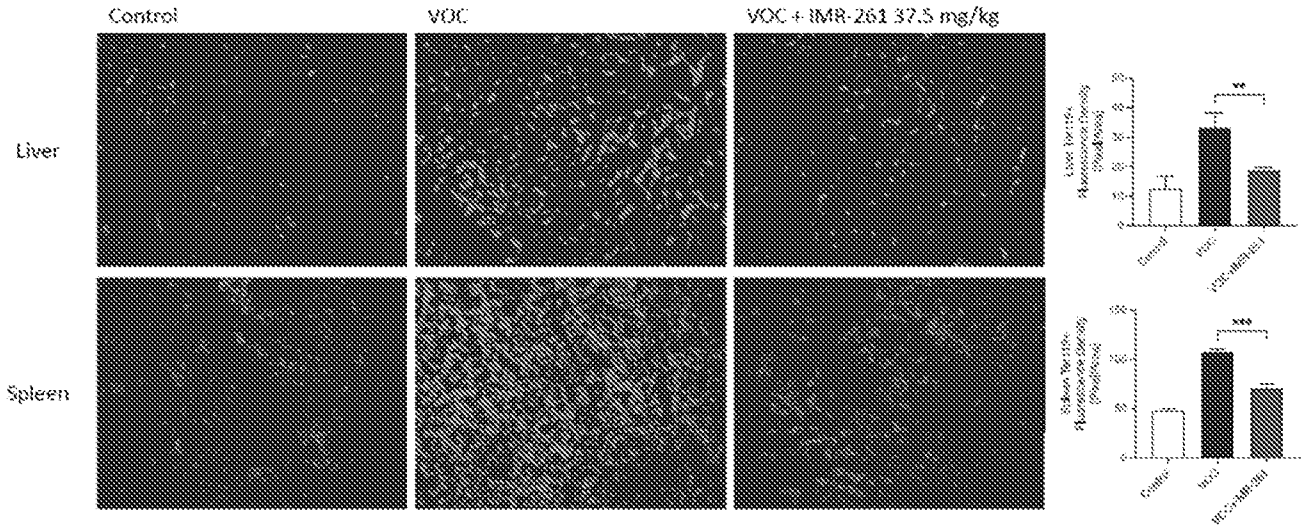


Fig. 10

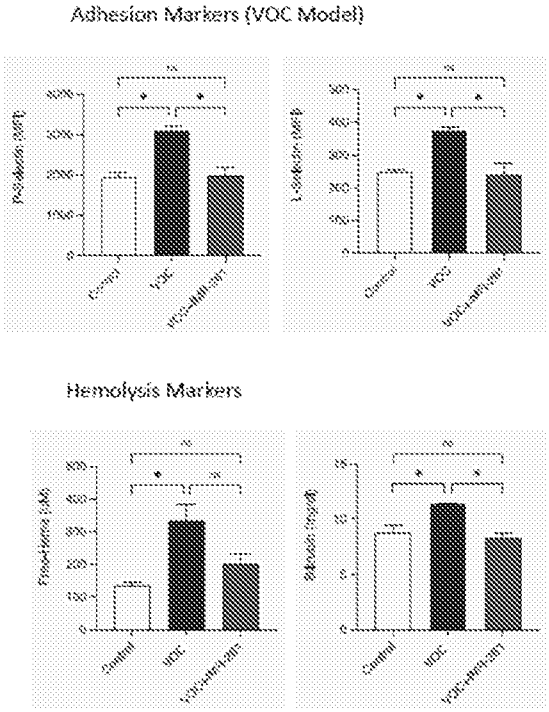


Fig. 11

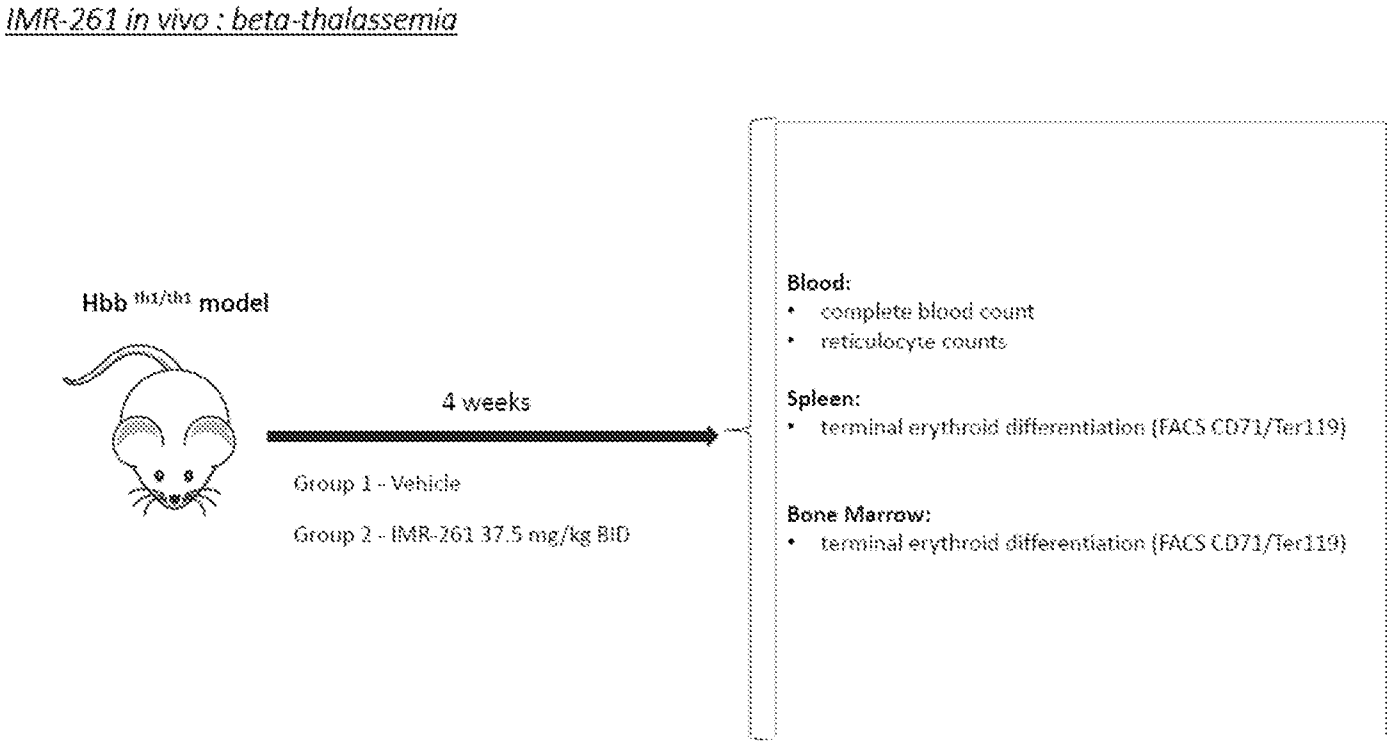

IMR-261 in vivo : beta-thalassemia
Hbb th3/th3 model
4 weeks
Group 1 - Vehicle
Group 2 - IMR-261 37.5 mg/kg BID
Blood:
• complete blood count
• reticulocyte counts
Spleen:
• terminal erythroid differentiation (FACS CD71/Ter119)
Bone Marrow:
• terminal erythroid differentiation (FACS CD71/Ter119)

Fig. 12

IMR-261 Hbb in vivo : biomarkers

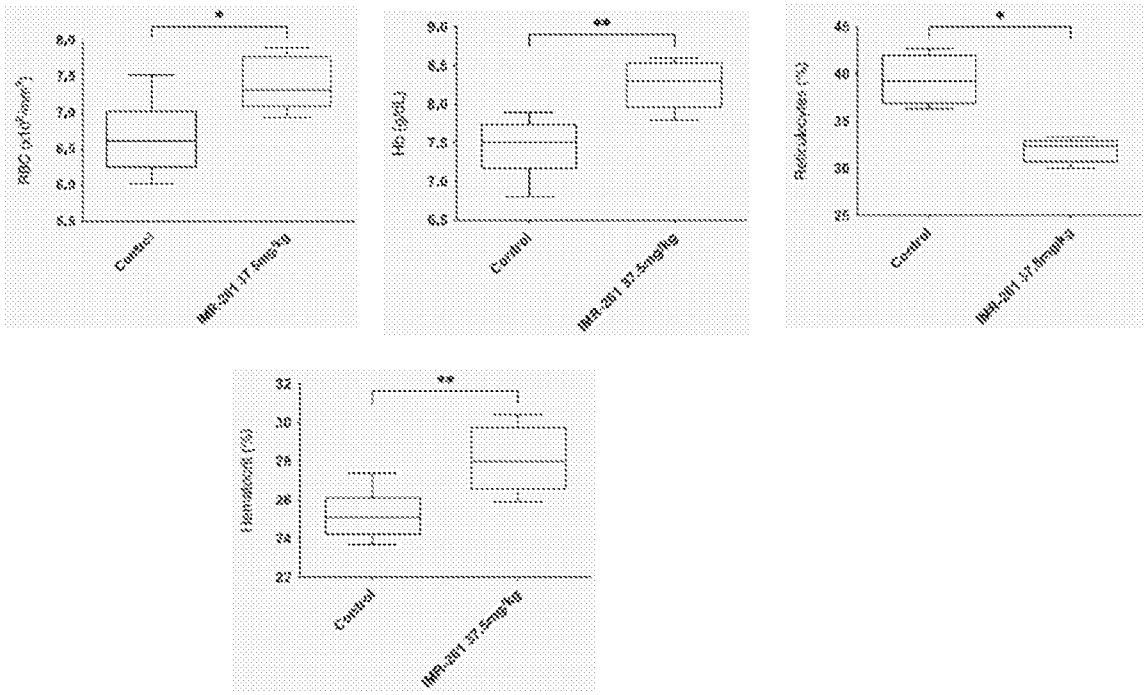
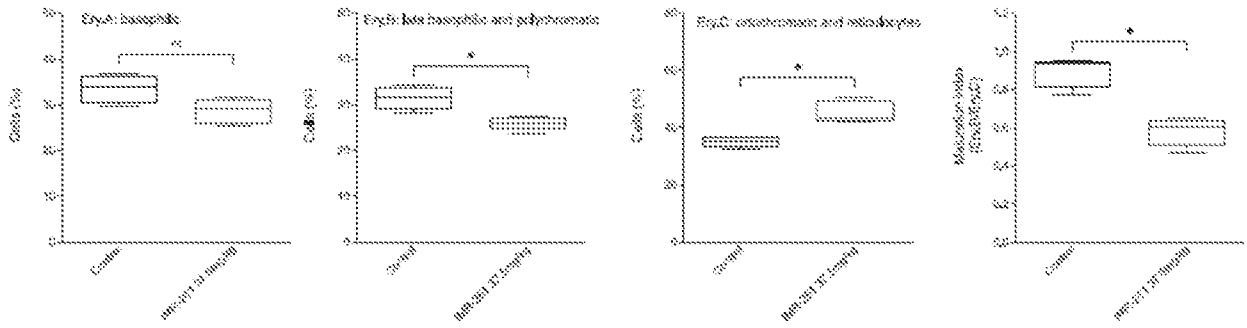


Fig. 13

Terminal erythroid differentiation

Spleen (stress erythropoiesis)



IMR-261 ameliorates ineffective erythropoiesis

Fig. 14

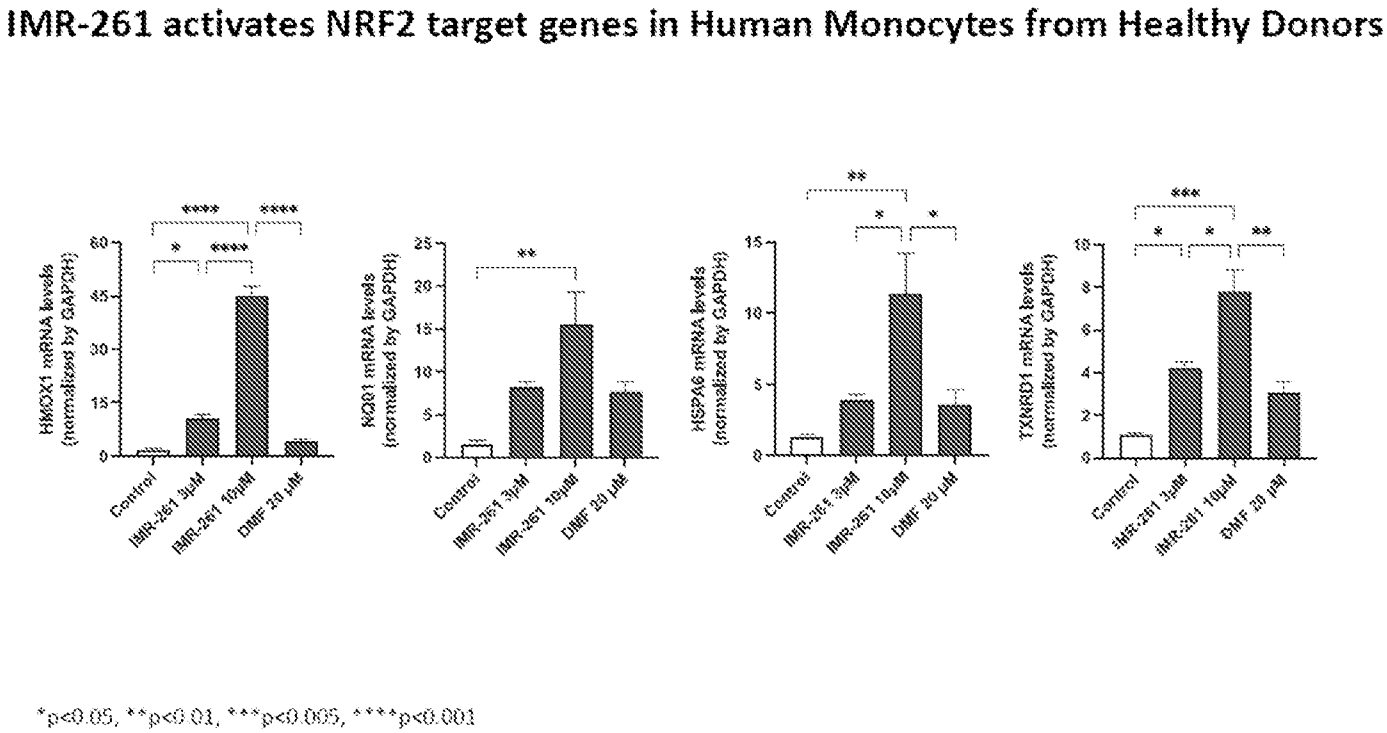

IMR-261 activates NRF2 target genes in Human Monocytes from Healthy Donors
HMOX1 mRNA levels (normalized by GAPDH)
NQO1 mRNA levels (normalized by GAPDH)
HSPA6 mRNA levels (normalized by GAPDH)
TXNRD1 mRNA levels (normalized by GAPDH)
Control
IMR-261 3µM
IMR-261 10µM
DMF 20 µM
*p<0.05, **p<0.01, ***p<0.005, ****p<0.001

Fig. 15

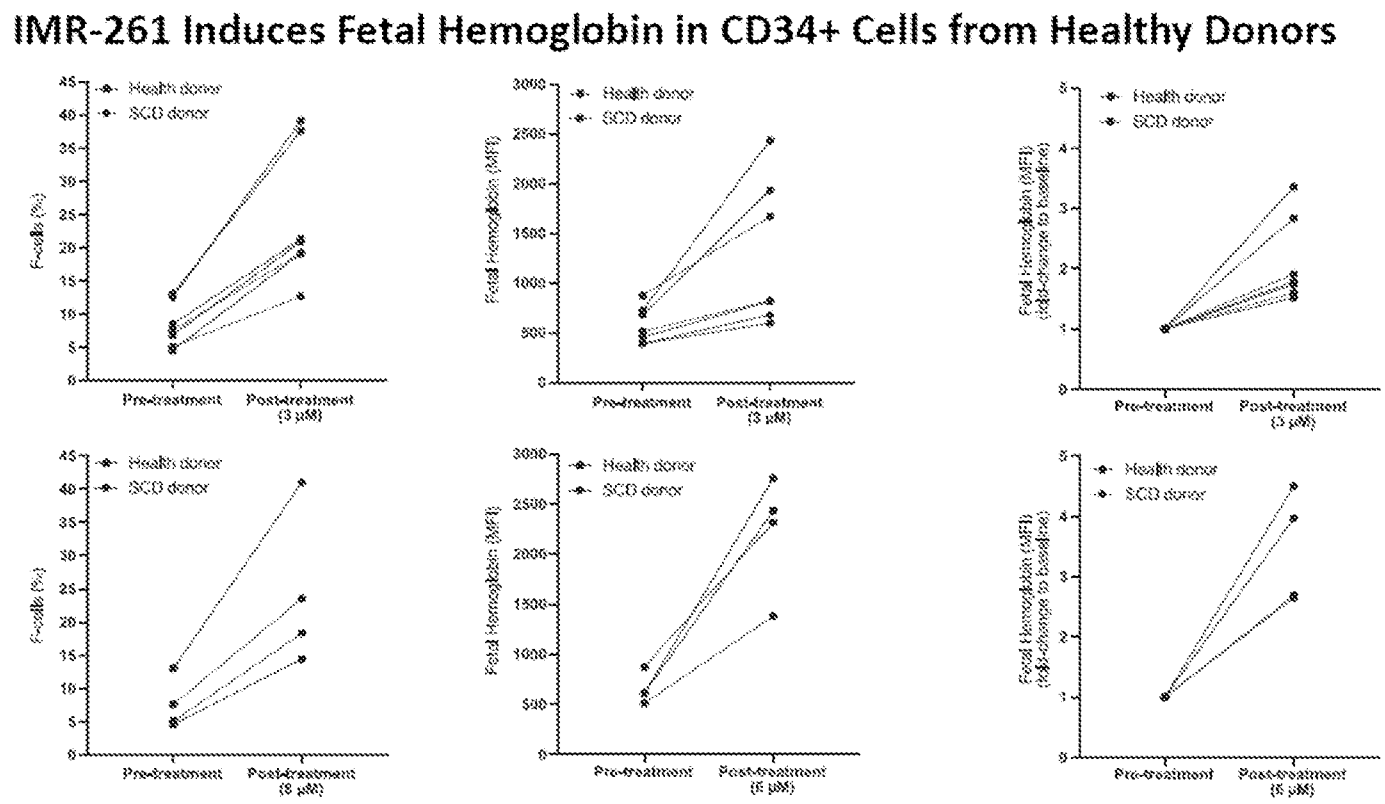
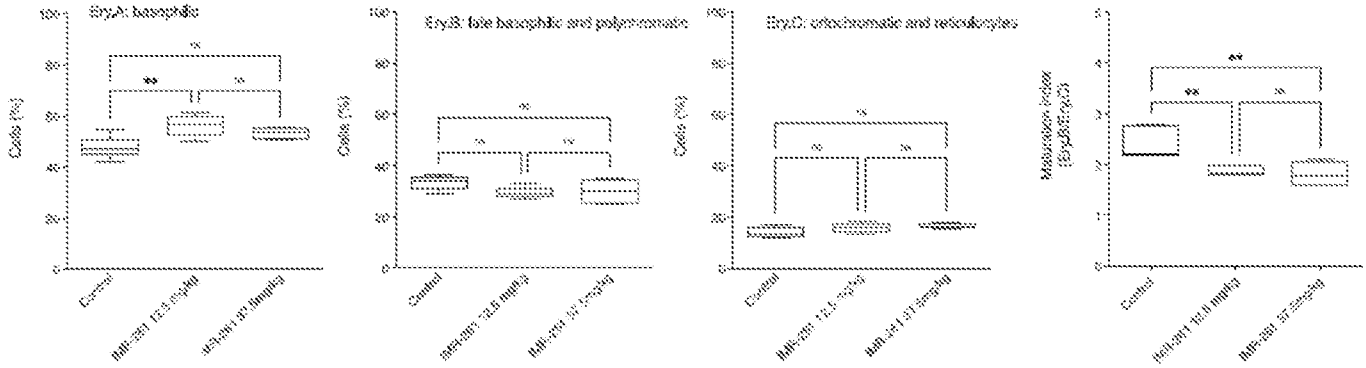

IMR-261 Induces Fetal Hemoglobin in CD34+ Cells from Healthy Donors
Health donor
SCD donor
Pre-treatment
Post-treatment
(3 μM)
(6 μM)
*p<0.05, **p<0.01, ***p<0.005, ****p<0.001, IMR-261 6 μM : N=4 (N=2 health subjects + N=2 SCD sample) due to decreased cell viability

Fig. 16

Terminal erythroid differentiation (Townes)

Bone Marrow

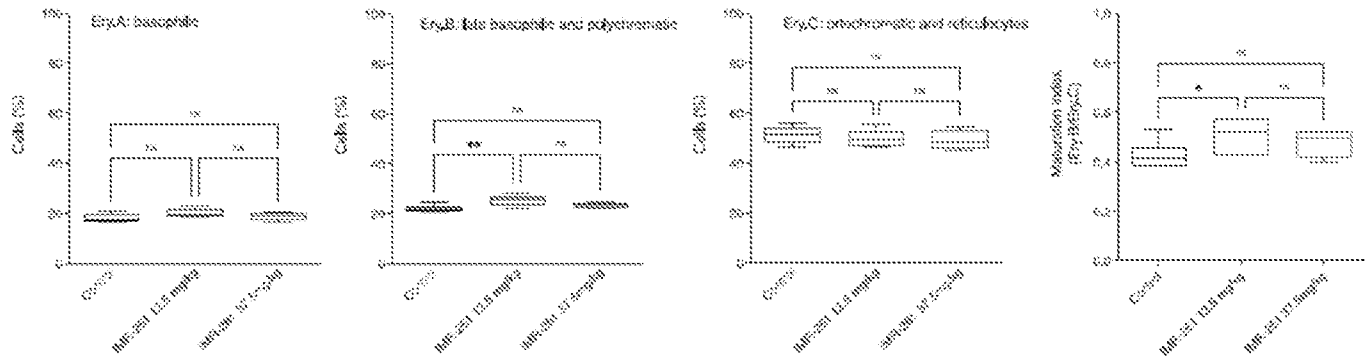


Small effect of IMR-261 on erythropoiesis (maturation of erythroblasts)

Fig. 17

Terminal erythroid differentiation (Townes)

Spleen (stress erythropoiesis)



No effects of IMR-261 on stress erythropoiesis

Fig. 18

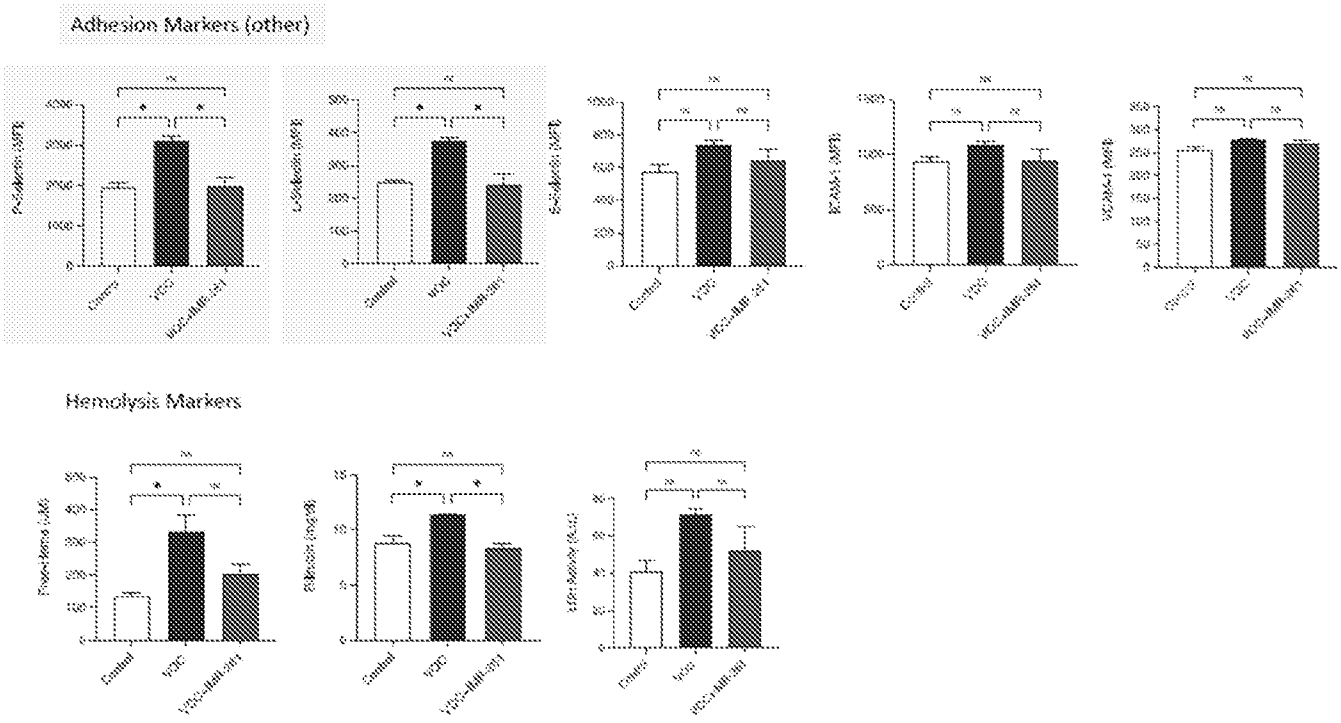


Fig. 19

IMR-261 Hbb: all markerts

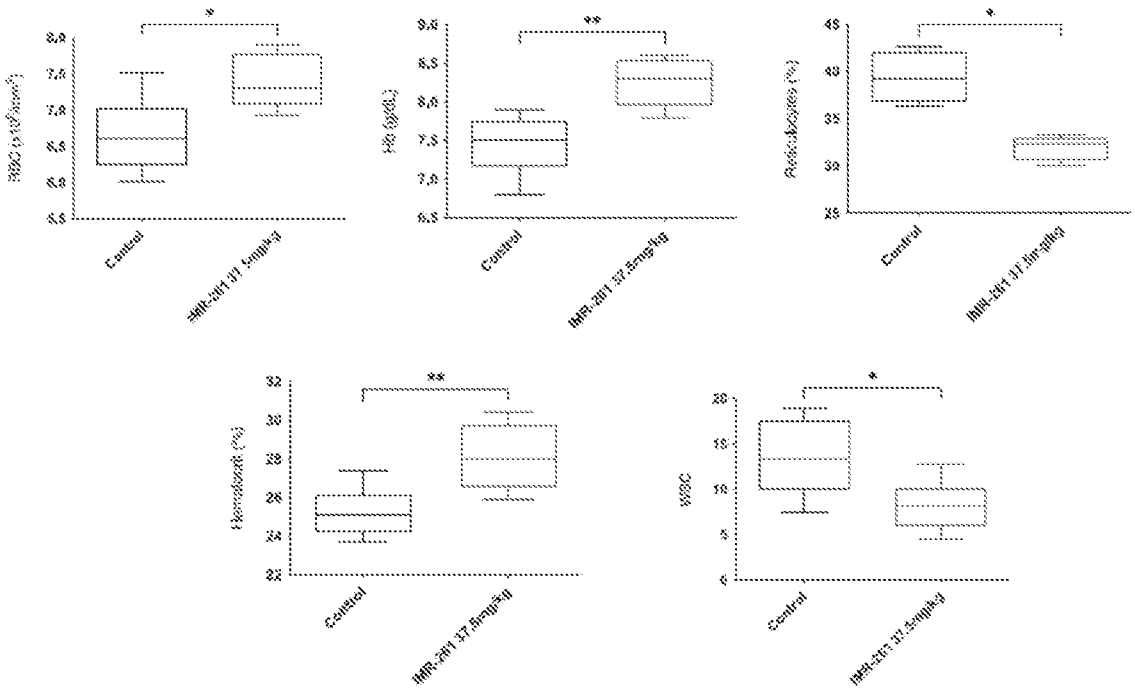
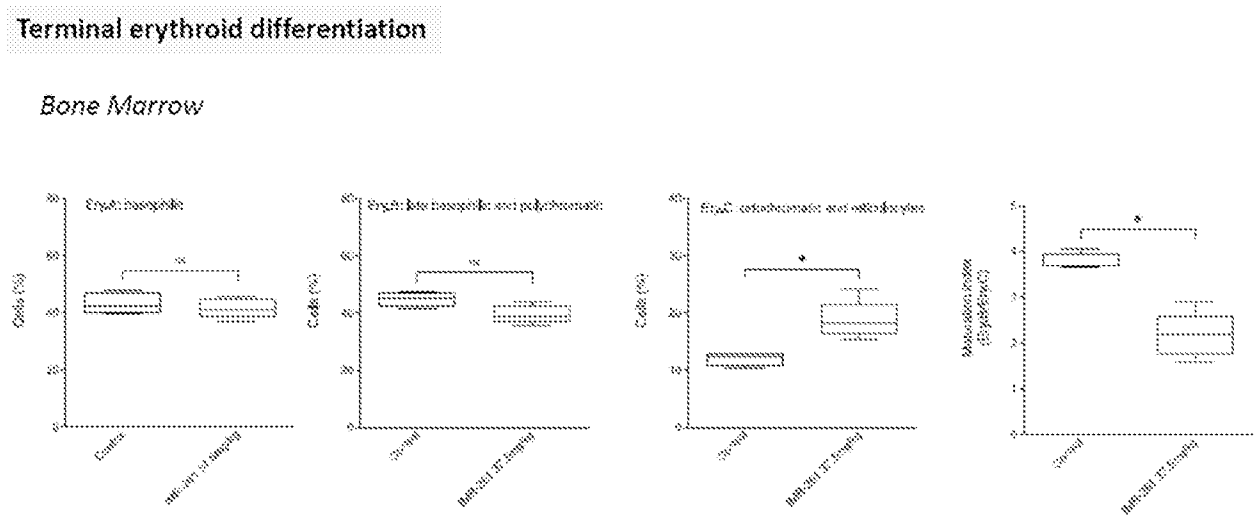


Fig. 20



IMR-261 ameliorates ineffective erythropoiesis

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/39217

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 31/201, A61K 31/231, A61K 31/202 (2022.01)
ADD. A61K 48/00 (2022.01)

CPC - INV. A61K 31/201, A61K 31/231, A61K 31/202
ADD. A61K 48/00

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)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2017/0095437 A1 (Complexa, Inc.) 06 April 2017 (06.04.2017); entire document, especially abstract	1, 4-5, 36-84
A	US 2018/0200387 A1 (CRISPR Therapeutics AG) 19 July 2018 (19.07.2018); entire document, especially abstract, [0089]	1, 4-5, 36-84
A	US 2010/0280100 A1 (COLLARD et al.) 04 November 2010 (01.11.2010); entire document	1, 4-5, 36-84
P/X	WO 2021/242758 A1 (IMARA INC.) 02 December 2021 (02.12.2021); entire document	1

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2022	DEC 06 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/39217

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

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

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

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

3. ☒ Claims Nos.: 85-90
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-84, directed to a method of treating a condition or disease comprising administering to the patient a therapeutically effective amount of 10-nitro-9(E)-octadec-9-enoic acid. The method will be searched to the extent that it encompasses the method comprising first choice of increasing HbF gene expression of instant claim 1. It is believed that claims 1, 4-5, and 36-84 read on this first named invention, and thus these claims will be searched without fee to the extent that they encompass the first species of instant claim 1, described above. Applicant is invited to elect additional method(s) wherein each additional method elected will require one additional invention fee. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines Applicants must specify the claims that encompass any additionally elected method.

****See Supplemental Box****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 4-5, 36-84

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box III Observations where unity of invention is lacking

Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be the method comprising increasing gamma-globin gene expression of instant claim 1 (i.e. claims 1, 14-15, 36-84).

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique method of treatment, which is not required by any other invention of Group I+.

Common technical features:

The inventions of Group I+ share the technical feature of a method of treating a condition or disease in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof. However, this shared technical feature does not provide a contribution over the prior art, because the shared technical feature is anticipated by US 2017/0095437 A1 to Complexa, Inc. (hereinafter "Complexa"). Complexa teaches a method of treating a condition or disease in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of 10-nitro-9(E)-octadec-9-enoic acid (Compound 1), or a pharmaceutically acceptable salt or solvate thereof (abstract, "Various embodiments of this invention are directed to pharmaceutical compositions and methods for treating diseases... The methods of various embodiments include administering an effective amount of 10-nitro-9(E)-octadec-9-enoic acid to treat such diseases").

As said method was known in the art at the time of the invention, this cannot be considered a special technical feature, that would otherwise unify the inventions of Group I+.

The inventions of Groups I+, thus lack unity under PCT Rule 13.

*Item 4 (contd): Claims 85-90 are held unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).