METHODS OF TREATING FABRY DISEASE IN PATIENTS HAVING RENAL IMPAIRMENT

TECHNICAL FIELD

[0001] Principles and embodiments of the present invention relate generally to the use of pharmacological chaperones for the treatment of Fabry disease, particularly in patients with varying degrees of renal impairment.

BACKGROUND

[0002] Many human diseases result from mutations that cause changes in the amino acid sequence of a protein which reduce its stability and may prevent it from folding properly. Proteins generally fold in a specific region of the cell known as the endoplasmic reticulum, or ER. The cell has quality control mechanisms that ensure that proteins are folded into their correct three-dimensional shape before they can move from the ER to the appropriate destination in the cell, a process generally referred to as protein trafficking. Misfolded proteins are often eliminated by the quality control mechanisms after initially being retained in the ER. In certain instances, misfolded proteins can accumulate in the ER before being eliminated. The retention of misfolded proteins in the ER interrupts their proper trafficking, and the resulting reduced biological activity can lead to impaired cellular function and ultimately to disease. In addition, the accumulation of misfolded proteins in the ER may lead to various types of stress on cells, which may also contribute to cellular dysfunction and disease.

[0003] Such mutations can lead to lysosomal storage disorders (LSDs), which are characterized by deficiencies of lysosomal enzymes due to mutations in the genes encoding the lysosomal enzymes. The resultant disease causes the pathologic accumulation of substrates of those enzymes, which include lipids, carbohydrates, and polysaccharides. Although there are many different mutant genotypes associated with each LSD, many of the mutations are missense mutations which can lead to the production of a less stable enzyme. These less stable enzymes are sometimes prematurely degraded by the ER-associated degradation pathway. This results in the enzyme deficiency in the lysosome, and the pathologic accumulation of substrate. Such mutant enzymes are sometimes referred to in the pertinent art as "folding mutants" or "conformational mutants."

[0004] Fabry Disease is a LSD caused by a mutation to the GLA gene, which encodes the enzyme α-galactosidase A (α-Gal A). α-Gal A is required for glycosphingolipid metabolism. The mutation causes the substrate globotriaosylceramide (Gb3, GL-3, or ceramide trihexoside) to accumulate in various tissues and organs. Males with Fabry disease are hemizygotes because the disease genes are encoded on the X chromosome. Fabry disease is estimated to affect 1 in 40,000 and 60,000 males, and occurs less frequently in females.

[0005] There have been several approaches to treatment of Fabry disease. One approved therapy for treating Fabry disease is enzyme replacement therapy (ERT), which typically involves intravenous, infusion of a purified form of the corresponding wild-type protein (Fabrazyme®, Genzyme Corp.). ERT has several drawbacks, however. One of the main complications with enzyme replacement therapy is rapid degradation of the infused protein, which leads to the need for numerous, costly high dose infusions. ERT has several additional caveats, such as difficulties with large-scale generation, purification, and storage of properly folded protein; obtaining glycosylated native protein; generation of an anti-protein immune response; and inability of protein to cross the blood-brain barrier to mitigate central nervous system pathologies (i.e., low bioavailability). In addition, replacement enzyme cannot penetrate the heart or kidney in sufficient amounts to reduce substrate accumulation in the renal podocytes or cardiac myocytes, which figure prominently in Fabry pathology.

[0006] Another approach to treating some enzyme deficiencies involves the use of small molecule inhibitors to reduce production of the natural substrate of deficient enzyme proteins, thereby ameliorating the pathology. This "substrate reduction" approach has been specifically described for a class of about 40 related enzyme disorders called lysosomal storage disorders that include glycosphingolipid storage disorders. The small molecule inhibitors proposed for use as therapy are specific for inhibiting the enzymes involved in synthesis of glycolipids, reducing the amount of cellular glycolipid that needs to be broken down by the deficient enzyme.

[0007] A third approach to treating Fabry disease has been treatment with what are called pharmacological chaperones (PCs). Such PCs include small molecule inhibitors of α-Gal A, which can bind to the α-Gal A to increase the stability of both mutant enzyme and the corresponding wild type.

[0008] One problem with current treatments is difficulty in treating patients exhibiting renal impairment, which is very common in Fabry patients and progresses with disease. On

average, it take between about 10-20 years for patients to decline from normal kidney function to severe renal impairment, with some countries reporting even faster declines. By some estimates, about 10% of Fabry patients receiving ERT may have moderate renal impairment. Another 25% of males and 5% of females receiving ERT have an estimated glomerular filtration rate (eGFR) of less than 30, corresponding to severe kidney impairment or even renal failure. Of these, about half have severe kidney impairment, and about half are on dialysis.

[0009] Unfortunately, renal impairment will progress despite ERT treatment. A patient having an eGFR of 30 may deteriorate to the point of needing dialysis in two to five years. About 30% of patients receiving ERT will end up on dialysis or needing a kidney transplant, depending on the start of ERT. The earlier ERT is commenced, the longer renal function may be preserved, but commencement of ERT may be delayed because Fabry disease is rare and often misdiagnosed.

[0010] Further, and as discussed above, ERT often does not sufficiently penetrate the kidneys to reduce substrate accumulation, thereby allowing further damage during disease progression. With PC treatment, the kidneys are often how the drug is cleared from the body, and renal impairment may affect drug pharmacokinetics and/or drug pharmacodynamics. Thus, there is still a need for a treatment of Fabry patients who have renal impairment.

SUMMARY

[0011] One aspect of the invention pertains to a method for treatment of Fabry disease in a patient having renal impairment, the method comprising administering to the patient about 100 mg to about 300 mg free base equivalent (FBE) of migalastat or salt thereof at a frequency of less than once every other day. In one or more embodiments, the patient has moderate renal impairment. In one or more embodiments, the patient has severe renal impairment. In some embodiments, the migalastat is in a solid dosage form. In one or more embodiments, the patient is administered about 123 mg FBE. In some embodiments, the patient is administered about 150 mg migalastat HCl. In one or more embodiments, the migalastat is administered orally. In one or more embodiments, the migalastat is administered for at least 28 days. In one or more embodiments, the migalastat is administered for at least 6 months. In one or more embodiments, the migalastat is administered for at least 12 months.

[0012] A second aspect of the invention pertains to a method for treatment of Fabry disease in a patient having renal impairment, the method comprising administering to the

patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every seven days. In some embodiments, the patient has moderate renal impairment. In one or more embodiments, the patient has severe renal impairment. In some embodiments, the migalastat is in a solid dosage form. In one or more embodiments, the patient is administered about 123 mg FBE. In some embodiments, the patient is administered about 150 mg migalastat HCl. In one or more embodiments, the migalastat is administered orally. In one or more embodiments, the migalastat is administered for at least 28 days. In one or more embodiments, the migalastat is administered for at least 6 months. In one or more embodiments, the migalastat is administered for at least 12 months.

[0013] A third aspect of the invention pertains to a method for treatment of Fabry disease in a patient having renal impairment, the method comprising administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a first frequency of once every other day for a first time period; and administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a second frequency of less than every other day for a second time period. In one or more embodiments, the second frequency is in a range of once every three days to once every seven days. In one or more embodiments, the second frequency is once every four days. In one or more embodiments, the second frequency is once every seven days. In one or more embodiments, the administration at the second frequency begins after a reduction in the patient's eGFR. In one or more embodiments, the reduction in eGFR is from $\geq$30 mL/min/1.73m^2 to < 30 mL/min/1.73m^2, i.e. a reduction in the patient's kidney function from mild or moderate renal impairment to severe renal impairment.

[0014] In some embodiments, the method further comprises:

(a) measuring lyso-Gb3 in one or more plasma samples from the patient;

(b) determining a first baseline lyso-Gb3 level during the first time period;

(c) measuring migalastat concentration, $AUC_{0-\infty}$ and/or C_{trough} in one or more plasma samples from the patient during the first time period; and

(d) beginning the administration at the second frequency after

(i) an increase above the first baseline lyso-Gb3 level, and

(ii) more than about 5 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period is measured, or

there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period.

[0015] In one or more embodiments, the method further comprises:

(a) measuring lyso-Gb3 in one or more plasma samples from the patient;

(b) determining a first baseline lyso-Gb3 level during the first time period;

(c) measuring migalastat concentration, $AUC_{0-\infty}$ and/or C_{trough} in one or more plasma samples from the patient during the first time period; and

(d) beginning the administration at the second frquency after

(i) an increase above the first baseline lyso-Gb3 level, and

(ii) more than about 5 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period is measured, or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period.

[0016] In some embodiments, the increase above the first baseline lyso-Gb3 level is at least about 30% and/or 2nM. In one or more embodiments, measuring migalastat comprising measuring migalastat concentration, and administration at the second frequency begins after more than about 10 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period. In some embodiments, measuring migalastat comprises measuring $AUC_{0-\infty}$ or C_{trough}, and administration at the second frequency begins after there is a greater than 2-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function. In one or more embodiments, the second frequency is once every four days, and the method further comprises administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a third frequency of once every seven days for a third time period.

[0017] In some embodiments, the method further comprises:

(a) measuring lyso-Gb3 in one or more plasma samples from the patient;

(b) determining a first baseline lyso-Gb3 level during first time period;

(c) measuring migalastat concentration, $AUC_{0-\infty}$ and/or C_{trough} in one or more plasma samples from the patient during the first time period;

(d) beginning the administration at the second frequency after

(i) an increase above the first baseline lyso-Gb3 level, and

(ii) more than about 5 ng/mL of migalastat is measured 96 hours after administration of the migalastat during the first time period is measured, or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period;

(e) determining a second baseline lyso-Gb3 level during the second time period; and

(f) beginning the administration at the third frequency after

(i) an increase above the second baseline lyso-Gb3 level, and

(ii) more than about 5 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the second time period is measured, or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the second time period.

[0018] In one or more embodiments, the increase above the first baseline lyso-Gb3 level is at least about 30% and/or 2nM. In some embodiments, more than about 10 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period. In one or more embodiments, the second frequency is once every seven days.

[0019] In some embodiments, the method further comprises:

(a) measuring lyso-Gb3 in one or more plasma samples from the patient;

(b) determining a first baseline lyso-Gb3 level during the first time period;

(c) measuring migalastat concentration, $AUC_{0-\infty}$ and/or C_{trough} in one or more plasma samples from the patient during the first time period; and

(d) beginning the administration at the second frequency after

(i) an increase above the first baseline lyso-Gb3 level, and

(ii) more than about 5 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period is measured, or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period.

[0020] In one or more embodiments, the increase above the first baseline lyso-Gb3 level is at least about 30% and/or 2nM. In some embodiments, more than about 10 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period.

[0021] In one or more embodiments, the patient has moderate renal impairment. In some embodiments, the patient has severe renal impairment. In one or more embodiments, the migalastat is in a solid dosage form. In some embodiments, he patient is administered about 123mg FBE. In one or more embodiments, the patient is administered about 150 mg migalastat HCl. In some embodiments, the migalastat is administered orally.

[0022] Another aspect of the invention pertains to the use of migalastat in the treatment of Fabry disease in a patient having renal impairment, wherein the migalastat is administered to a Fabry disease patient having renal impairment in an amount of about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every four or seven days. In one or more embodiments, the frequency is once every four days. In some embodiments, the frequency is once every seven days. In one or more embodiments, the patient has moderate renal impairment. In some embodiments, the patient has severe renal impairment. In one or more embodiments, the migalastat is in a solid dosage form. In some embodiments, the patient is administered about 123 mg FBE. In one or more embodiments, the patient is administered about 150 mg migalastat HCl. In some embodiments, the migalastat is administered orally.

[0023] Various embodiments are listed below. It will be understood that the embodiments listed below may be combined not only as listed below, but in other suitable combinations in accordance with the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIG. 1A shows the migalastat plasma concentrations of non-Fabry patients with varying degrees of renal impairment as a function of CL_{CR};

[0025] FIG. 1B shows the migalastat plasma concentrations of non-Fabry patients with varying degrees of renal impairment as a function of time post-dose;

[0026] FIG. 1C shows the migalastat area under the curve (AUC) of non-Fabry patients with varying degrees of renal impairment;

[0027] FIGS. 2A-D show migalastat concentration as a function of time for various dosing regimens and degrees of renal impairment;

[0028] FIGS. 3A-B show accumulation ratio and migalastat concentration for various dosing regimens;

[0029] FIG. 4 shows migalastat $AUC_{0-\infty}$ and migalastat concentration after 48 hours in non-Fabry patients with varying degrees of renal impairment as a function;

[0030] FIG. 5 shows plasma migalastat concentration after 48 hours as a function of $eGFR_{MDRD}$ non-Fabry patients with varying degrees of renal impairment and two Fabry patients with renal impairment;

[0031] FIG. 6 shows plasma migalastat $AUC_{0-\infty}$ for non-Fabry patients with varying degrees of renal impairment and two Fabry patients with renal impairment;

[0032] FIGS. 7A-D show simulated median and observed migalastat concentration versus time in normal, severe, mild and moderate renal impairment subjects, respectively;

[0033] FIGS. 8A-D show migalastat C_{max}, AUC, C_{min} and C_{48h}, respectively, for normal, mild, moderate and severe renal impairment subjects;

[0034] FIGS. 9A-D show the steady state prediction for QOD for normal, severe, mild and moderate renal impairment subjects, respectively;

[0035] FIGS. 10A-D show migalastat C_{max}, AUC, C_{min} and C_{48h}, respectively, for normal, mild, moderate and severe renal impairment subjects;

[0036] FIG. 11A shows migalastat concentration after administration of 100 mg migalastat over 96 hours in a patient with moderate renal impairment;

[0037] FIG. 11B shows migalastat concentration after administration of 150 mg migalastat over 48 hours in a patient with normal kidney function;

[0038] FIGS. 12A-D show migalastat C_{max}, AUC, C_{min} and C_{48h}, respectively, for normal and moderate renal impairment subjects;

[0039] FIGS. 13A-E shows the full DNA sequence of human wild type GLA gene (SEQ ID NO: 1);

[0040] FIG. 14 shows the wild type GLA protein (SEQ ID NO: 2); and

[0041] FIG. 15 shows the lyso-Gb3 and eGFR of patient P3 over time.

DETAILED DESCRIPTION

[0042] Before describing several exemplary embodiments of the invention, it is to be understood that the invention is not limited to the details of construction or process steps set

forth in the following description. The invention is capable of other embodiments and of being practiced or being carried out in various ways.

[0043] Various aspects of the present invention pertain to particular dosing regimens of migalastat or a salt thereof for Fabry patients having renal impairment. Migalastat is a pharmacological chaperone used in the treatment of Fabry disease. This pharmacological chaperone is usually cleared from the body by the kidneys. However, patients who have renal impairment (a common problem for Fabry patients) may not be able to clear the migalastat from the body, and it was not previously known how patients with both Fabry disease and renal impairment would respond to migalastat therapy. Because pharmacological chaperones are also inhibitors, balancing the enzyme-enhancing and inhibitory effects of pharmacological chaperones such as migalastat is very difficult. Moreover, due to the complex interactions between Fabry disease and renal function and the lack of knowledge on the role of a pharmacological chaperone, migalastat dosing for Fabry patients with renal impairment is difficult to ascertain without significant clinical data and/or computer modeling.

[0044] Accordingly, one aspect of the invention pertains to a method for treatment of Fabry disease in a patient having renal impairment. In exemplary embodiments, the method comprises administering migalastat or a salt thereof every two, three, four, five, six or seven days. Although specific reference is made to administering every four or seven days, the methods and uses disclosed herein can also be used with other intermittent dosing regimens, such as every three, five or six days, based on, for example, the state of a patient's kidney.

[0045] In one or more embodiments, the method comprises administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every four days. In some embodiments, the method comprises administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every seven days. In some embodiments, the method comprises administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every four days for a first time period and then administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every seven days for a second time period. The patient may have mild, moderate or severe renal impairment.

[0046] Another aspect of the invention pertains to a use of migalastat in the treatment of Fabry disease in a patient having renal impairment, wherein the migalastat is administered to a Fabry disease patient having renal impairment in an amount of about 100 mg to about 300

mg FBE of migalastat or salt thereof at a frequency of once every four or seven days. The patient may have mild, moderate or severe renal impairment. In one or more embodiments, the patient has moderate or severe renal impairment. In specific embodiments, the patient has moderate renal impairment. In other specific embodiments, the patient has severe renal impairment.

[0047] Definitions

[0048] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them.

[0049] The term "Fabry disease" refers to an X-linked inborn error of glycosphingolipid catabolism due to deficient lysosomal α-galactosidase A activity. This defect causes accumulation of globotriaosylceramide (ceramide trihexoside) and related glycosphingolipids in vascular endothelial lysosomes of the heart, kidneys, skin, and other tissues.

[0050] The term "atypical Fabry disease" refers to patients with primarily cardiac manifestations of the α-Gal A deficiency, namely progressive globotriaosylceramide (GL-3) accumulation in myocardial cells that leads to significant enlargement of the heart, particularly the left ventricle.

[0051] A "carrier" is a female who has one X chromosome with a defective α-Gal A gene and one X chromosome with the normal gene and in whom X chromosome inactivation of the normal allele is present in one or more cell types. A carrier is often diagnosed with Fabry disease.

[0052] A "patient" refers to a subject who has been diagnosed with or is suspected of having a particular disease. The patient may be human or animal.

[0053] A "Fabry disease patient" refers to an individual who has been diagnosed with or suspected of having Fabry disease and has a mutated α-Gal A as defined further below. Characteristic markers of Fabry disease can occur in male hemizygotes and female carriers with the same prevalence, although females typically are less severely affected.

[0054] Human α-galactosidase A (α-Gal A) refers to an enzyme encoded by the human GLA gene. The full DNA sequence of α-Gal A, including introns and exons, is available in

GenBank Accession No. X14448.1 and shown in SEQ ID NO: 1 and FIGS. 13A-E. The human α-Gal A enzyme consists of 429 amino acids and is available in GenBank Accession Nos. X14448.1 and U78027.1 and shown in SEQ ID NO: 2 and FIG. 14.

[0055] The term "mutant protein" includes a protein which has a mutation in the gene encoding the protein which results in the inability of the protein to achieve a stable conformation under the conditions normally present in the ER. The failure to achieve a stable conformation results in a substantial amount of the enzyme being degraded, rather than being transported to the lysosome. Such a mutation is sometimes called a "conformational mutant." Such mutations include, but are not limited to, missense mutations, and in-frame small deletions and insertions.

[0056] As used herein in one embodiment, the term "mutant α-Gal A" includes an α-Gal A which has a mutation in the gene encoding α-Gal A which results in the inability of the enzyme to achieve a stable conformation under the conditions normally present in the ER. The failure to achieve a stable conformation results in a substantial amount of the enzyme being degraded, rather than being transported to the lysosome.

[0057] As used herein, the term "specific pharmacological chaperone" ("SPC") or "pharmacological chaperone" ("PC") refers to any molecule including a small molecule, protein, peptide, nucleic acid, carbohydrate, etc. that specifically binds to a protein and has one or more of the following effects: (i) enhances the formation of a stable molecular conformation of the protein; (ii) induces trafficking of the protein from the ER to another cellular location, preferably a native cellular location, i.e., prevents ER-associated degradation of the protein; (iii) prevents aggregation of misfolded proteins; and/or (iv) restores or enhances at least partial wild-type function and/or activity to the protein. A compound that specifically binds to e.g., α-Gal A, means that it binds to and exerts a chaperone effect on the enzyme and not a generic group of related or unrelated enzymes. More specifically, this term does not refer to endogenous chaperones, such as BiP, or to non-specific agents which have demonstrated non-specific chaperone activity against various proteins, such as glycerol, DMSO or deuterated water, i.e., chemical chaperones. In one or more embodiments of the present invention, the PC may be a reversible competitive inhibitor.

[0058] A "competitive inhibitor" of an enzyme can refer to a compound which structurally resembles the chemical structure and molecular geometry of the enzyme substrate to bind the enzyme in approximately the same location as the substrate. Thus, the inhibitor

competes for the same active site as the substrate molecule, thus increasing the Km. Competitive inhibition is usually reversible if sufficient substrate molecules are available to displace the inhibitor, i.e., competitive inhibitors can bind reversibly. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site.

[0059] As used herein, the term "specifically binds" refers to the interaction of a pharmacological chaperone with a protein such as α-Gal A, specifically, an interaction with amino acid residues of the protein that directly participate in contacting the pharmacological chaperone. A pharmacological chaperone specifically binds a target protein, e.g., α-Gal A, to exert a chaperone effect on the protein and not a generic group of related or unrelated proteins. The amino acid residues of a protein that interact with any given pharmacological chaperone may or may not be within the protein's "active site." Specific binding can be evaluated through routine binding assays or through structural studies, e.g., co-crystallization, NMR, and the like. The active site for α-Gal A is the substrate binding site.

[0060] "Deficient α-Gal A activity" refers to α-Gal A activity in cells from a patient which is below the normal range as compared (using the same methods) to the activity in normal individuals not having or suspected of having Fabry or any other disease (especially a blood disease).

[0061] As used herein, the terms "enhance α-Gal A activity" or "increase α-Gal A activity" refer to increasing the amount of α-Gal A that adopts a stable conformation in a cell contacted with a pharmacological chaperone specific for the α-Gal A, relative to the amount in a cell (preferably of the same cell-type or the same cell, e.g., at an earlier time) not contacted with the pharmacological chaperone specific for the α-Gal A. This term also refers to increasing the trafficking of α-Gal A to the lysosome in a cell contacted with a pharmacological chaperone specific for the α-Gal A, relative to the trafficking of α-Gal A not contacted with the pharmacological chaperone specific for the protein. These terms refer to both wild-type and mutant α-Gal A. In one embodiment, the increase in the amount of α-Gal A in the cell is measured by measuring the hydrolysis of an artificial substrate in lysates from cells that have been treated with the PC. An increase in hydrolysis is indicative of increased α-Gal A activity.

[0062] The term "α-Gal A activity" refers to the normal physiological function of a wild-type α-Gal A in a cell. For example, α-Gal A activity includes hydrolysis of GL-3.

[0063] A "responder" is an individual diagnosed with or suspected of having a lysosomal storage disorder, such, for example Fabry disease, whose cells exhibit sufficiently increased α-Gal A activity, respectively, and/or amelioration of symptoms or improvement in surrogate markers, in response to contact with a PC. Non-limiting examples of improvements in surrogate markers for Fabry are lyso-Gb3 and those disclosed in US Patent Application Publication No. US 2010-0113517, which is hereby incorporated by reference in its entirety.

[0064] Non-limiting examples of improvements in surrogate markers for Fabry disease disclosed in US 2010/0113517 include increases in α-Gal A levels or activity in cells (*e.g.*, fibroblasts) and tissue; reductions in of GL-3 accumulation; decreased plasma concentrations of homocysteine and vascular cell adhesion molecule-1 (VCAM-1); decreased GL-3 accumulation within myocardial cells and valvular fibrocytes; reduction in plasma globotriaosylsphingosine (lyso-Gb3); reduction in cardiac hypertrophy (especially of the left ventricle), amelioration of valvular insufficiency, and arrhythmias; amelioration of proteinuria; decreased urinary concentrations of lipids such as CTH, lactosylceramide, ceramide, and increased urinary concentrations of glucosylceramide and sphingomyelin; the absence of laminated inclusion bodies (Zebra bodies) in glomerular epithelial cells; improvements in renal function; mitigation of hypohidrosis; the absence of angiokeratomas; and improvements hearing abnormalities such as high frequency sensorineural hearing loss progressive hearing loss, sudden deafness, or tinnitus. Improvements in neurological symptoms include prevention of transient ischemic attack (TIA) or stroke; and amelioration of neuropathic pain manifesting itself as acroparaesthesia (burning or tingling in extremities). Another type of clinical marker that can be assessed for Fabry disease is the prevalence of deleterious cardiovascular manifestations. Common cardiac-related signs and symptoms of Fabry disease include left ventricular hypertrophy, valvular disease (especially mitral valve prolapse and/or regurgitation), premature coronary artery disease, angina, myocardial infarction, conduction abnormalities, arrhythmias, congestive heart failure.

[0065] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a human. In some embodiments, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans. The term "carrier" in reference to a

pharmaceutical carrier refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, 18th Edition, or other editions.

[0066] The term "enzyme replacement therapy" or "ERT" refers to the introduction of a non-native, purified enzyme into an individual having a deficiency in such enzyme. The administered protein can be obtained from natural sources or by recombinant expression (as described in greater detail below). The term also refers to the introduction of a purified enzyme in an individual otherwise requiring or benefiting from administration of a purified enzyme, e.g., suffering from enzyme insufficiency. The introduced enzyme may be a purified, recombinant enzyme produced in vitro, or protein purified from isolated tissue or fluid, such as, e.g., placenta or animal milk, or from plants.

[0067] As used herein, the term "isolated" means that the referenced material is removed from the environment in which it is normally found. Thus, an isolated biological material can be free of cellular components, i.e., components of the cells in which the material is found or produced. In the case of nucleic acid molecules, an isolated nucleic acid includes a PCR product, an mRNA band on a gel, a cDNA, or a restriction fragment. In another embodiment, an isolated nucleic acid is preferably excised from the chromosome in which it may be found, and more preferably is no longer joined to non-regulatory, non-coding regions, or to other genes, located upstream or downstream of the gene contained by the isolated nucleic acid molecule when found in the chromosome. In yet another embodiment, the isolated nucleic acid lacks one or more introns. Isolated nucleic acids include sequences inserted into plasmids, cosmids, artificial chromosomes, and the like. Thus, in a specific embodiment, a recombinant nucleic acid is an isolated nucleic acid. An isolated protein may be associated with other proteins or nucleic acids, or both, with which it associates in the cell, or with cellular membranes if it is a membrane-associated protein. An isolated organelle, cell, or tissue is removed from the anatomical site in which it is found in an organism. An isolated material may be, but need not be, purified.

[0068] The terms "about" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements.

Typical, exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably within 10- or 5-fold, and more preferably within 2-fold of a given value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

[0069] As used herein, the term "free base equivalent" or "FBE" refers to the amount of migalastat present in the migalastat or salt thereof. In other words, the term "FBE" means either an amount of migalastat free base, or the equivalent amount of migalastat free base that is provided by a salt of migalastat. For example, due to the weight of the hydrochloride salt, 150 mg of migalastat hydrochloride only provides as much migalastat as 123 mg of the free base form of migalastat. Other salts are expected to have different conversion factors, depending on the molecular weight of the salt.

[0070] The term "migalastat" encompasses migalastat free base or a pharmaceutically acceptable salt thereof (e.g., migalastat HCl), unless specifically indicated to the contrary.

[0071] Fabry Disease

[0072] Fabry disease is a rare, progressive and devastating X-linked lysosomal storage disorder. Mutations in the GLA gene result in a deficiency of the lysosomal enzyme, α-Gal A, which is required for glycosphingolipid metabolism. Beginning early in life, the reduction in α-Gal A activity results in an accumulation of glycosphingolipids, including GL-3 and plasma lyso-Gb3, and leads to the symptoms and life-limiting sequelae of Fabry disease, including pain, gastrointestinal symptoms, renal failure, cardiomyopathy, cerebrovascular events, and early mortality. Early initiation of therapy and lifelong treatment provide an opportunity to slow disease progression and prolong life expectancy.

[0073] Fabry disease encompasses a spectrum of disease severity and age of onset, although it has traditionally been divided into 2 main phenotypes, "classic" and "late-onset". The classic phenotype has been ascribed primarily to males with undetectable to low α-Gal A activity and earlier onset of renal, cardiac and/or cerebrovascular manifestations. The late-onset phenotype has been ascribed primarily to males with higher residual α-Gal A activity and later onset of these disease manifestations. Heterozygous female carriers typically express the

late-onset phenotype but depending on the pattern of X-chromosome inactivation may also display the classic phenotype.

[0074] More than 800 Fabry disease-causing GLA mutations have been identified. Approximately 60% are missense mutations, resulting in single amino acid substitutions in the α-Gal A enzyme. Missense GLA mutations often result in the production of abnormally folded and unstable forms of α-Gal A and the majority are associated with the classic phenotype. Normal cellular quality control mechanisms in the endoplasmic reticulum block the transit of these abnormal proteins to lysosomes and target them for premature degradation and elimination. Many missense mutant forms are targets for migalastat, an α-Gal A-specific pharmacological chaperone.

[0075] The clinical manifestations of Fabry disease span a broad spectrum of severity and roughly correlate with a patient's residual α-GAL levels. The majority of currently treated patients are referred to as classic Fabry disease patients, most of whom are males. These patients experience disease of various organs, including the kidneys, heart and brain, with disease symptoms first appearing in adolescence and typically progressing in severity until death in the fourth or fifth decade of life. A number of recent studies suggest that there are a large number of undiagnosed males and females that have a range of Fabry disease symptoms, such as impaired cardiac or renal function and strokes, that usually first appear in adulthood. Individuals with this type of Fabry disease, referred to as later-onset Fabry disease, tend to have higher residual α-GAL levels than classic Fabry disease patients. Individuals with later-onset Fabry disease typically first experience disease symptoms in adulthood, and often have disease symptoms focused on a single organ, such as enlargement of the left ventricle or progressive kidney failure. In addition, later-onset Fabry disease may also present in the form of strokes of unknown cause.

[0076] Fabry patients have progressive kidney impairment, and untreated patients exhibit end-stage renal impairment by the fifth decade of life. Deficiency in α-Gal A activity leads to accumulation of globotriaosylceramide (Gb3) and related glycosphingolipids in many cell types including cells in the kidney. Gb3 accumulates in podocytes, epithelial cells and the tubular cells of the distal tubule and loop of Henle. Impairment in kidney function can manifest as proteinuria and reduced glomerular filtration rate.

[0077] Because Fabry disease can cause progressive worsening in renal function, it is important to understand the pharmacokinetics (PK) of potential therapeutic agents in

individuals with renal impairment and particularly so for therapeutic agents that are predominantly cleared by renal excretion. Impairment of renal function may lead to accumulation of the therapeutic agent to levels that become toxic.

[0078] Because Fabry disease is rare, involves multiple organs, has a wide age range of onset, and is heterogeneous, proper diagnosis is a challenge. Awareness is low among health care professionals and misdiagnoses are frequent. Diagnosis of Fabry disease is most often confirmed on the basis of decreased α-Gal A activity in plasma or peripheral leukocytes (WBCs) once a patient is symptomatic, coupled with mutational analysis. In females, diagnosis is even more challenging since the enzymatic identification of carrier females is less reliable due to random X-chromosomal inactivation in some cells of carriers. For example, some obligate carriers (daughters of classically affected males) have α-Gal A enzyme activities ranging from normal to very low activities. Since carriers can have normal α-Gal A enzyme activity in leukocytes, only the identification of an α-Gal A mutation by genetic testing provides precise carrier identification and/or diagnosis.

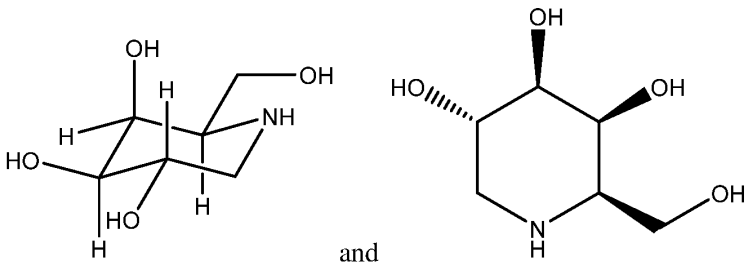
[0079] Mutant forms of α-galactosidase A are considered to be amenable to migalastat are defined as showing a relative increase (+10 μM migalastat) of ≥1.20-fold and an absolute increase (+ 10 μM migalastat) of ≥ 3.0% wild-type (WT) when the mutant form of α-galactosidase A is expressed in HEK-293 cells (referred to as the "HEK assay") according to Good Laboratory Practice (GLP)-validated in vitro assay (GLP HEK or Migalastat Amenability Assay). Such mutations are also referred to herein as "HEK assay amenable" mutations.

[0080] Previous screening methods have been provided that assess enzyme enhancement prior to the initiation of treatment. For example, an assay using HEK-293 cells has been utilized in clinical trials to predict whether a given mutation will be responsive to pharmacological chaperone (*e.g.*, migalastat) treatment. In this assay, cDNA constructs are created. The corresponding α-Gal A mutant forms are transiently expressed in HEK-293 cells. Cells are then incubated ± migalastat (17 nM to 1 mM) for 4 to 5 days. After, α-Gal A levels are measured in cell lysates using a synthetic fluorogenic substrate (4-MU-α-Gal) or by western blot. This has been done for known disease-causing missense or small in-frame insertion/deletion mutations. Mutations that have previously been identified as responsive to a PC (*e.g.* migalastat) using these methods are listed in US Patent No. 8,592,362, which is hereby incorporated by reference in its entirety.

[0081] Pharmacological Chaperones

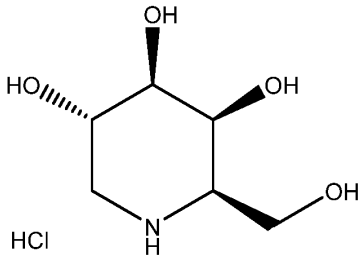
[0082] The binding of small molecule inhibitors of enzymes associated with LSDs can increase the stability of both mutant enzyme and the corresponding wild-type enzyme (see U.S. Pat. Nos. 6,274,597; 6,583,158; 6,589,964; 6,599,919; 6,916,829, and 7,141,582 all incorporated herein by reference). In particular, administration of small molecule derivatives of glucose and galactose, which are specific, selective competitive inhibitors for several target lysosomal enzymes, effectively increased the stability of the enzymes in cells in vitro and, thus, increased trafficking of the enzymes to the lysosome. Thus, by increasing the amount of enzyme in the lysosome, hydrolysis of the enzyme substrates is expected to increase. The original theory behind this strategy was as follows: since the mutant enzyme protein is unstable in the ER (Ishii et al., *Biochem. Biophys. Res. Comm.* 1996; 220: 812-815), the enzyme protein is retarded in the normal transport pathway (ER→Golgi apparatus→endosomes→lysosome) and prematurely degraded. Therefore, a compound which binds to and increases the stability of a mutant enzyme, may serve as a "chaperone" for the enzyme and increase the amount that can exit the ER and move to the lysosomes. In addition, because the folding and trafficking of some wild-type proteins is incomplete, with up to 70% of some wild-type proteins being degraded in some instances prior to reaching their final cellular location, the chaperones can be used to stabilize wild-type enzymes and increase the amount of enzyme which can exit the ER and be trafficked to lysosomes.

[0083] In one or more embodiments, the pharmacological chaperone comprises migalastat or a salt thereof. The compound migalastat, also known as 1-deoxygalactonojirimycin (1-DGJ) or (2R,3S,4R,5S)-2-(hydroxymethyl) piperdine-3,4,5-triol is a compound having the following chemical formula:



Migalastat free base

[0084] As discussed herein, pharmaceutically acceptable salts of migalastat may also be used in the present invention. When a salt of migalastat is used, the dosage of the salt will be adjusted so that the dose of migalastat received by the patient is equivalent to the amount which would have been received had the migalastat free base been used. One example of a pharmaceutically acceptable salt of migalastat is migalastat HCl:



Migalastat HCl

[0085] Migalastat is a low molecular weight iminosugar and is an analogue of the terminal galactose of GL-3. *In vitro* and *in vivo* pharmacologic studies have demonstrated that migalastat acts as a pharmacological chaperone, selectively and reversibly binding, with high affinity, to the active site of wild-type (WT) α-Gal A and specific mutant forms of α Gal A, the genotypes of which are referred to as HEK assay amenable mutations. Migalastat binding stabilizes these mutant forms of α-Gal A in the endoplasmic reticulum facilitating their proper trafficking to lysosomes where dissociation of migalastat allows α-Gal A to reduce the level of GL-3 and other substrates. Approximately 30-50% of patients with Fabry disease have HEK assay amenable mutations; the majority of which are associated with the classic phenotype of the disease. A list of HEK assay amenable mutations includes at least those mutations listed in Table 1 below. In one or more embodiments, if a double mutation is present on the same chromosome (males and females), that patient is considered HEK assay amenable if the double mutation is present in one entry in Table 1 (e.g., D55V/Q57L). In some embodiments, if a double mutation is present on different chromosomes (only in females) that patient is considered HEK assay amenable if either one of the individual mutations is present in Table 1. In addition to Table 1 below, HEK assay amenable mutations can also be found in the summary of product characteristics and/or prescribing information for GALAFOLD™ in various countries in which GALAFOLD™ is approved for use, or at the website

www.galafoldamenabilitytable.com, each of which is hereby incorporated by reference in its entirety.

Table 1: Amenable mutations

5

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.7C>G	c.C7G	L3V
c.8T>C	c.T8C	L3P
c.[11G>T; 620A>C]	c.G11T/A620C	R4M/Y207S
c.13A>G	c.A13G	N5D
c.15C>G	c.C15G	N5K
c.16C>A	c.C16A	P6T
c.16C>T	c.C16T	P6S
c.17C>A	c.C17A	P6Q
c.17C>G	c.C17G	P6R
c.17C>T	c.C17T	P6L
c.19G>A	c.G19A	E7K
c.20A>T	c.A20T	E7V
c.21A>T	c.A21T	E7D
c.22C>A	c.C22A	L8I
c.23T>A	c.T23A	L8Q
c.23T>C	c.T23C	L8P
c.25C>T	c.C25T	H9Y
c.26A>G	c.A26G	H9R
c.26A>T	c.A26T	H9L
c.27T>A	c.T27A	H9Q
c.28C>A	c.C28A	L10M
c.28C>G	c.C28G	L10V
c.29T>A	c.T29A	L10Q

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.29T>C	c.T29C	L10P
c.29T>G	c.T29G	L10R
c.31G>A	c.G31A	G11S
c.31G>C	c.G31C	G11R
c.31G>T	c.G31T	G11C
c.32G>A	c.G32A	G11D
c.32G>T	c.G32T	G11V
c.34T>A	c.T34A	C12S
c.34T>C	c.T34C	C12R
c.34T>G	c.T34G	C12G
c.35G>A	c.G35A	C12Y
c.37G>A	c.G37A	A13T
c.37G>C	c.G37C	A13P
c.38C>A	c.C38A	A13E
c.38C>G	c.C38G	A13G
c.40C>G	c.C40G	L14V
c.40C>T	c.C40T	L14F
c.41T>A	c.T41A	L14H
c.43G>A	c.G43A	A15T
c.44C>G	c.C44G	A15G
c.49C>A	c.C49A	R17S
c.49C>G	c.C49G	R17G
c.49C>T	c.C49T	R17C
c.50G>A	c.G50A	R17H
c.50G>C	c.G50C	R17P
c.52T>A	c.T52A	F18I
c.53T>G	c.T53G	F18C
c.54C>G	c.C54G	F18L

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.58G>C	c.G58C	A20P
c.59C>A	c.C59A	A20D
c.59C>G	c.C59G	A20G
c.62T>A	c.T62A	L21H
c.64G>A	c.G64A	V22I
c.64G>C	c.G64C	V22L
c.64G>T	c.G64T	V22F
c.65T>C	c.T65C	V22A
c.65T>G	c.T65G	V22G
c.67T>A	c.T67A	S23T
c.67T>C	c.T67C	S23P
c.70T>C or c.70T>A	c.T70C or c.T70A	W24R
c.70T>G	c.T70G	W24G
c.71G>C	c.G71C	W24S
c.72G>C or c.72G>T	c.G72C or c.G72T	W24C
c.73G>C	c.G73C	D25H
c.77T>A	c.T77A	I26N
c.79C>A	c.C79A	P27T
c.79C>G	c.C79G	P27A
c.79C>T	c.C79T	P27S
c.80C>T	c.C80T	P27L
c.82G>C	c.G82C	G28R
c.82G>T	c.G82T	G28W
c.83G>A	c.G83A	G28E
c.85G>C	c.G85C	A29P
c.86C>A	c.C86A	A29D
c.86C>G	c.C86G	A29G
c.86C>T	c.C86T	A29V

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.88A>G	c.A88G	R30G
c.94C>A	c.C94A	L32M
c.94C>G	c.C94G	L32V
c.95T>A	c.T95A	L32Q
c.95T>C	c.T95C	L32P
c.95T>G	c.T95G	L32R
c.97G>C	c.G97C	D33H
c.97G>T	c.G97T	D33Y
c.98A>C	c.A98C	D33A
c.98A>G	c.A98G	D33G
c.98A>T	c.A98T	D33V
c.99C>G	c.C99G	D33E
c.100A>C	c.A100C	N34H
c.100A>G	c.A100G	N34D
c.101A>C	c.A101C	N34T
c.101A>G	c.A101G	N34S
c.102T>G or c.102T>A	c.T102G or c.T102A	N34K
c.103G>C or c.103G>A	c.G103C or c.G103A	G35R
c.104G>A	c.G104A	G35E
c.104G>C	c.G104C	G35A
c.104G>T	c.G104T	G35V
c.106T>A	c.T106A	L36M
c.106T>G	c.T106G	L36V
c.107T>C	c.T107C	L36S
c.107T>G	c.T107G	L36W
c.108G>C or c.108G>T	c.G108C or c.G108T	L36F
c.109G>A	c.G109A	A37T
c.109G>T	c.G109T	A37S

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.110C>A	c.C110A	A37E
c.110C>G	c.C110G	A37G
c.110C>T	c.C110T	A37V
c.112A>G	c.A112G	R38G
c.112A>T	c.A112T	R38W
c.113G>T	c.G113T	R38M
c.114G>C	c.G114C	R38S
c.115A>G	c.A115G	T39A
c.115A>T	c.A115T	T39S
c.116C>A	c.C116A	T39K
c.116C>G	c.C116G	T39R
c.116C>T	c.C116T	T39M
c.121A>G	c.A121G	T41A
c.122C>A	c.C122A	T41N
c.122C>G	c.C122G	T41S
c.122C>T	c.C122T	T41I
c.124A>C or c.124A>T	c.A124C or c.A124T	M42L
c.124A>G	c.A124G	M42V
c.125T>A	c.T125A	M42K
c.125T>C	c.T125C	M42T
c.125T>G	c.T125G	M42R
c.126G>A or c.126G>C or c.126G>T	c.G126A or c.G126C or c.G126T	M42I
c.128G>C	c.G128C	G43A
c.133C>A	c.C133A	L45M
c.133C>G	c.C133G	L45V
c.136C>A	c.C136A	H46N
c.136C>G	c.C136G	H46D

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.137A>C	c.A137C	H46P
c.138C>G	c.C138G	H46Q
c.142G>C	c.G142C	E48Q
c.143A>C	c.A143C	E48A
c.149T>A	c.T149A	F50Y
c.151A>G	c.A151G	M51V
c.152T>A	c.T152A	M51K
c.152T>C	c.T152C	M51T
c.152T>G	c.T152G	M51R
c.153G>A or c.153G>T or c.153G>C	c.G153A or c.G153T or c.G153C	M51I
c.157A>C	c.A157C	N53H
c.[157A>C; 158A>T]	c.A157C/A158T	N53L
c.157A>G	c.A157G	N53D
c.157A>T	c.A157T	N53Y
c.158A>C	c.A158C	N53T
c.158A>G	c.A158G	N53S
c.158A>T	c.A158T	N53I
c.159C>G or c.159C>A	c.C159G or c.C159A	N53K
c.160C>G	c.C160G	L54V
c.160C>T	c.C160T	L54F
c.161T>A	c.T161A	L54H
c.161T>C	c.T161C	L54P
c.161T>G	c.T161G	L54R
c.163G>C	c.G163C	D55H
c.163G>T	c.G163T	D55Y
c.164A>C	c.A164C	D55A
c.164A>G	c.A164G	D55G

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.164A>T	c.A164T	D55V
c.[164A>T; 170A>T]	c.A164T/A170T	D55V/Q57L
c.165C>G	c.C165G	D55E
c.167G>A	c.G167A	C56Y
c.167G>T	c.G167T	C56F
c.168C>G	c.C168G	C56W
c.170A>G	c.A170G	Q57R
c.170A>T	c.A170T	Q57L
c.172G>A	c.G172A	E58K
c.175G>A	c.G175A	E59K
c.175G>C	c.G175C	E59Q
c.176A>C	c.A176C	E59A
c.176A>G	c.A176G	E59G
c.176A>T	c.A176T	E59V
c.177G>C	c.G177C	E59D
c.178C>A	c.C178A	P60T
c.178C>G	c.C178G	P60A
c.178C>T	c.C178T	P60S
c.179C>A	c.C179A	P60Q
c.179C>G	c.C179G	P60R
c.179C>T	c.C179T	P60L
c.182A>T	c.A182T	D61V
c.183T>A	c.T183A	D61E
c.184_185insTAG	c.184_185insTAG	S62delinsLA
c.184T>C	c.T184C	S62P
c.184T>G	c.T184G	S62A
c.185C>A	c.C185A	S62Y
c.185C>G	c.C185G	S62C

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.185C>T	c.C185T	S62F
c.190A>C	c.A190C	I64L
c.190A>G	c.A190G	I64V
c.193A>G	c.A193G	S65G
c.193A>T	c.A193T	S65C
c.195T>A	c.T195A	S65R
c.196G>A	c.G196A	E66K
c.197A>G	c.A197G	E66G
c.197A>T	c.A197T	E66V
c.198G>C	c.G198C	E66D
c.199A>C	c.A199C	K67Q
c.199A>G	c.A199G	K67E
c.200A>C	c.A200C	K67T
c.200A>T	c.A200T	K67M
c.201G>C	c.G201C	K67N
c.202C>A	c.C202A	L68I
c.205T>A	c.T205A	F69I
c.206T>A	c.T206A	F69Y
c.207C>A or c.207C>G	c.C207A or c.C207G	F69L
c.208A>T	c.A208T	M70L
c.209T>A	c.T209A	M70K
c.209T>G	c.T209G	M70R
c.210G>C	c.G210C	M70I
c.211G>C	c.G211C	E71Q
c.212A>C	c.A212C	E71A
c.212A>G	c.A212G	E71G
c.212A>T	c.A212T	E71V
c.213G>C	c.G213C	E71D

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.214A>G	c.A214G	M72V
c.214A>T	c.A214T	M72L
c.215T>C	c.T215C	M72T
c.216G>A or c.216G>T or c.216G>C	c.G216A or c.G216T or c.G216C	M72I
c.217G>A	c.G217A	A73T
c.217G>T	c.G217T	A73S
c.218C>T	c.C218T	A73V
c.220G>A	c.G220A	E74K
c.221A>G	c.A221G	E74G
c.221A>T	c.A221T	E74V
c.222G>C	c.G222C	E74D
c.223C>T	c.C223T	L75F
c.224T>C	c.T224C	L75P
c.226A>G	c.A226G	M76V
c.227T>C	c.T227C	M76T
c.229G>A	c.G229A	V77I
c.229G>C	c.G229C	V77L
c.232T>C	c.T232C	S78P
c.233C>T	c.C233T	S78L
c.235G>A	c.G235A	E79K
c.235G>C	c.G235C	E79Q
c.236A>C	c.A236C	E79A
c.236A>G	c.A236G	E79G
c.236A>T	c.A236T	E79V
c.237A>T	c.A237T	E79D
c.238G>A	c.G238A	G80S
c.238G>T	c.G238T	G80C

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.239G>A	c.G239A	G80D
c.239G>C	c.G239C	G80A
c.239G>T	c.G239T	G80V
c.242G>T	c.G242T	W81L
c.244A>G	c.A244G	K82E
c.245A>C	c.A245C	K82T
c.245A>G	c.A245G	K82R
c.245A>T	c.A245T	K82M
c.246G>C	c.G246C	K82N
c.247G>A	c.G247A	D83N
c.248A>C	c.A248C	D83A
c.248A>G	c.A248G	D83G
c.248A>T	c.A248T	D83V
c.249T>A	c.T249A	D83E
c.250G>A	c.G250A	A84T
c.250G>C	c.G250C	A84P
c.250G>T	c.G250T	A84S
c.251C>A	c.C251A	A84E
c.251C>G	c.C251G	A84G
c.251C>T	c.C251T	A84V
c.253G>A	c.G253A	G85S
c.[253G>A; 254G>A]	c.G253A/G254A	G85N
c.[253G>A; 254G>T; 255T>G]	c.G253A/G254T/T255G	G85M
c.253G>C	c.G253C	G85R
c.253G>T	c.G253T	G85C
c.254G>A	c.G254A	G85D
c.254G>C	c.G254C	G85A

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.257A>T	c.A257T	Y86F
c.260A>G	c.A260G	E87G
c.261G>C or c.261G>T	c.G261C or c.G261T	E87D
c.262T>A	c.T262A	Y88N
c.262T>C	c.T262C	Y88H
c.263A>C	c.A263C	Y88S
c.263A>G	c.A263G	Y88C
c.265C>G	c.C265G	L89V
c.265C>T	c.C265T	L89F
c.271A>C	c.A271C	I91L
c.271A>T	c.A271T	I91F
c.272T>C	c.T272C	I91T
c.272T>G	c.T272G	I91S
c.273T>G	c.T273G	I91M
c.286A>G	c.A286G	M96V
c.286A>T	c.A286T	M96L
c.287T>C	c.T287C	M96T
c.288G>A or c.288G>T or c.288G>C	c.G288A or c.G288T or c.G288C	M96I
c.289G>A	c.G289A	A97T
c.289G>C	c.G289C	A97P
c.289G>T	c.G289T	A97S
c.290C>A	c.C290A	A97D
c.290C>T	c.C290T	A97V
c.293C>A	c.C293A	P98H
c.293C>G	c.C293G	P98R
c.293C>T	c.C293T	P98L
c.295C>G	c.C295G	Q99E

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.296A>C	c.A296C	Q99P
c.296A>G	c.A296G	Q99R
c.296A>T	c.A296T	Q99L
c.301G>C	c.G301C	D101H
c.302A>C	c.A302C	D101A
c.302A>G	c.A302G	D101G
c.302A>T	c.A302T	D101V
c.303T>A	c.T303A	D101E
c.304T>A	c.T304A	S102T
c.304T>C	c.T304C	S102P
c.304T>G	c.T304G	S102A
c.305C>T	c.C305T	S102L
c.310G>A	c.G310A	G104S
c.311G>A	c.G311A	G104D
c.311G>C	c.G311C	G104A
c.311G>T	c.G311T	G104V
c.313A>G	c.A313G	R105G
c.314G>A	c.G314A	R105K
c.314G>C	c.G314C	R105T
c.314G>T	c.G314T	R105I
c.316C>A	c.C316A	L106I
c.316C>G	c.C316G	L106V
c.316C>T	c.C316T	L106F
c.317T>A	c.T317A	L106H
c.317T>C	c.T317C	L106P
c.319C>A	c.C319A	Q107K
c.319C>G	c.C319G	Q107E
c.320A>G	c.A320G	Q107R

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.321G>C	c.G321C	Q107H
c.322G>A	c.G322A	A108T
c.323C>A	c.C323A	A108E
c.323C>T	c.C323T	A108V
c.325G>A	c.G325A	D109N
c.325G>C	c.G325C	D109H
c.325G>T	c.G325T	D109Y
c.326A>C	c.A326C	D109A
c.326A>G	c.A326G	D109G
c.327C>G	c.C327G	D109E
c.328C>A	c.C328A	P110T
c.334C>G	c.C334G	R112G
c.335G>A	c.G335A	R112H
c.335G>T	c.G335T	R112L
c.337T>A	c.T337A	F113I
c.337T>C or c.339T>A or c.339T>G	c.T337C or c.T339A or c.T339G	F113L
c.337T>G	c.T337G	F113V
c.338T>A	c.T338A	F113Y
c.341C>T	c.C341T	P114L
c.343C>A	c.C343A	H115N
c.343C>G	c.C343G	H115D
c.346G>C	c.G346C	G116R
c.350T>C	c.T350C	I117T
c.351T>G	c.T351G	I117M
c.352C>T	c.C352T	R118C
c.361G>A	c.G361A	A121T
c.362C>T	c.C362T	A121V

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.367T>A	c.T367A	Y123N
c.367T>G	c.T367G	Y123D
c.368A>C	c.A368C	Y123S
c.368A>G	c.A368G	Y123C
c.368A>T	c.A368T	Y123F
c.370G>A	c.G370A	V124I
c.371T>G	c.T371G	V124G
c.373C>A	c.C373A	H125N
c.373C>G	c.C373G	H125D
c.373C>T	c.C373T	H125Y
c.374A>G	c.A374G	H125R
c.374A>T	c.A374T	H125L
c.376A>G	c.A376G	S126G
c.376A>T	c.A376T	S126C
c.377G>T	c.G377T	S126I
c.379A>G	c.A379G	K127E
c.383G>A	c.G383A	G128E
c.383G>C	c.G383C	G128A
c.385C>G	c.C385G	L129V
c.388A>C	c.A388C	K130Q
c.389A>T	c.A389T	K130M
c.390G>C	c.G390C	K130N
c.391C>G	c.C391G	L131V
c.397A>C	c.A397C	I133L
c.397A>G	c.A397G	I133V
c.397A>T	c.A397T	I133F
c.398T>C	c.T398C	I133T
c.399T>G	c.T399G	I133M

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.[399T>G; 434T>C]	c.T399G/T434C	I133M/F145S
c.403G>A	c.G403A	A135T
c.403G>T	c.G403T	A135S
c.404C>A	c.C404A	A135E
c.404C>G	c.C404G	A135G
c.404C>T	c.C404T	A135V
c.406G>A	c.G406A	D136N
c.407A>C	c.A407C	D136A
c.407A>T	c.A407T	D136V
c.408T>A or c.408T>G	c.T408A or c.T408G	D136E
c.409G>A	c.G409A	V137I
c.409G>C	c.G409C	V137L
c.410T>A	c.T410A	V137D
c.410T>C	c.T410C	V137A
c.410T>G	c.T410G	V137G
c.413G>C	c.G413C	G138A
c.415A>C	c.A415C	N139H
c.415A>T	c.A415T	N139Y
c.416A>G	c.A416G	N139S
c.416A>T	c.A416T	N139I
c.417T>A	c.T417A	N139K
c.418A>C	c.A418C	K140Q
c.418A>G	c.A418G	K140E
c.419A>C	c.A419C	K140T
c.419A>G	c.A419G	K140R
c.419A>T	c.A419T	K140I
c.420A>T	c.A420T	K140N
c.421A>T	c.A421T	T141S

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.427G>A	c.G427A	A143T
c.428C>A	c.C428A	A143E
c.428C>G	c.C428G	A143G
c.428C>T	c.C428T	A143V
c.430G>A	c.G430A	G144S
c.430G>C	c.G430C	G144R
c.430G>T	c.G430T	G144C
c.431G>A	c.G431A	G144D
c.431G>C	c.G431C	G144A
c.431G>T	c.G431T	G144V
c.433T>G	c.T433G	F145V
c.434T>A	c.T434A	F145Y
c.434T>C	c.T434C	F145S
c.434T>G	c.T434G	F145C
c.435C>G	c.C435G	F145L
c.436C>A	c.C436A	P146T
c.436C>G	c.C436G	P146A
c.436C>T	c.C436T	P146S
c.437C>A	c.C437A	P146H
c.437C>G	c.C437G	P146R
c.437C>T	c.C437T	P146L
c.440G>C	c.G440C	G147A
c.442A>G	c.A442G	S148G
c.442A>T	c.A442T	S148C
c.443G>C	c.G443C	S148T
c.446T>G	c.T446G	F149C
c.449G>A	c.G449A	G150E
c.449G>T	c.G449T	G150V

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.451T>G	c.T451G	Y151D
c.452A>C	c.A452C	Y151S
c.452A>G	c.A452G	Y151C
c.454T>A	c.T454A	Y152N
c.454T>C	c.T454C	Y152H
c.454T>G	c.T454G	Y152D
c.455A>C	c.A455C	Y152S
c.455A>G	c.A455G	Y152C
c.455A>T	c.A455T	Y152F
c.457G>A	c.G457A	D153N
c.457G>C	c.G457C	D153H
c.457G>T	c.G457T	D153Y
c.458A>C	c.A458C	D153A
c.458A>T	c.A458T	D153V
c.465T>A or c.465T>G	c.T465A or c.T465G	D155E
c.466G>A	c.G466A	A156T
c.466G>T	c.G466T	A156S
c.467C>G	c.C467G	A156G
c.467C>T	c.C467T	A156V
c.469C>A	c.C469A	Q157K
c.469C>G	c.C469G	Q157E
c.470A>C	c.A470C	Q157P
c.470A>T	c.A470T	Q157L
c.471G>C or c.471G>T	c.G471C or c.G471T	Q157H
c.472A>G	c.A472G	T158A
c.472A>T	c.A472T	T158S
c.473C>A	c.C473A	T158N
c.473C>T	c.C473T	T158I

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.475T>A	c.T475A	F159I
c.475T>G	c.T475G	F159V
c.476T>A	c.T476A	F159Y
c.476T>G	c.T476G	F159C
c.477T>A	c.T477A	F159L
c.478G>A	c.G478A	A160T
c.478G>T	c.G478T	A160S
c.479C>A	c.C479A	A160D
c.479C>G	c.C479G	A160G
c.479C>T	c.C479T	A160V
c.481G>A	c.G481A	D161N
c.481G>C	c.G481C	D161H
c.481G>T	c.G481T	D161Y
c.482A>T	c.A482T	D161V
c.484T>G	c.T484G	W162G
c.485G>C	c.G485C	W162S
c.490G>A	c.G490A	V164I
c.490G>T	c.G490T	V164L
c.491T>C	c.T491C	V164A
c.493G>A	c.G493A	D165N
c.493G>C	c.G493C	D165H
c.494A>C	c.A494C	D165A
c.494A>G	c.A494G	D165G
c.495T>A	c.T495A	D165E
c.496_497delinsTC	c.496_497delinsTC	L166S
c.496C>A	c.C496A	L166M
c.496C>G	c.C496G	L166V
c.[496C>G; 497T>G]	c.C496G/T497G	L166G

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.497T>A	c.T497A	L166Q
c.499C>A	c.C499A	L167I
c.499C>G	c.C499G	L167V
c.505T>A	c.T505A	F169I
c.505T>G	c.T505G	F169V
c.506T>A	c.T506A	F169Y
c.506T>C	c.T506C	F169S
c.506T>G	c.T506G	F169C
c.507T>A	c.T507A	F169L
c.511G>A	c.G511A	G171S
c.512G>C	c.G512C	G171A
c.512G>T	c.G512T	G171V
c.517T>C	c.T517C	Y173H
c.518A>C	c.A518C	Y173S
c.518A>G	c.A518G	Y173C
c.518A>T	c.A518T	Y173F
c.520T>C	c.T520C	C174R
c.520T>G	c.T520G	C174G
c.523G>C	c.G523C	D175H
c.523G>T	c.G523T	D175Y
c.524A>G	c.A524G	D175G
c.524A>T	c.A524T	D175V
c.525C>G or c.525C>A	c.C525G or c.C525A	D175E
c.526A>T	c.A526T	S176C
c.528T>A	c.T528A	S176R
c.529T>A	c.T529A	L177M
c.529T>G	c.T529G	L177V
c.530T>C	c.T530C	L177S

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.530T>G	c.T530G	L177W
c.531G>C	c.G531C	L177F
c.532G>A	c.G532A	E178K
c.532G>C	c.G532C	E178Q
c.533A>C	c.A533C	E178A
c.533A>G	c.A533G	E178G
c.538T>A	c.T538A	L180M
c.538T>G	c.T538G	L180V
c.539T>C	c.T539C	L180S
c.539T>G	c.T539G	L180W
c.540G>C or c.540G>T	c.G540C or c.G540T	L180F
c.541G>A	c.G541A	A181T
c.541G>C	c.G541C	A181P
c.542C>T	c.C542T	A181V
c.544G>T	c.G544T	D182Y
c.545A>C	c.A545C	D182A
c.545A>G	c.A545G	D182G
c.545A>T	c.A545T	D182V
c.546T>A	c.T546A	D182E
c.548G>A	c.G548A	G183D
c.548G>C	c.G548C	G183A
c.550T>A	c.T550A	Y184N
c.550T>C	c.T550C	Y184H
c.551A>C	c.A551C	Y184S
c.551A>G	c.A551G	Y184C
c.551A>T	c.A551T	Y184F
c.553A>C	c.A553C	K185Q
c.553A>G	c.A553G	K185E

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.554A>C	c.A554C	K185T
c.554A>T	c.A554T	K185M
c.555G>C	c.G555C	K185N
c.556C>A	c.C556A	H186N
c.556C>G	c.C556G	H186D
c.556C>T	c.C556T	H186Y
c.557A>T	c.A557T	H186L
c.558C>G	c.C558G	H186Q
c.559_564dup	c.559_564dup	p.M187_S188dup
c.559A>T	c.A559T	M187L
c.559A>G	c.A559G	M187V
c.560T>C	c.T560C	M187T
c.561G>T or c.561G>A or c.561G>C	c.G561T or c.G561A or c.G561C	M187I
c.562T>A	c.T562A	S188T
c.562T>C	c.T562C	S188P
c.562T>G	c.T562G	S188A
c.563C>A	c.C563A	S188Y
c.563C>G	c.C563G	S188C
c.563C>T	c.C563T	S188F
c.565T>G	c.T565G	L189V
c.566T>C	c.T566C	L189S
c.567G>C or c.567G>T	c.G567C or c.G567T	L189F
c.568G>A	c.G568A	A190T
c.568G>T	c.G568T	A190S
c.569C>A	c.C569A	A190D
c.569C>G	c.C569G	A190G
c.569C>T	c.C569T	A190V

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.571C>A	c.C571A	L191M
c.571C>G	c.C571G	L191V
c.572T>A	c.T572A	L191Q
c.574A>C	c.A574C	N192H
c.574A>G	c.A574G	N192D
c.575A>C	c.A575C	N192T
c.575A>G	c.A575G	N192S
c.576T>A	c.T576A	N192K
c.577A>G	c.A577G	R193G
c.577A>T	c.A577T	R193W
c.578G>C	c.G578C	R193T
c.578G>T	c.G578T	R193M
c.580A>C	c.A580C	T194P
c.580A>G	c.A580G	T194A
c.580A>T or c.581C>G	c.A580T or c.C581G	T194S
c.581C>A	c.C581A	T194N
c.581C>T	c.C581T	T194I
c.583G>A	c.G583A	G195S
c.583G>C	c.G583C	G195R
c.583G>T	c.G583T	G195C
c.584G>T	c.G584T	G195V
c.586A>G	c.A586G	R196G
c.587G>A	c.G587A	R196K
c.587G>C	c.G587C	R196T
c.587G>T	c.G587T	R196I
c.589A>G	c.A589G	S197G
c.589A>T	c.A589T	S197C
c.590G>A	c.G590A	S197N

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.590G>C	c.G590C	S197T
c.590G>T	c.G590T	S197I
c.593T>C	c.T593C	I198T
c.593T>G	c.T593G	I198S
c.594T>G	c.T594G	I198M
c.595G>A	c.G595A	V199M
c.595G>C	c.G595C	V199L
c.596T>A	c.T596A	V199E
c.596T>C	c.T596C	V199A
c.596T>G	c.T596G	V199G
c.598T>A	c.T598A	Y200N
c.599A>C	c.A599C	Y200S
c.599A>G	c.A599G	Y200C
c.601T>A	c.T601A	S201T
c.601T>G	c.T601G	S201A
c.602C>A	c.C602A	S201Y
c.602C>G	c.C602G	S201C
c.602C>T	c.C602T	S201F
c.607G>C	c.G607C	E203Q
c.608A>C	c.A608C	E203A
c.608A>G	c.A608G	E203G
c.608A>T	c.A608T	E203V
c.609G>C or c.609G>T	c.G609C or c.G609T	E203D
c.610T>G	c.T610G	W204G
c.611G>C	c.G611C	W204S
c.611G>T	c.G611T	W204L
c.613C>A	c.C613A	P205T
c.613C>T	c.C613T	P205S

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.614C>T	c.C614T	P205L
c.616C>A	c.C616A	L206I
c.616C>G	c.C616G	L206V
c.616C>T	c.C616T	L206F
c.617T>A	c.T617A	L206H
c.617T>G	c.T617G	L206R
c.619T>C	c.T619C	Y207H
c.620A>C	c.A620C	Y207S
c.620A>T	c.A620T	Y207F
c.623T>A	c.T623A	M208K
c.623T>G	c.T623G	M208R
c.625T>A	c.T625A	W209R
c.625T>G	c.T625G	W209G
c.627G>C	c.G627C	W209C
c.628C>A	c.C628A	P210T
c.628C>T	c.C628T	P210S
c.629C>A	c.C629A	P210H
c.629C>T	c.C629T	P210L
c.631T>C	c.T631C	F211L
c.631T>G	c.T631G	F211V
c.632T>A	c.T632A	F211Y
c.632T>C	c.T632C	F211S
c.632T>G	c.T632G	F211C
c.635A>C	c.A635C	Q212P
c.636A>T	c.A636T	Q212H
c.637A>C	c.A637C	K213Q
c.637A>G	c.A637G	K213E
c.638A>G	c.A638G	K213R

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.638A>T	c.A638T	K213M
c.640C>A	c.C640A	P214T
c.640C>G	c.C640G	P214A
c.640C>T	c.C640T	P214S
c.641C>A	c.C641A	P214H
c.641C>G	c.C641G	P214R
c.641C>T	c.C641T	P214L
c.643A>C	c.A643C	N215H
c.643A>G	c.A643G	N215D
c.643A>T	c.A643T	N215Y
c.644A>C	c.A644C	N215T
c.644A>G	c.A644G	N215S
c.[644A>G; 937G>T]	c.A644G/G937T	N215S/D313Y
c.644A>T	c.A644T	N215I
c.645T>A	c.T645A	N215K
c.646T>A	c.T646A	Y216N
c.646T>C	c.T646C	Y216H
c.646T>G	c.T646G	Y216D
c.647A>C	c.A647C	Y216S
c.647A>G	c.A647G	Y216C
c.647A>T	c.A647T	Y216F
c.649A>C	c.A649C	T217P
c.649A>G	c.A649G	T217A
c.649A>T	c.A649T	T217S
c.650C>A	c.C650A	T217K
c.650C>G	c.C650G	T217R
c.650C>T	c.C650T	T217I
c.652G>A	c.G652A	E218K

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.652G>C	c.G652C	E218Q
c.653A>C	c.A653C	E218A
c.653A>G	c.A653G	E218G
c.653A>T	c.A653T	E218V
c.654A>T	c.A654T	E218D
c.655A>C	c.A655C	I219L
c.655A>T	c.A655T	I219F
c.656T>A	c.T656A	I219N
c.656T>C	c.T656C	I219T
c.656T>G	c.T656G	I219S
c.657C>G	c.C657G	I219M
c.659G>A	c.G659A	R220Q
c.659G>C	c.G659C	R220P
c.659G>T	c.G659T	R220L
c.661C>A	c.C661A	Q221K
c.661C>G	c.C661G	Q221E
c.662A>C	c.A662C	Q221P
c.662A>G	c.A662G	Q221R
c.662A>T	c.A662T	Q221L
c.663G>C	c.G663C	Q221H
c.664T>A	c.T664A	Y222N
c.664T>C	c.T664C	Y222H
c.664T>G	c.T664G	Y222D
c.665A>C	c.A665C	Y222S
c.665A>G	c.A665G	Y222C
c.670A>C	c.A670C	N224H
c.671A>C	c.A671C	N224T
c.671A>G	c.A671G	N224S

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.673C>G	c.C673G	H225D
c.679C>G	c.C679G	R227G
c.682A>C	c.A682C	N228H
c.682A>G	c.A682G	N228D
c.683A>C	c.A683C	N228T
c.683A>G	c.A683G	N228S
c.683A>T	c.A683T	N228I
c.685T>A	c.T685A	F229I
c.686T>A	c.T686A	F229Y
c.686T>C	c.T686C	F229S
c.687T>A or c.687T>G	c.T687A or c.T687G	F229L
c.688G>C	c.G688C	A230P
c.689C>A	c.C689A	A230D
c.689C>G	c.C689G	A230G
c.689C>T	c.C689T	A230V
c.694A>C	c.A694C	I232L
c.694A>G	c.A694G	I232V
c.695T>C	c.T695C	I232T
c.696T>G	c.T696G	I232M
c.698A>C	c.A698C	D233A
c.698A>G	c.A698G	D233G
c.698A>T	c.A698T	D233V
c.699T>A	c.T699A	D233E
c.703T>A	c.T703A	S235T
c.703T>G	c.T703G	S235A
c.710A>T	c.A710T	K237I
c.712A>G	c.A712G	S238G
c.712A>T	c.A712T	S238C

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.713G>A	c.G713A	S238N
c.713G>C	c.G713C	S238T
c.713G>T	c.G713T	S238I
c.715A>T	c.A715T	I239L
c.716T>C	c.T716C	I239T
c.717A>G	c.A717G	I239M
c.718A>G	c.A718G	K240E
c.719A>G	c.A719G	K240R
c.719A>T	c.A719T	K240M
c.720G>C or c.720G>T	c.G720C or c.G720T	K240N
c.721A>T	c.A721T	S241C
c.722G>C	c.G722C	S241T
c.722G>T	c.G722T	S241I
c.724A>C	c.A724C	I242L
c.724A>G	c.A724G	I242V
c.724A>T	c.A724T	I242F
c.725T>A	c.T725A	I242N
c.725T>C	c.T725C	I242T
c.725T>G	c.T725G	I242S
c.726C>G	c.C726G	I242M
c.727T>A	c.T727A	L243M
c.727T>G	c.T727G	L243V
c.728T>C	c.T728C	L243S
c.728T>G	c.T728G	L243W
c.729G>C or c.729G>T	c.G729C or c.G729T	L243F
c.730G>A	c.G730A	D244N
c.730G>C	c.G730C	D244H
c.730G>T	c.G730T	D244Y

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.731A>C	c.A731C	D244A
c.731A>G	c.A731G	D244G
c.731A>T	c.A731T	D244V
c.732C>G	c.C732G	D244E
c.733T>G	c.T733G	W245G
c.735G>C	c.G735C	W245C
c.736A>G	c.A736G	T246A
c.737C>A	c.C737A	T246K
c.737C>G	c.C737G	T246R
c.737C>T	c.C737T	T246I
c.739T>A	c.T739A	S247T
c.739T>G	c.T739G	S247A
c.740C>A	c.C740A	S247Y
c.740C>G	c.C740G	S247C
c.740C>T	c.C740T	S247F
c.742T>G	c.T742G	F248V
c.743T>A	c.T743A	F248Y
c.743T>G	c.T743G	F248C
c.744T>A	c.T744A	F248L
c.745A>C	c.A745C	N249H
c.745A>G	c.A745G	N249D
c.745A>T	c.A745T	N249Y
c.746A>C	c.A746C	N249T
c.746A>G	c.A746G	N249S
c.746A>T	c.A746T	N249I
c.747C>G or c.747C>A	c.C747G or c.C747A	N249K
c.748C>A	c.C748A	Q250K
c.748C>G	c.C748G	Q250E

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.749A>C	c.A749C	Q250P
c.749A>G	c.A749G	Q250R
c.749A>T	c.A749T	Q250L
c.750G>C	c.G750C	Q250H
c.751G>A	c.G751A	E251K
c.751G>C	c.G751C	E251Q
c.752A>G	c.A752G	E251G
c.752A>T	c.A752T	E251V
c.754A>G	c.A754G	R252G
c.757A>G	c.A757G	I253V
c.757A>T	c.A757T	I253F
c.758T>A	c.T758A	I253N
c.758T>C	c.T758C	I253T
c.758T>G	c.T758G	I253S
c.760-762delGTT or c.761-763del	c.760_762delGTT or c.761_763del	p.V254del
c.760G>T	c.G760T	V254F
c.761T>A	c.T761A	V254D
c.761T>C	c.T761C	V254A
c.761T>G	c.T761G	V254G
c.763G>A	c.G763A	D255N
c.763G>C	c.G763C	D255H
c.763G>T	c.G763T	D255Y
c.764A>C	c.A764C	D255A
c.764A>T	c.A764T	D255V
c.765T>A	c.T765A	D255E
c.766G>C	c.G766C	V256L
c.767T>A	c.T767A	V256D

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.767T>G	c.T767G	V256G
c.769G>A	c.G769A	A257T
c.769G>C	c.G769C	A257P
c.769G>T	c.G769T	A257S
c.770C>G	c.C770G	A257G
c.770C>T	c.C770T	A257V
c.772G>C or c.772G>A	c.G772C or c.G772A	G258R
c.773G>A	c.G773A	G258E
c.773G>T	c.G773T	G258V
c.775C>A	c.C775A	P259T
c.775C>G	c.C775G	P259A
c.775C>T	c.C775T	P259S
c.776C>A	c.C776A	P259Q
c.776C>G	c.C776G	P259R
c.776C>T	c.C776T	P259L
c.778G>T	c.G778T	G260W
c.779G>A	c.G779A	G260E
c.779G>C	c.G779C	G260A
c.781G>A	c.G781A	G261S
c.781G>C	c.G781C	G261R
c.781G>T	c.G781T	G261C
c.782G>C	c.G782C	G261A
c.787A>C	c.A787C	N263H
c.788A>C	c.A788C	N263T
c.788A>G	c.A788G	N263S
c.790G>A	c.G790A	D264N
c.790G>C	c.G790C	D264H
c.790G>T	c.G790T	D264Y

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.793C>G	c.C793G	P265A
c.794C>A	c.C794A	P265Q
c.794C>T	c.C794T	P265L
c.799A>G	c.A799G	M267V
c.799A>T	c.A799T	M267L
c.800T>C	c.T800C	M267T
c.802T>A	c.T802A	L268I
c.804A>T	c.A804T	L268F
c.805G>A	c.G805A	V269M
c.805G>C	c.G805C	V269L
c.806T>C	c.T806C	V269A
c.808A>C	c.A808C	I270L
c.808A>G	c.A808G	I270V
c.809T>C	c.T809C	I270T
c.809T>G	c.T809G	I270S
c.810T>G	c.T810G	I270M
c.811G>A	c.G811A	G271S
c.[811G>A; 937G>T]	c.G811A/G937T	G271S/D313Y
c.812G>A	c.G812A	G271D
c.812G>C	c.G812C	G271A
c.814A>G	c.A814G	N272D
c.818T>A	c.T818A	F273Y
c.823C>A	c.C823A	L275I
c.823C>G	c.C823G	L275V
c.827G>A	c.G827A	S276N
c.827G>C	c.G827C	S276T
c.829T>G	c.T829G	W277G
c.830G>T	c.G830T	W277L

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.831G>T or c.831G>C	c.G831T or c.G831C	W277C
c.832A>T	c.A832T	N278Y
c.833A>T	c.A833T	N278I
c.835C>G	c.C835G	Q279E
c.838C>A	c.C838A	Q280K
c.839A>G	c.A839G	Q280R
c.839A>T	c.A839T	Q280L
c.840A>T or c.840A>C	c.A840T or c.A840C	Q280H
c.841G>C	c.G841C	V281L
c.842T>A	c.T842A	V281E
c.842T>C	c.T842C	V281A
c.842T>G	c.T842G	V281G
c.844A>G	c.A844G	T282A
c.844A>T	c.A844T	T282S
c.845C>T	c.C845T	T282I
c.847C>G	c.C847G	Q283E
c.848A>T	c.A848T	Q283L
c.849G>C	c.G849C	Q283H
c.850A>G	c.A850G	M284V
c.850A>T	c.A850T	M284L
c.851T>C	c.T851C	M284T
c.852G>C	c.G852C	M284I
c.853G>A	c.G853A	A285T
c.854C>G	c.C854G	A285G
c.854C>T	c.C854T	A285V
c.856C>G	c.C856G	L286V
c.856C>T	c.C856T	L286F
c.857T>A	c.T857A	L286H

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.860G>T	c.G860T	W287L
c.862G>C	c.G862C	A288P
c.862G>T	c.G862T	A288S
c.863C>G	c.C863G	A288G
c.863C>T	c.C863T	A288V
c.865A>C	c.A865C	I289L
c.865A>G	c.A865G	I289V
c.866T>C	c.T866C	I289T
c.866T>G	c.T866G	I289S
c.868A>C or c.868A>T	c.A868C or c.A868T	M290L
c.868A>G	c.A868G	M290V
c.869T>C	c.T869C	M290T
c.870G>A or c.870G>C or c.870G>T	c.G870A or c.G870C or c.G870T	M290I
c.871G>A	c.G871A	A291T
c.871G>T	c.G871T	A291S
c.872C>G	c.C872G	A291G
c.874G>T	c.G874T	A292S
c.875C>G	c.C875G	A292G
c.877C>A	c.C877A	P293T
c.880T>A	c.T880A	L294I
c.880T>G	c.T880G	L294V
c.881T>C	c.T881C	L294S
c.882A>T	c.A882T	L294F
c.883T>A	c.T883A	F295I
c.883T>G	c.T883G	F295V
c.884T>A	c.T884A	F295Y
c.884T>C	c.T884C	F295S

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.884T>G	c.T884G	F295C
c.886A>G	c.A886G	M296V
c.886A>T or c.886A>C	c.A886T or c.A886C	M296L
c.887T>C	c.T887C	M296T
c.888G>A or c.888G>T or c.888G>C	c.G888A or c.G888T or c.G888C	M296I
c.889T>A	c.T889A	S297T
c.892A>G	c.A892G	N298D
c.893A>C	c.A893C	N298T
c.893A>G	c.A893G	N298S
c.893A>T	c.A893T	N298I
c.895G>A	c.G895A	D299N
c.895G>C	c.G895C	D299H
c.897C>G or c.897C>A	c.C897G or c.C897A	D299E
c.898C>A	c.C898A	L300I
c.898C>G	c.C898G	L300V
c.898C>T	c.C898T	L300F
c.899T>C	c.T899C	L300P
c.901C>G	c.C901G	R301G
c.902G>A	c.G902A	R301Q
c.902G>C	c.G902C	R301P
c.902G>T	c.G902T	R301L
c.904C>A	c.C904A	H302N
c.904C>G	c.C904G	H302D
c.904C>T	c.C904T	H302Y
c.905A>T	c.A905T	H302L
c.907A>G	c.A907G	I303V
c.907A>T	c.A907T	I303F

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.908T>A	c.T908A	I303N
c.908T>C	c.T908C	I303T
c.908T>G	c.T908G	I303S
c.911G>A	c.G911A	S304N
c.911G>C	c.G911C	S304T
c.911G>T	c.G911T	S304I
c.916C>G	c.C916G	Q306E
c.917A>C	c.A917C	Q306P
c.917A>T	c.A917T	Q306L
c.919G>A	c.G919A	A307T
c.919G>C	c.G919C	A307P
c.919G>T	c.G919T	A307S
c.920C>A	c.C920A	A307D
c.920C>G	c.C920G	A307G
c.920C>T	c.C920T	A307V
c.922A>C	c.A922C	K308Q
c.922A>G	c.A922G	K308E
c.923A>G	c.A923G	K308R
c.923A>T	c.A923T	K308I
c.924A>T or c.924A>C	c.A924T or c.A924C	K308N
c.925G>A	c.G925A	A309T
c.925G>C	c.G925C	A309P
c.926C>A	c.C926A	A309D
c.926C>T	c.C926T	A309V
c.928C>A	c.C928A	L310I
c.928C>G	c.C928G	L310V
c.928C>T	c.C928T	L310F
c.931C>A	c.C931A	L311I

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.931C>G	c.C931G	L311V
c.934C>A	c.C934A	Q312K
c.934C>G	c.C934G	Q312E
c.935A>G	c.A935G	Q312R
c.935A>T	c.A935T	Q312L
c.936G>T or c.936G>C	c.G936T or c.G936C	Q312H
c.937G>T	c.G937T	D313Y
c.[937G>T; 1232G>A]	c.G937T/G1232A	D313Y/G411D
c.938A>G	c.A938G	D313G
c.938A>T	c.A938T	D313V
c.939T>A	c.T939A	D313E
c.940A>G	c.A940G	K314E
c.941A>C	c.A941C	K314T
c.941A>T	c.A941T	K314M
c.942G>C	c.G942C	K314N
c.943G>A	c.G943A	D315N
c.943G>C	c.G943C	D315H
c.943G>T	c.G943T	D315Y
c.944A>C	c.A944C	D315A
c.944A>G	c.A944G	D315G
c.944A>T	c.A944T	D315V
c.946G>A	c.G946A	V316I
c.946G>C	c.G946C	V316L
c.947T>C	c.T947C	V316A
c.947T>G	c.T947G	V316G
c.949A>C	c.A949C	I317L
c.949A>G	c.A949G	I317V
c.950T>C	c.T950C	I317T

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.951T>G	c.T951G	I317M
c.952G>A	c.G952A	A318T
c.952G>C	c.G952C	A318P
c.953C>A	c.C953A	A318D
c.953C>T	c.C953T	A318V
c.955A>T	c.A955T	I319F
c.956T>C	c.T956C	I319T
c.957C>G	c.C957G	I319M
c.958A>C	c.A958C	N320H
c.959A>C	c.A959C	N320T
c.959A>G	c.A959G	N320S
c.959A>T	c.A959T	N320I
c.961C>A	c.C961A	Q321K
c.962A>G	c.A962G	Q321R
c.962A>T	c.A962T	Q321L
c.963G>C or c.963G>T	c.G963C or c.G963T	Q321H
c.964G>A	c.G964A	D322N
c.964G>C	c.G964C	D322H
c.965A>C	c.A965C	D322A
c.965A>T	c.A965T	D322V
c.966C>A or c.966C>G	c.C966A or c.C966G	D322E
c.967C>A	c.C967A	P323T
c.968C>G	c.C968G	P323R
c.970T>G	c.T970G	L324V
c.971T>G	c.T971G	L324W
c.973G>A	c.G973A	G325S
c.973G>C	c.G973C	G325R
c.973G>T	c.G973T	G325C

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.974G>C	c.G974C	G325A
c.974G>T	c.G974T	G325V
c.976A>C	c.A976C	K326Q
c.976A>G	c.A976G	K326E
c.977A>C	c.A977C	K326T
c.977A>G	c.A977G	K326R
c.977A>T	c.A977T	K326M
c.978G>C or c.978G>T	c.G978C or c.G978T	K326N
c.979C>G	c.C979G	Q327E
c.980A>C	c.A980C	Q327P
c.980A>T	c.A980T	Q327L
c.981A>T	c.A981T	Q327H
c.983G>C	c.G983C	G328A
c.985T>A	c.T985A	Y329N
c.985T>C	c.T985C	Y329H
c.985T>G	c.T985G	Y329D
c.986A>G	c.A986G	Y329C
c.986A>T	c.A986T	Y329F
c.988C>A	c.C988A	Q330K
c.988C>G	c.C988G	Q330E
c.989A>C	c.A989C	Q330P
c.989A>G	c.A989G	Q330R
c.990G>C	c.G990C	Q330H
c.991C>G	c.C991G	L331V
c.992T>A	c.T992A	L331H
c.992T>C	c.T992C	L331P
c.992T>G	c.T992G	L331R
c.994A>G	c.A994G	R332G

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.995G>C	c.G995C	R332T
c.995G>T	c.G995T	R332I
c.996A>T	c.A996T	R332S
c.997C>G	c.C997G	Q333E
c.998A>C	c.A998C	Q333P
c.998A>T	c.A998T	Q333L
c.1000G>C	c.G1000C	G334R
c.1001G>A	c.G1001A	G334E
c.1001G>T	c.G1001T	G334V
c.1003G>T	c.G1003T	D335Y
c.1004A>C	c.A1004C	D335A
c.1004A>G	c.A1004G	D335G
c.1004A>T	c.A1004T	D335V
c.1005C>G	c.C1005G	D335E
c.1006A>G	c.A1006G	N336D
c.1006A>T	c.A1006T	N336Y
c.1007A>C	c.A1007C	N336T
c.1007A>G	c.A1007G	N336S
c.1007A>T	c.A1007T	N336I
c.1009T>G	c.T1009G	F337V
c.1010T>A	c.T1010A	F337Y
c.1010T>C	c.T1010C	F337S
c.1010T>G	c.T1010G	F337C
c.1011T>A	c.T1011A	F337L
c.1012G>A	c.G1012A	E338K
c.1013A>C	c.A1013C	E338A
c.1013A>G	c.A1013G	E338G
c.1013A>T	c.A1013T	E338V

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1014A>T	c.A1014T	E338D
c.1015G>A	c.G1015A	V339M
c.1016T>A	c.T1016A	V339E
c.1016T>C	c.T1016C	V339A
c.1021G>C	c.G1021C	E341Q
c.1022A>C	c.A1022C	E341A
c.1027C>A	c.C1027A	P343T
c.1027C>G	c.C1027G	P343A
c.1027C>T	c.C1027T	P343S
c.1028C>T	c.C1028T	P343L
c.1030C>G	c.C1030G	L344V
c.1030C>T	c.C1030T	L344F
c.1031T>G	c.T1031G	L344R
c.1033T>C	c.T1033C	S345P
c.1036G>T	c.G1036T	G346C
c.1037G>A	c.G1037A	G346D
c.1037G>C	c.G1037C	G346A
c.1037G>T	c.G1037T	G346V
c.1039T>A	c.T1039A	L347I
c.1043C>A	c.C1043A	A348D
c.1046G>C	c.G1046C	W349S
c.1046G>T	c.G1046T	W349L
c.1047G>C	c.G1047C	W349C
c.1048G>A	c.G1048A	A350T
c.1048G>T	c.G1048T	A350S
c.1049C>G	c.C1049G	A350G
c.1049C>T	c.C1049T	A350V
c.1052T>A	c.T1052A	V351E

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1052T>C	c.T1052C	V351A
c.1054G>A	c.G1054A	A352T
c.1054G>T	c.G1054T	A352S
c.1055C>G	c.C1055G	A352G
c.1055C>T	c.C1055T	A352V
c.1057A>T	c.A1057T	M353L
c.1058T>A	c.T1058A	M353K
c.1058T>C	c.T1058C	M353T
c.1061T>A	c.T1061A	I354K
c.1061T>G	c.T1061G	I354R
c.1063A>C	c.A1063C	N355H
c.1063A>G	c.A1063G	N355D
c.1063A>T	c.A1063T	N355Y
c.1064A>G	c.A1064G	N355S
c.1066C>G	c.C1066G	R356G
c.1066C>T	c.C1066T	R356W
c.1067G>A	c.G1067A	R356Q
c.1067G>C	c.G1067C	R356P
c.1067G>T	c.G1067T	R356L
c.1069C>G	c.C1069G	Q357E
c.1072G>C	c.G1072C	E358Q
c.1073A>C	c.A1073C	E358A
c.1073A>G	c.A1073G	E358G
c.1074G>T or c.1074G>C	c.G1074T or c.G1074C	E358D
c.1075A>C	c.A1075C	I359L
c.1075A>G	c.A1075G	I359V
c.1075A>T	c.A1075T	I359F

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1076T>A	c.T1076A	I359N
c.1076T>C	c.T1076C	I359T
c.1076T>G	c.T1076G	I359S
c.1078G>A	c.G1078A	G360S
c.1078G>C	c.G1078C	G360R
c.1078G>T	c.G1078T	G360C
c.1079G>A	c.G1079A	G360D
c.1079G>C	c.G1079C	G360A
c.1082G>A	c.G1082A	G361E
c.1082G>C	c.G1082C	G361A
c.1084C>A	c.C1084A	P362T
c.1084C>G	c.C1084G	P362A
c.1084C>T	c.C1084T	P362S
c.1085C>A	c.C1085A	P362H
c.1085C>G	c.C1085G	P362R
c.1085C>T	c.C1085T	P362L
c.1087C>A	c.C1087A	R363S
c.1087C>G	c.C1087G	R363G
c.1087C>T	c.C1087T	R363C
c.1088G>A	c.G1088A	R363H
c.1088G>T	c.G1088T	R363L
c.1090T>C	c.T1090C	S364P
c.1091C>G	c.C1091G	S364C
c.1093T>A	c.T1093A	Y365N
c.1093T>G	c.T1093G	Y365D
c.1094A>C	c.A1094C	Y365S
c.1094A>T	c.A1094T	Y365F
c.1096A>C	c.A1096C	T366P

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1096A>T	c.A1096T	T366S
c.1097C>A	c.C1097A	T366N
c.1097C>T	c.C1097T	T366I
c.1099A>C	c.A1099C	I367L
c.1099A>T	c.A1099T	I367F
c.1101C>G	c.C1101G	I367M
c.1102G>A	c.G1102A	A368T
c.1102G>C	c.G1102C	A368P
c.1103C>G	c.C1103G	A368G
c.1105G>A	c.G1105A	V369I
c.1105G>C	c.G1105C	V369L
c.1105G>T	c.G1105T	V369F
c.1106T>C	c.T1106C	V369A
c.1106T>G	c.T1106G	V369G
c.1108G>A	c.G1108A	A370T
c.1108G>C	c.G1108C	A370P
c.1109C>A	c.C1109A	A370D
c.1109C>G	c.C1109G	A370G
c.1109C>T	c.C1109T	A370V
c.1111T>A	c.T1111A	S371T
c.1112C>G	c.C1112G	S371C
c.1117G>A	c.G1117A	G373S
c.1117G>T	c.G1117T	G373C
c.1118G>C	c.G1118C	G373A
c.1120A>G	c.A1120G	K374E
c.1121A>C	c.A1121C	K374T
c.1121A>G	c.A1121G	K374R
c.1121A>T	c.A1121T	K374I

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1123G>C	c.G1123C	G375R
c.1124G>A	c.G1124A	G375E
c.1124G>C	c.G1124C	G375A
c.1126G>A	c.G1126A	V376M
c.1126G>C	c.G1126C	V376L
c.1127T>A	c.T1127A	V376E
c.1127T>G	c.T1127G	V376G
c.1129G>A	c.G1129A	A377T
c.1129G>C	c.G1129C	A377P
c.1129G>T	c.G1129T	A377S
c.1130C>G	c.C1130G	A377G
c.1135A>G	c.A1135G	N379D
c.1136A>C	c.A1136C	N379T
c.1136A>T	c.A1136T	N379I
c.1137T>A	c.T1137A	N379K
c.1138C>A	c.C1138A	P380T
c.1138C>G	c.C1138G	P380A
c.1139C>A	c.C1139A	P380H
c.1139C>G	c.C1139G	P380R
c.1139C>T	c.C1139T	P380L
c.1142C>A	c.C1142A	A381D
c.1147T>A	c.T1147A	F383I
c.1148T>A	c.T1148A	F383Y
c.1148T>G	c.T1148G	F383C
c.1150A>T	c.A1150T	I384F
c.1151T>C	c.T1151C	I384T
c.1152C>G	c.C1152G	I384M
c.1153A>G	c.A1153G	T385A

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1154C>T	c.C1154T	T385I
c.1156C>A	c.C1156A	Q386K
c.1157A>T	c.A1157T	Q386L
c.1158G>C	c.G1158C	Q386H
c.1159C>A	c.C1159A	L387I
c.1159C>T	c.C1159T	L387F
c.1160T>A	c.T1160A	L387H
c.1160T>G	c.T1160G	L387R
c.1162C>A	c.C1162A	L388I
c.1162C>G	c.C1162G	L388V
c.1162C>T	c.C1162T	L388F
c.1163T>A	c.T1163A	L388H
c.1163T>G	c.T1163G	L388R
c.1168G>A	c.G1168A	V390M
c.1171A>C	c.A1171C	K391Q
c.1171A>G	c.A1171G	K391E
c.1172A>C	c.A1172C	K391T
c.1172A>G	c.A1172G	K391R
c.1172A>T	c.A1172T	K391I
c.1173A>T	c.A1173T	K391N
c.1174A>G	c.A1174G	R392G
c.1174A>T	c.A1174T	R392W
c.1175G>A	c.G1175A	R392K
c.1175G>C	c.G1175C	R392T
c.1175G>T	c.G1175T	R392M
c.1177A>C	c.A1177C	K393Q
c.1177A>G	c.A1177G	K393E
c.1178A>C	c.A1178C	K393T

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1179G>C	c.G1179C	K393N
c.1180C>A	c.C1180A	L394I
c.1181T>A	c.T1181A	L394Q
c.1181T>C	c.T1181C	L394P
c.1181T>G	c.T1181G	L394R
c.1183G>C	c.G1183C	G395R
c.1184G>A	c.G1184A	G395E
c.1184G>C	c.G1184C	G395A
c.1186T>A	c.T1186A	F396I
c.1186T>G	c.T1186G	F396V
c.1187T>G	c.T1187G	F396C
c.1188C>G	c.C1188G	F396L
c.1189T>A	c.T1189A	Y397N
c.1189T>C	c.T1189C	Y397H
c.1190A>C	c.A1190C	Y397S
c.1190A>G	c.A1190G	Y397C
c.1190A>T	c.A1190T	Y397F
c.1192G>A	c.G1192A	E398K
c.1192G>C	c.G1192C	E398Q
c.1193A>G	c.A1193G	E398G
c.1195T>A	c.T1195A	W399R
c.1195T>G	c.T1195G	W399G
c.1198A>C	c.A1198C	T400P
c.1198A>G	c.A1198G	T400A
c.1198A>T	c.A1198T	T400S
c.1199C>A	c.C1199A	T400N
c.1199C>T	c.C1199T	T400I
c.1201T>A	c.T1201A	S401T

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1201T>G	c.T1201G	S401A
c.1202_1203insGACTTC	c.1202_1203insGACTTC	p.T400_S401dup
c.1202C>T	c.C1202T	S401L
c.1204A>G	c.A1204G	R402G
c.1204A>T	c.A1204T	R402W
c.1205G>C	c.G1205C	R402T
c.1205G>T	c.G1205T	R402M
c.1206G>C	c.G1206C	R402S
c.1207T>G	c.T1207G	L403V
c.1208T>C	c.T1208C	L403S
c.1209A>T	c.A1209T	L403F
c.1210A>G	c.A1210G	R404G
c.1211G>A	c.G1211A	R404K
c.1211G>C	c.G1211C	R404T
c.1211G>T	c.G1211T	R404I
c.1212A>T	c.A1212T	R404S
c.1213A>G	c.A1213G	S405G
c.1216C>G	c.C1216G	H406D
c.1217A>T	c.A1217T	H406L
c.1218C>G	c.C1218G	H406Q
c.1219A>T	c.A1219T	I407L
c.1220T>C	c.T1220C	I407T
c.1221A>G	c.A1221G	I407M
c.1222A>C	c.A1222C	N408H
c.1222A>G	c.A1222G	N408D
c.1222A>T	c.A1222T	N408Y
c.1223A>C	c.A1223C	N408T

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1225C>A	c.C1225A	P409T
c.1225C>G	c.C1225G	P409A
c.1225C>T	c.C1225T	P409S
c.1226C>T	c.C1226T	P409L
c.1228A>G	c.A1228G	T410A
c.1228A>T	c.A1228T	T410S
c.1229C>T	c.C1229T	T410I
c.1231G>A	c.G1231A	G411S
c.1231G>T	c.G1231T	G411C
c.1232G>A	c.G1232A	G411D
c.1232G>C	c.G1232C	G411A
c.1232G>T	c.G1232T	G411V
c.1234A>C	c.A1234C	T412P
c.1234A>G	c.A1234G	T412A
c.1234A>T	c.A1234T	T412S
c.1235C>A	c.C1235A	T412N
c.1235C>T	c.C1235T	T412I
c.1237G>A	c.G1237A	V413I
c.1237G>T	c.G1237T	V413F
c.1238T>G	c.T1238G	V413G
c.1240T>G	c.T1240G	L414V
c.1242G>C	c.G1242C	L414F
c.1243C>A	c.C1243A	L415I
c.1244T>A	c.T1244A	L415H
c.1246C>G	c.C1246G	Q416E
c.1247A>T	c.A1247T	Q416L
c.1248G>C	c.G1248C	Q416H
c.1249C>A	c.C1249A	L417I

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1252G>A	c.G1252A	E418K
c.1252G>C	c.G1252C	E418Q
c.1253A>C	c.A1253C	E418A
c.1253A>G	c.A1253G	E418G
c.1254A>T	c.A1254T	E418D
c.1255A>G	c.A1255G	N419D
c.1255A>T	c.A1255T	N419Y
c.1256A>C	c.A1256C	N419T
c.1256A>G	c.A1256G	N419S
c.1256A>T	c.A1256T	N419I
c.1258A>C	c.A1258C	T420P
c.1258A>T	c.A1258T	T420S
c.1259C>A	c.C1259A	T420K
c.1259C>G	c.C1259G	T420R
c.1261A>G	c.A1261G	M421V
c.1261A>T	c.A1261T	M421L
c.1262T>A	c.T1262A	M421K
c.1262T>C	c.T1262C	M421T
c.1262T>G	c.T1262G	M421R
c.1263G>C	c.G1263C	M421I
c.1265A>C	c.A1265C	Q422P
c.1267A>T	c.A1267T	M423L
c.1268T>A	c.T1268A	M423K
c.1268T>C	c.T1268C	M423T
c.1269G>C	c.G1269C	M423I
c.1271C>T	c.C1271T	S424L
c.1275A>C	c.A1275C	L425F
c.1279G>A	c.G1279A	D427N

Table 1

Nucleotide change	Nucleotide change	Protein sequence change
c.1286T>G	c.T1286G	L429R

[0086] Kidney Function in Fabry Patients

[0087] Progressive decline in renal function is a major complication of Fabry disease. For example, patients associated with a classic Fabry phenotype exhibit progressive renal impairment that can lead to dialysis or renal transplantation.

[0088] A frequently used method in the art to assess kidney function is the glomerular filtration rate (GFR). Generally, the GFR is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time. Clinically, estimates of GFR are made based upon the clearance of creatinine from serum. GFR can be estimated by collecting urine to determine the amount of creatinine that was removed from the blood over a given time interval. Age, body size and gender may also be factored in. The lower the GFR number, the more advanced kidney damage is.

[0089] Some studies indicate that untreated Fabry patients experience an average decline in GFR between 7.0 and 18.9 mL/min/1.73 m^2 per year, while patients receiving an enzyme replacement therapy (ERT) may experience an average decline in GFR between 2.0 and 2.7 mL/min/1.73 m^2 per year, although more rapid declines may occur in patients with more significant proteinuria or with more severe chronic kidney disease. Thus, even with patients receiving therapy there is a need to determine an appropriate dose of the therapeutic to account for a patient's developing impairment of renal function. Adjustment of the dose can be used to avoid an accumulation of the therapeutic to a level that is outside the therapeutic index or to a level where the patient experiences toxicity.

[0090] An estimated GFR (eGFR) is calculated from serum creatinine using an isotope dilution mass spectrometry (IDMS) traceable equation. Two of the most commonly used equations for estimating glomerular filtration rate (GFR) from serum creatinine are the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and the Modification of Diet in Renal Disease (MDRD) Study equation. Both the MDRD Study and CKD-EPI equations include variables for age, gender, and race, which may allow providers to observe that CKD is

present despite a serum creatinine concentration that appears to fall within or just above the normal reference interval.

[0091] The CKD-EPI equation uses a 2-slope "spline" to model the relationship between GFR and serum creatinine, age, sex, and race. CKD-EPI equation expressed as a single equation:

$GFR = 141 \times \min(S_{cr}/\kappa, 1)\alpha \times \max(S_{cr}/\kappa, 1)\text{-}1.209 \times 0.993^{Age} \times 1.018$ [if female] $\times$ 1.159 [if black]

where:

S_{cr} is serum creatinine in mg/dL,

κ is 0.7 for females and 0.9 for males,

α is -0.329 for females and -0.411 for males,

min indicates the minimum of S_{cr}/κ or 1, and

max indicates the maximum of S_{cr}/κ or 1.

[0092] The following is the IDMS-traceable MDRD Study equation (for creatinine methods calibrated to an IDMS reference method):

$GFR\ (mL/min/1.73\ m^2) = 175 \times (S_{cr})^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female) $\times$ (1.212 if African American)

[0093] The equation does not require weight or height variables because the results are reported normalized to 1.73 m^2 body surface area, which is an accepted average adult surface area. The equation has been validated extensively in Caucasian and African American populations between the ages of 18 and 70 with impaired kidney function (eGFR < 60 mL/min/1.73 m^2) and has shown good performance for patients with all common causes of kidney disease.

[0094] One method for estimating the creatinine clearance rate (eC_{Cr}) is using the Cockcroft-Gault equation, which in turn estimates GFR in ml/min:

Creatinine Clearance (ml/min) = [(140-Age) x Mass(kg)*] ÷ 72 x Serum Creatinine (mg/dL)

[* multiplied by 0.85 if female]

[0095] The Cockcroft-Gault equation is the equation suggested for use by the Food and Drug Administration for renal impairment studies. It is common for the creatinine clearance calculated by the Cockcroft-Gault formula to be normalized for a body surface area of 1.73 m^2. Therefore, this equation can be expressed as the estimated eGFR in mL/min/1.73 m^2. The

normal range of GFR, adjusted for body surface area, is 100-130 ml/min/1.73m^2 in men and 90-120 ml/min/1.73m^2 in women younger than the age of 40.

[0096] The severity of chronic kidney disease has been defined in six stages (see also Table 2): (Stage 0) Normal kidney function – GFR above 90 mL/min/1.73 m^2 and no proteinuria; (Stage 1) – GFR above 90 mL/min/1.73 m^2 with evidence of kidney damage; (Stage 2) (mild) – GFR of 60 to 89 mL/min/1.73 m^2 with evidence of kidney damage; (Stage 3) (moderate) – GFR of 30 to 59 mL/min/1.73 m^2; (Stage 4) (severe) – GFR of 15 to 29 mL/min/1.73 m^2; (Stage 5) kidney failure - GFR less than 15 mL/min/1.73 m^2. Table 2 below shows the various kidney disease stages with corresponding GFR levels.

Table 2:

Chronic Kidney Disease Stage	GFR level (mL/min/1.73 m^2)
Stage 1 (Normal)	≥ 90
Stage 2 (Mild)	60 – 89
Stage 3 (Moderate)	30 – 59
Stage 4 (Severe)	15 – 29
Stage 5 (Kidney Failure)	< 15

[0097] Dosing, Formulation and Administration

[0098] One or more of the dosing regimens described herein are particularly suitable for Fabry patients who have some degree of renal impairment. Amicus Therapeutics has sponsored two Phase 3 studies using migalastat 150 mg every other day (QOD) in Fabry patients. FACETS (011, NCT00925301) was a 24-month trial, including a 6-month double-blind, placebo-controlled period, in 67 enzyme replacement therapy (ERT)-naive patients. ATTRACT (012, NCT01218659) was an active-controlled, 18-month trial in 57 ERT-experienced patients with a 12-month open-label extension (OLE). Both the FACETS and ATTRACT studies included patients having an estimated glomerular filtration rate (eGFR) of ≥30ml/min/1.73m^2. Accordingly, both studies included Fabry patients with normal renal

function as well as patients with mild and moderate renal impairment, but neither study included patients with severe renal impairment.

[0099] The Phase 3 studies of migalastat treatment of Fabry patients established that 150 mg every other day slowed the progression of the disease as shown by surrogate markers. However, in some embodiments, the migalastat dosing regimen may be adjusted in some Fabry patients because these patients can experience kidney deterioration. With a slowing in the ability to clear the drug from the body there can be an increasing exposure to the patient to the drug. Thus, in some embodiments a dose adjustment protocol is provided to inform physicians of the best dose taking into consideration the current clearance profile from the body. Dose adjustment is particularly difficult with a chaperone because it is an inhibitor, and a delicate balance must be reached such that the chaperone is present in amounts great enough to be therapeutic, but also not so great that the chaperone inhibits enzyme function (which would exacerbate the disease). As such, it is difficult to predict correct dosing, which is further complicated in patients who have reduced capacity to clear the migalastat.

[00100] Accordingly, in one or more embodiments, the Fabry patient with renal impairment is administered about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every other day, once every three days, once every four days, once every five days, once every six days or once every seven days. In one or more embodiments, the migalastat or salt thereof is administered at a frequency of once every other day (also referred to as "QOD" or "Q48H"), every four days (also referred to as "Q4D" or "Q96H") or every seven days (also referred to as "Q7D" or "Q168H"). In some embodiments, the Fabry patient with renal impairment is administered about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every four days. In other embodiments, the Fabry patient with renal impairment is administered about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every seven days. In some embodiments, dosing regimens of longer intervals (e.g. every three days to every seven days) may be begun after, or as an adjustment to, a dosing regimen of about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every other day.

[00101] In various embodiments, the doses described herein pertain to migalastat hydrochloride or an equivalent dose of migalastat or a salt thereof other than the hydrochloride salt. In some embodiments, these doses pertain to the free base of migalastat. In alternate embodiments, these doses pertain to a salt of migalastat. In further embodiments, the salt of

migalastat is migalastat hydrochloride. The administration of migalastat or a salt of migalastat is referred to herein as "migalastat therapy".

[00102] The effective amount of migalastat or salt thereof can be in the range from about 100 mg FBE to about 300 mg FBE. Exemplary doses include about 100 mg FBE, about 105 mg FBE, about 110 mg FBE, about 115 mg FBE, about 120 mg FBE, about 123 mg FBE, about 125 mg FBE, about 130 mg FBE, about 135 mg FBE, about 140 mg FBE, about 145 mg FBE, about 150 mg FBE, about 155 mg FBE, about 160 mg FBE, about 165 mg FBE, about 170 mg FBE, about 175 mg FBE, about 180 mg FBE, about 185 mg FBE, about 190 mg FBE, about 195 mg FBE, about 200 mg FBE, about 205 mg FBE, about 210 mg FBE, about 215 mg FBE, about 220 mg FBE, about 225 mg FBE, about 230 mg FBE, about 235 mg FBE, about 240 mg FBE, about 245 mg FBE, about 250 mg FBE, about 255 mg FBE, about 260 mg FBE, about 265 mg FBE, about 270 mg FBE, about 275 mg FBE, about 280 mg FBE, about 285 mg FBE, about 290 mg FBE, about 295 mg FBE or about 300 mg FBE.

[00103] Again, it is noted that 150 mg of migalastat hydrochloride is equivalent to 123 mg of the free base form of migalastat. Thus, in one or more embodiments, the dose is 150 mg of migalastat hydrochloride or an equivalent dose of migalastat or a salt thereof other than the hydrochloride salt, administered at a frequency of once every other day. As set forth above, this dose is referred to as 123 mg FBE of migalastat. In further embodiments, the dose is 150 mg of migalastat hydrochloride administered at a frequency of once every other day. In other embodiments, the dose is 123 mg of the migalastat free base administered at a frequency of once every other day.

[00104] In various embodiments, the effective amount is about 122 mg, about 128 mg, about 134 mg, about 140 mg, about 146 mg, about 150 mg, about 152 mg, about 159 mg, about 165 mg, about 171 mg, about 177 mg, about 183 mg, about 189 mg, about 195 mg, about 201 mg, about 207 mg, about 213 mg, about 220 mg, about 226 mg, about 232 mg, about 238 mg, about 244 mg, about 250 mg, about 256 mg, about 262 mg, about 268 mg, about 274 mg, about 280 mg, about 287 mg, about 293 mg, about 299 mg, about 305 mg, about 311 mg, about 317 mg, about 323 mg, about 329 mg, about 335 mg, about 341 mg, about 348 mg, about 354 mg, about 360 mg or about 366 mg of migalastat hydrochloride.

[00105] Thus, in one or more embodiments, the dose is 150 mg migalastat hydrochloride or an equivalent dose of migalastat or a salt thereof other than the hydrochloride salt, administered at a frequency of once every four days or once every seven days. In further

embodiments, the dose is 150 mg migalastat hydrochloride administered every four days. In other embodiments, the dose is 150 mg 1 migalastat hydrochloride administered every seven days. In other embodiments, the dose is 123 mg of migalastat free base administered at a frequency of once every other day, once every four days or once every seven days. Longer dosing intervals (e.g. every three to seven days) may be useful with a higher degree of renal impairment compared to a dosing frequency of every other day. Such longer dosing intervals include every three, four, five, six or seven days.

[00106] In some embodiments, dosing intervals may include any dosing interval with more than 48 hours between doses. For example, dosing intervals may include dosing every 72, 96, 120, 144, or 168 hours.

[00107] In some embodiments, dosing intervals may include administration less than 3.5 times per week on average. For example, dosing may occur 3 times per week, 2 times per week or once per week on average. In some embodiments, dosing may occur, on average, less than or equal to about 2.3 times per week, less than or equal to about 1.75 times per week, less than or equal to about 1.4 times per week, or less than or equal to about 1.167 times per week.

[00108] In some embodiments, dosing intervals may be irregular. For example, dosing intervals may include administration every Monday, Wednesday and Friday, without administration on Tuesday, Thursday, Saturday or Sunday. Similarly, dosing intervals may include administration every Monday and Thursday, without administration on other days.

[00109] The administration of migalastat may be for a certain period of time. In one or more embodiments, the migalastat is administered for a duration of at least 28 days, such as at least 30, 60 or 90 days or at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20 or 24 months or at least 1, 2 or 3 years. In various embodiments, the migalastat therapy is long-term migalastat therapy of at least 6 months, such as at least 6, 7, 8, 9, 10, 11, 12, 16, 20 or 24 months or at least 1, 2 or 3 years.

[00110] Administration of migalastat according to the present invention may be in a formulation suitable for any route of administration, but is preferably administered in an oral dosage form such as a tablet, capsule or solution. As one example, the patient is orally administered capsules each containing 25 mg, 50 mg, 75 mg, 100 mg or 150 mg migalastat hydrochloride (i.e. 1-deoxygalactonojirimycin hydrochloride) or an equivalent dose of migalastat or a salt thereof other than the hydrochloride salt.

[00111] In some embodiments, the PC (*e.g.*, migalastat or salt thereof) is administered orally. In one or more embodiments, the PC (*e.g.*, migalastat or salt thereof) is administered by injection. The PC may be accompanied by a pharmaceutically acceptable carrier, which may depend on the method of administration.

[00112] In one embodiment of the invention, the chaperone compound is administered as monotherapy, and can be in a form suitable for any route of administration, including e.g., orally in the form tablets or capsules or liquid, or in sterile aqueous solution for injection. In other embodiments, the PC is provided in a dry lyophilized powder to be added to the formulation of the replacement enzyme during or immediately after reconstitution to prevent enzyme aggregation in vitro prior to administration.

[00113] When the chaperone compound is formulated for oral administration, the tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or another suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active chaperone compound.

[00114] The pharmaceutical formulations of the chaperone compound suitable for parenteral/injectable use generally include sterile aqueous solutions (where water soluble), or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and

must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, benzyl alcohol, sorbic acid, and the like. In many cases, it will be reasonable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monosterate and gelatin.

[00115] Sterile injectable solutions are prepared by incorporating the purified enzyme and the chaperone compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter or terminal sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

[00116] The formulation can contain an excipient. Pharmaceutically acceptable excipients which may be included in the formulation are buffers such as citrate buffer, phosphate buffer, acetate buffer, bicarbonate buffer, amino acids, urea, alcohols, ascorbic acid, and phospholipids; proteins, such as serum albumin, collagen, and gelatin; salts such as EDTA or EGTA, and sodium chloride; liposomes; polyvinylpyrollidone; sugars, such as dextran, mannitol, sorbitol, and glycerol; propylene glycol and polyethylene glycol (e.g., PEG-4000, PEG-6000); glycerol; glycine or other amino acids; and lipids. Buffer systems for use with the formulations include citrate; acetate; bicarbonate; and phosphate buffers. Phosphate buffer is a preferred embodiment.

[00117] The route of administration of the chaperone compound may be oral (preferably) or parenteral, including intravenous, subcutaneous, intra-arterial, intraperitoneal, ophthalmic, intramuscular, buccal, rectal, vaginal, intraorbital, intracerebral, intradermal,

intracranial, intraspinal, intraventricular, intrathecal, intracisternal, intracapsular, intrapulmonary, intranasal, transmucosal, transdermal, or via inhalation.

[00118] Administration of the above-described parenteral formulations of the chaperone compound may be by periodic injections of a bolus of the preparation, or may be administered by intravenous or intraperitoneal administration from a reservoir which is external (e.g., an i.v. bag) or internal (e.g., a bioerodable implant).

[00119] Embodiments relating to pharmaceutical formulations and administration may be combined with any of the other embodiments of the invention, for example embodiments relating to a method of treating a patient with Fabry disease, a method of enhancing α-galactosidase A in a patient diagnosed with or suspected of having Fabry disease, use of a pharmacological chaperone for α-galactosidase A for the manufacture of a medicament for treating a patient diagnosed with Fabry disease or to a pharmacological chaperone for α-galactosidase A for use in treating a patient diagnosed with Fabry disease as well as embodiments relating to amenable mutations, the PCs and suitable dosages thereof.

[00120] In one or more embodiments, chaperone is administered in combination with enzyme replacement therapy. Enzyme replacement therapy increases the amount of protein by exogenously introducing wild-type or biologically functional enzyme by way of infusion. This therapy has been developed for many genetic disorders, including lysosomal storage disorders such as Fabry disease, as referenced above. After the infusion, the exogenous enzyme is expected to be taken up by tissues through non-specific or receptor-specific mechanism. In general, the uptake efficiency is not high, and the circulation time of the exogenous protein is short. In addition, the exogenous protein is unstable and subject to rapid intracellular degradation as well as having the potential for adverse immunological reactions with subsequent treatments. In one or more embodiments, the chaperone is administered at the same time as replacement enzyme. In some embodiments, the chaperone is co-formulated with the replacement enzyme.

[00121] In one or more embodiments, a patient is switched from enzyme replace therapy (ERT) to migalastat therapy. In some embodiments, a patient on ERT is identified, the patient's ERT is discontinued, and the patient begins receiving migalastat therapy. The migalastat therapy can be in accordance with any of the methods described herein. In various embodiments, the patient has some degree of renal impairment, such as mild, moderate or severe renal impairment.

[00122] Monitoring Lyso-Gb3 and Migalastat Levels

[00123] Lyso-Gb3 (globotriaosylsphingosine) can be monitored to determine whether substrate is being cleared from the body of a Fabry patient. Higher levels of lyso-Gb3 correlate with higher levels of substrate. If a patient is being successfully treated, then lyso-Gb3 levels are expected to drop. One dosing regimen for Fabry disease is administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a frequency of once every other day.

[00124] Over time, the levels of lyso-Gb3 may rise which can be due to either disease progression and/or decreasing ability of the kidneys to clear migalastat from the patient's body. Lyso-Gb3 levels will rise when the level of migalastat is too high because at higher levels the migalastat acts as an inhibitor of α-Gal A, thus preventing the enzyme from binding to the target substrate. Individuals with normal kidney function will generally clear a 150 mg dose of migalastat hydrochloride by 48 hours (*i.e.*, C_{48h} to below a level of quantification of about 5 ng/mL). In cases of severe kidney impairment, C_{48h} may be 250 or even above 300 ng/mL. It is thought that high levels of migalastat are due to impaired kidney function because migalastat does not have other known interactions that would otherwise result in high levels.

[00125] Accordingly, another aspect of the invention pertains to method for treatment of Fabry disease in a patient having renal impairment. In one or more embodiments, the method comprises administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a first frequency of once every other day for a first time period; and administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a longer dosing interval (e.g., once every three to seven days) for a second time period. In some embodiments, the dosing frequency is adjusted after measuring lyso-Gb3 and/or migalastat levels. In some embodiments, the dosing frequency is adjusted after a change in the patient's kidney function (e.g. eGFR). For example, the dosing frequency can be adjusted as the patient's eGFR indicates a change from mild renal impairment to moderate renal impairment or a change from moderate renal impairment to severe renal impairment.

[00126] In some embodiments, the migalastat or salt thereof is administered at a first frequency for a first time period, and then administered at a second frequency for a second time period. The first frequency is greater (i.e., more frequent) than the second frequency. The first frequency and the second frequency may be any dosing interval disclosed herein. In some embodiments, the first frequency is every other day and the second frequency is every

three days, every four days, every five days, every six days or every seven days. In some embodiments, the first frequency is every four days and the second frequency is every five days, every six days, or every seven days.

[00127] In some embodiments, the migalastat or salt thereof is administered at a first frequency for a first time period, then administered at a second frequency for a second time period, and then administered at a third frequency for a third time period. The first frequency is greater (i.e., more frequent) than the second frequency, and the second frequency is greater than the third frequency. For example, in some embodiments, the migalastat or salt thereof is administered at a first frequency of once every other day for a first time period, then the migalastat or salt thereof is administered at a second frequency of once every four days for a second time period, and then the migalastat or salt thereof is administered at a third frequency of once every seven days for a third time period.

[00128] In some embodiments, the dosing frequency is adjusted in response to a reduction in the patient's eGFR. In exemplary embodiments, when the patient's eGFR is reduced below 30 mL/min/1.73 m^2, the dosing frequency can be adjusted from every other day to every four days. In exemplary embodiments, when the patient's eGFR is reduced below 20 mL/min/1.73 m^2, the dosing frequency can be adjusted from every four days to every seven days. Other adjustments in dosing frequency can be made from one dosing interval to a longer dosing interval as described above. In some embodiments, the patient suffers from severe renal impairment.

[00129] In some embodiments, the method further comprises measuring migalastat levels. In one or more embodiments, migalastat concentration (*e.g.*, ng/mL) is measured. In some embodiments, the total area under the curve ($AUC_{0-\infty}$) is measured. In one or more embodiments, the lowest concentration the migalastat reaches before the next dose (C_{trough}) is measured. C_{trough} for QOD will be the concentration at 48 hours (C_{48h}). C_{trough} for Q4D will be the concentration at 96 hours (C_{96}). Similarly, C_{trough} for Q7D will be the concentration at 168 hours (C_{168}). In one or more embodiments, the targeted C_{trough} values are at or near below the level of quantitation (BLQ). Such C_{trough} values indicate that the migalastat is being cleared from the body at an appropriate rate (*i.e.*, is almost completely cleared before administration of the next dose).

[00130] Migalastat levels can be measured via methods known in the art. For example, if measuring migalastat from tissue samples, tissue aliquots may be homogenized (7 µL water

per 1 mg tissue) using a homogenizer (*e.g.*, FastPrep-24 from MP Biomedical, Irvine, CA). Microcentrifuge tubes containing 100 µl of the tissue homogenate or 50 µl of plasma may then be spiked with 500 ng/mL 13C d2-AT1001 HCl internal standard (manufactured by MDS Pharma Services). A 600 µl volume of 5 mM HCl in 95/5 MeOH:H_2O can then be added and the tubes vortexed for 2 minutes, followed by centrifugation at 21000 x g for 10 minutes at room temperature. The supernatants may then be collected into a clean, 96-well plate, diluted with 5 mM HCl in dH_2O and applied to a 96-well solid phase extraction (SPE) plate (Waters Corp., Milford MA). After several wash steps and elution into a clean, 96-well plate, the extracts may be dried down under N_2 and reconstituted with mobile phase A. Migalastat levels can then be determined by liquid chromatography – tandem mass spectroscopy (LC-MS/MS) (*e.g.*, LC: Shimadzu; MS/MS: ABSciex API 5500 MS/MS). The liquid chromatography can be conducted using an ACN:water:formate binary mobile phase system (mobile phase A: 5 mM ammonium formate, 0.5% formic acid in 95:5 ACN:water; mobile phase B: 5 mM ammonium formate, 0.5% formic acid in 5:47.5:47.5 ACN:MeOH:water) with a flow rate of 0.7 mL/minute on an Halo HILIC column (150x4.6 mm, 2.7 µm) (Advanced Materials Technology, Inc.). MS/MS analysis may be carried out under APCi positive ion mode. The same procedure may be followed for migalastat determination in plasma except without homogenization. The following precursor ion→product ion transitions may be monitored: mass/charge (m/z) 164.1→m/z 80.1 for migalastat and m/z 167.1→m/z 83.1 for the internal standard. A 12-point calibration curve and quality control samples may be prepared. The ratio of the area under the curve for migalastat to that of the internal standard is then determined and final concentrations of migalastat in each sample calculated using the linear least squares fit equation applied to the calibration curve. To derive approximate molar concentrations, one gram of tissue may be estimated as one mL of volume.

[00131] Migalastat concentration can be measured from plasma samples at various times to monitor clearance from the body. A clinically relevant increase in C_{trough} suggests significant accumulation of plasma migalastat concentration. If the migalastat is not cleared from the body enough prior to the next dose administration, then the levels of migalastat can build up, possibly leading to an inhibitory effect. Thus, in one or more embodiments, a change in the dosing frequency occurs after a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0-fold increase in C_{trough} compared to normal renal function C_{trough}. In one or more embodiments, the C_{trough} of normal renal function at is BLQ. In some

embodiments, BLQ is 5 ng/mL of migalastat. A person with normal kidney function will generally clear 150 mg of migalastat HCl in 48 hours. Thus, a patient that is currently on a dosing QOD regimen of 150 mg of migalastat HCl should reach BLQ by 48 hours, which is also the C_{trough} value. If values above BLQ are measured at 48 hours in a patient on a QOD dosing regimen, then this may indicate a need to change the dosing interval. Accordingly, in one or more embodiments, the C_{trough} value of a patient with renal impairment (C_{48h} if on a QOD regimen, C_{96} if on a Q4D regimen or C_{168} if on a Q7D regimen) will be compared with C_{trough} of a person with normal renal function (C_{48h}).

[00132] In one or more embodiments, a change in the dosing frequency occurs after a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0-fold increase in $AUC_{0-\infty}$ compared to normal renal function $AUC_{0-\infty}$.

[00133] In some embodiments, samples may be taken at 0, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and/or 168 hours after administration. In some embodiments, the migalastat concentration 48 hours after administration is measured. In some embodiments, the administration of the second time period is begun after more than about 5, 10, 15, 20, 25, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175 or 200 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period is measured.

[00134] In further embodiments, the method further comprises measuring lyso-Gb3 in one or more plasma samples from the patient. A first baseline lyso-Gb3 level may be determined during the first time period. As used herein, "baseline lyso-Gb3 level" refers to the lowest plasma lyso-Gb3 value measured during a given time period or dosing regimen. Thus, if the lyso-Gb3 levels go up significantly from the baseline lyso-Gb3 levels, this may indicate kidney disease progression and/or improper clearance of migalastat. Thus, in further embodiments, the administration of the second time period is begun after an increase (*e.g.*, of at least about 20, 25, 30, 33, 35, 40, 45 or 50% and/or 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5 or 3 nM) above the first baseline lyso-Gb3 level is measured. A 33% and/or 2 nM increase from baseline in plasma lyso-Gb3 has been deemed clinically relevant based upon Phase 3 data in Fabry patients signaling either inhibition-induced migalastat exposure from decline in renal function and/or progression of disease condition. Lyso-Gb3 levels may be measured at varying frequencies (*e.g.*, about once every 2, 3, 4 or 5 months). It is thought that it takes about 3 months for a baseline lyso-Gb3 level to be established once a dosing regimen has been started.

[00135] Lyso-Gb3 can be measured via methods known in the art using validated assays. As with migalastat, lyso-Gb3 levels may be determined using liquid chromatography – tandem mass spectroscopy (LC-MS/MS) (*e.g.*, LC: Shimadzu; MS/MS: ABSciex API 5500 MS/MS). For example, one process of measuring plasma lyso-Gb3 is described in Hamler, Rick, *et al.* "Accurate quantitation of plasma globotriaosylsphingosine (lyso-Gb3) in normal individuals and Fabry disease patients by liquid chromatography–tandem mass spectrometry (LC–MS/MS)." *Molecular Genetics and Metabolism*, Volume 114.2 (2015):S51. In one or more embodiments, lyso-Gb3 is measured in samples from a patient's urine.

[00136] Thus, in one exemplary embodiment, the method comprises

administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a first frequency of once every other day for a first time period;

administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a second frequency of once every four or seven days for a second time period;

measuring lyso-Gb3 in one or more plasma samples from the patient;

determining a first baseline lyso-Gb3 level during the first time period;

measuring migalastat concentration, $AUC_{0-\infty}$ and/or C_{trough} in one or more plasma samples during the first time period; and

beginning the administration at the second frequency after

(i) an increase above the first baseline lyso-Gb3 level, and/or

(ii) more than about 5 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period, or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period..

[00137] In further embodiments, the administration of the second time period may begin after an increase above the first baseline lyso-Gb3 level is at least about 30, or 33% and/or 2nM and/or more than about 50 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period is measured. In some embodiments, the administration of the second time period may begin after an increase above the first baseline lyso-Gb3 level is at least about 30, or 33% and/or 2nM and/or more than about 50 ng/mL of

migalastat is measured 48 hours after administration of the migalastat during the first time period is measured, or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period.

[00138] In further embodiments, dosing from every other day is adjusted to every four days, and then further adjusted to every seven days. In such embodiments, the frequency of the second time period is once every four days, and the method further comprises administering to the patient about 100 mg to about 300 mg FBE of migalastat or salt thereof at a third frequency of once every seven days for a third time period. In yet further embodiments, the method may further comprise

measuring lyso-Gb3 in one or more plasma samples from the patient;

determining a first baseline lyso-Gb3 level during first time period;

measuring migalastat concentration, $AUC_{0-\infty}$ and/or C_{trough} in one or more plasma samples from the patient during the first time period;

beginning the administration of the second time period after

(i) an increase above the first baseline lyso-Gb3 level, and

(ii) more than about 5 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the first time period; , or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period

determining a second baseline lyso-Gb3 level during the second time period; and

beginning the administration of the third time period after

(i) an increase above the second baseline lyso-Gb3 level, and

(ii) more than about 5 ng/mL of migalastat is measured 48 hours after administration of the migalastat during the second time period, or there is a greater than 1.5-fold increase in $AUC_{0-\infty}$ and/or C_{trough} compared to normal renal function during the first time period.

[00139] In other embodiments, the dosing from every other day is adjusted directly to every seven days without first adjusting to four days.

[00140] In an exemplary embodiment, a Fabry patient may be receiving 150 mg of migalastat HCl every other day. If upon measuring plasma lyso-Gb3 levels, the following are measured: (1) an increase in plasma lyso-Gb3 relative to the baseline level for the current dose regimen (*e.g.*, at least a 30 or 33% increase); and/or (2) an increase of at least 2 nM in plasma lyso-Gb3 relative to the baseline level for the current dose regimen, the dosing regimen may be changed to once every four or seven days. If the patient's migalastat levels are also high, then the regimen may also be changed to once every four or seven days. Such high levels of migalastat could be a measurement of the $AUC_{0-\infty}$ and/or C_{trough} that is higher compared to normal renal function during the first time period (*e.g.*, 1.5 or 2-fold increase).

[00141] Once the dose regimen has been changed, a new plasma lyso-Gb3 baseline level will be established. Any new dose regimen modifications will be based on a comparison to the subject's most current baseline level. For example, a new baseline level may be established as follows: if a subject has a decrease in plasma lyso-Gb3 relative to their previous measurement, a confirmatory retest may take place. If the confirmatory value is also lower than their previous measurement, the average of the 2 values will be the subject's new baseline level. If the retest is NOT lower than the subject's previous measurement, the previous measurement will continue as the current baseline level until the next visit.

[00142] Reference throughout this specification to "one embodiment," "certain embodiments," "various embodiments," "one or more embodiments" or "an embodiment" means that a particular feature, structure, material, or characteristic described in connection with the embodiment is included in at least one embodiment of the invention. Thus, the appearances of the phrases such as "in one or more embodiments," "in certain embodiments," "in various embodiments," "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily referring to the same embodiment of the invention. Furthermore, the particular features, structures, materials, or characteristics may be combined in any suitable manner in one or more embodiments.

[00143] Although the invention herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the principles and applications of the present invention. It will be apparent to those skilled in the art that various modifications and variations can be made to the method and apparatus of the present invention without departing from the spirit and scope of the invention. Thus, it is

intended that the present invention include modifications and variations that are within the scope of the appended claims and their equivalents.

[00144] Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

EXAMPLES

[00145] Example 1: Pharmacokinetics of Migalastat in Non-Fabry Patients with Renal Impairment

[00146] A phase 1 trial was conducted to study the pharmacokinetics and safety of migalastat HCl in non-Fabry subjects with renal impairment. The results are reported in Johnson, et al. "An Open-Label Study to Determine the Pharmacokinetics and Safety of Migalastat HCl in Subjects With Impaired Renal Function and Healthy Subjects with Normal Renal Function." *American College of Clinical Pharmacology* 4.4 (2015): 256–261, and is also described here. A single 150 mg migalastat HCl dose was administered to subjects with mild, moderate, and severe renal impairment, and normal renal function. The eGFR estimated by the Cockcroft-Gault equation per the FDA Guidance for renal impairment studies.

[00147] Volunteers were enrolled into two cohorts stratified for renal function calculated using the Cockcroft–Gault equation for creatinine clearance (CLcr). Subjects were assigned to groups based on an estimated CLcr at screening as calculated using the Cockcroft-Gault equation. For each subject, the following plasma migalastat PK parameters were determined by noncompartmental analysis with WinNonlin® software (Pharsight Corporation, Version 5.2).

C_{max} maximum observed concentration

t_{max} time to maximum concentration

AUC_{0-t} area under the concentration-time curve from Hour 0 to the last measurable concentration, calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations

$AUC_{0-\infty}$ area under the concentration-time curve extrapolated to infinity, calculated using the formula:

$AUC0\text{-}\infty = AUC0\text{-}t + Ct/\lambda Z$

where Ct is the last measurable concentration and λZ is the apparent terminal elimination rate constant

λz apparent terminal elimination rate constant, where λZ is the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase

$t_{1/2}$ apparent terminal elimination half-life (whenever possible), where t1/2 = (ln2)/ λZ

CL/F oral clearance, calculated as Dose/AUC0-∞

V_d/F oral volume of distribution, calculated as Dose/ AUC0-∞·λZ

C_{48h} concentration at 48 hours postdose

[00148] Pharmacokinetic parameters determined were: area under the concentration–time curve (AUC) from time zero to the last measurable concentration postdose (AUC_{0-t}) and extrapolated to infinity ($AUC_{0-\infty}$), maximum observed concentration (C_{max}), time to C_{max} (t_{max}), concentration at 48 hours postdose (C_{48h}), terminal elimination half-life ($t_{1/2}$), oral clearance (CL/F), and apparent terminal elimination rate constant (λz) (ClinicalTrials.gov registration: NCT01730469).

[00149] Study subjects were defined as having renal impairment if creatinine clearance (CLcr) was less than 90 mL/min (i.e. CLcr <90 mL/min) as determined using the Cockcroft-Gault formula. Subjects were grouped according to degree of renal dysfunction: mild (CLcr ≥60 and <90 mL/min), moderate (CLcr ≥30 and <60 mL/min), or severe (CLcr ≥15 and <30 mL/min)

[00150] The plasma and urine pharmacokinetics of migalastat have been studied in healthy volunteers and Fabry patients with normal to mildly impaired renal function. In the single-dose studies, migalastat had a moderate rate of absorption reaching maximum concentrations in approximately 3 hours (range, 1 to 6 hrs) after oral administration over the dose range studied. Mean C_{max} and AUC0-t values increased in a dose-proportional manner following oral doses from 75 mg to 1250 mg migalastat. The mean elimination half-lives (t1/2) ranged from 3.04 to 4.79 hours. Mean percent of the dose recovered in urine from doses evaluated in the single ascending dose (SAD) study were 32.2%, 43.0%, 49.3%, and 48.5% for the 25 mg, 75 mg, 225 mg, and 675 mg dose groups, respectively. In multiple ascending dose studies, only minimal accumulation of plasma migalastat was observed. In a TQT study, migalastat was negative for effect on cardiac repolarization at 150 mg and 1250 mg single

doses (Johnson et al. "Pharmacokinetics and Safety of Migalastat HCl and Effects on Agalsidase Activity in Healthy Volunteers." *Clin Pharmacol Drug Dev.* 2013 Apr; 2(2):120-32 2013).

[00151] In this single dose renal impairment study conducted in non-Fabry subjects, plasma concentrations of single-dose migalastat HCl 150 mg increased with increasing degree of renal failure compared to subjects with normal renal function. Following a single oral dose of migalastat HCl 150 mg, mean plasma migalastat $AUC_{0-\infty}$ increased in subjects with mild, moderate, or severe renal impairment by 1.2-fold, 1.8-fold, and 4.5-fold, respectively, compared to healthy control subjects. Increases in plasma migalastat 150 mg $AUC_{0-\infty}$ values were statistically significant in subjects with moderate or severe renal impairment but not in subjects with mild renal impairment following single-dose administration compared to subjects with normal renal function. Migalastat tmax was slightly delayed in the severe group; C_{max} was not increased across any of the groups following a single oral dose of migalastat HCl 150 mg in subjects with varying degrees of renal impairment compared to healthy control subjects. Plasma migalastat C_{48h} levels were elevated in subjects with moderate (predominantly from subjects with CrCL <50ml/min) and severe renal impairment compared with healthy control subjects. The $t_{1/2}$ of migalastat in plasma increased as the degree of renal impairment increased (arithmetic mean [min, max]: 6.4 [3.66, 9.47], 7.7 [3.81, 13.8], 22.2 [6.74, 48.3], and 32.3 [24.6, 48.0] h) in subjects with normal renal function and those with mild, moderate, or severe renal impairment, respectively. Mean CL/F decreased with increasing degree of renal failure and ranged from 12.1 to 2.7 L/hr from mild to severe renal impairment (Johnson et al. 2014).

[00152] Migalastat clearance decreased with increasing renal impairment, resulting in increases in migalastat HCl plasma $t_{1/2}$, $AUC_{0-\infty}$, and C_{48h} compared with subjects with normal renal function. Incidence of adverse events was comparable across all renal function groups.

[00153] Following a single oral dose of 150 mg migalastat HCl plasma exposure (expressed as AUC_{0-t}) increased as the degree of renal impairment increased. Figure 1A shows an increase in migalastat AUC_{0-t} values as CLcr values decrease. Figure 1B shows the mean (SE) plasma migalastat concentration-time profiles for each renal function group. BLQ values were entered as zero and included in the calculation of means.

[00154] As demonstrated in Figure 1C, as renal impairment worsens, plasma migalastat AUC_{0-t} values increase in a nonlinear manner. Results demonstrated that, as renal impairment worsened, the clearance of plasma migalastat decreased, resulting in prolonged $t_{1/2}$, higher C_{48h}

values, and higher overall plasma exposure ($AUC_{0-\infty}$), in particular in subjects with severe renal impairment. Migalastat is primarily excreted unchanged in urine. Thus, an increase in plasma migalastat exposure is consistent with worsening renal impairment.

[00155] Conclusions: Plasma migalastat clearance decreased as degree of renal impairment increased

[00156] A summary of the PK results are shown in Table 3 below.

Table 3:

PK		Renal Function Group			
Parameter	**Units**	**Normal (N=8)**	**Mild (N=8)**	**Moderate (N=8)**	**Severe (N=8)**
AUC_{0-t}	(ng·hr/mL)	12306 (27.9)	14389 (31.1)	22126 (42.8)	53070 (27.0)
$AUC_{0-\infty}$	(ng·hr/mL)	12397 (27.7)	14536 (30.7)	22460 (42.2)	56154 (24.9)
C_{max}	(ng/mL)	2100 (26.0)	2191 (28.8)	1868 (32.1)	2078 (45.5)
t_{max}	(hr)	2.50 (1.50, 3.00)	2.50 (1.50, 4.00)	3.00 (1.50, 4.00)	4.27 (3.00, 8.00)
$t_{½}$	(hr)	6.42 (1.93)	7.66 (3.02)	22.2 (14.2)	32.3 (7.35)
CL/F	(L/hr)	12.1 (27.7)	10.3 (30.7)	6.68 (42.2)	2.67 (24.9)
C_{48h}	(ng/mL)	5.70 (3.63)	9.34 (7.57)	64.5 (68.1)	334 (126)

[00157] Example 2: Multiple Dose Simulations on Renal Impairment Subjects

[00158] In the renal impairment study of Example 1, consistent increases in area under the curve (AUC) and trough concentration of migalastat at 48 hours post-dose following QOD dosing (C_{48h}) of 2- to 4-fold were observed at eGFR values ≤ 35 mL/min relative to subjects with normal renal function.

[00159] A population PK model was developed to predict exposures and time above IC50 in Fabry patients with varying degrees of renal impairment. Various dosing regimens were assessed to develop an understanding of migalastat exposure in patients with different ranges of renal impairment (≥30, 20-30, <20 mL/min/1.73m^2). The dosing regimens evaluated included 150 mg every other day (QOD), 150 mg every 4 days (Q4D), and 150 mg once weekly (Q7D).

[00160] A model-based dose finding approach was used to predict appropriate migalastat dosing in a Fabry patient sub-population, namely Fabry patients with renal impairment. In general, dose optimization goals to which model-based drug development (MBDD) methods can be applied include: (1) predicting first-in-human dose; (2) finding the

dose or dose range that best balances safety and efficacy; (3) finding best dose frequency; (4) finding promising combinations for co-administered drugs; (5) accounting for realistic subject behavior, including adherence; (6) maximizing early phase learning to strengthen dose confirmation.

[00161] This example provides computer simulations of dosing the renal impairment subjects of Example 1. The key assumption was exposure characterized in non-Fabry subjects with renal impairment is the same as in Fabry patients with renal impairment. The software program was WinNonlin version 5.2 or higher. The conditions of the model are described below. 11 subjects who had BSA-adjusted $eGFR_{Cockcroft\text{-}Gault} \leq 35$ mL/min/1.73m^2 were included in the modeling exercise; 3 had moderate renal impairment, but were ≥ 30 mL/min/1.73m^2 and ≤ 35 mL/min/1.73m^2, and 8 were ≥ 14 mL/min/1.73m^2 and < 30 mL/min/1.73m^2. Steady state was assumed by 7th dose.

[00162] Four regimens with 150 mg migalastat HCl were simulated: QOD (every other day or 48 hrs), Q3D (every 3rd day or 72 hrs), Q4D (every 4th day or 96 hrs), and Q7D (every 7th day/once a week or 168 hrs).

[00163] A 2-compartment model was used to estimate volume of distribution (V_d) and elimination rate constants from single dose data. These estimates were inputted into each molecular dose simulation regimen.

[00164] FIGS. 2A-D show the mean simulation plots for each regimen. Table 4 below shows the exposures and accumulation ratios. Based on AUCs, MD simulations suggest accumulation is minimal ($\leq 5\%$) for Q7D dosing. The highest exposure of migalastat in a Fabry patient was recorded as 53035 ng*hr/mL, who received a single dose of 450 mg.

[00165] Table 5 below shows the $C_{min,ss}$ for a 150 mg regimen. Based on $C_{min,ss}$, MD simulations for Q7D are similar to PPK C_{min} (8.70 ng/mL) for most subjects.

[00166] FIGS. 3A-B show the R_{ac} and C_{min} values across simulated regimens. FIG. 3A shows the QOD regimen has greater accumulation of migalastat, then Q3D, Q4D lesser yet, and Q7D has virtually none in severe renal impairment. FIG. 3B also shows this trend, but for C_{48h} concentration.

[00167] FIG. 4 shows AUC versus C_{48h} from Example 1. This stick plot provides a visual correlation of AUC to C_{48h} concentration across all levels of renal function, and demonstrates the two values are well visually correlated.

[00168] Tables 6-7 are provided below showing a summary of the population PK modeling and time above IC_{50} (inhibition).

[00169] Based on predicted exposure data, clinical trial simulations suggest that a Q4D regimen would provide exposures similar to subjects with normal renal function for those with eGFR between >30 and <40 mL/min/1.73m^2 and a Q7D regimen would provide exposures similar to subjects with normal renal function for those with eGFR between >20 and <30 mL/min/1.73m^2.

[00170] This modelling predicts the slower removal of migalastat based upon the level of kidney impairment and adjusts the frequency of dosing to bring the level of migalastat below the level where it would inhibit enzyme activity.

[00171] Simulated migalastat exposures following Q7D in subjects with eGFR <20 mL/min/1.73m^2 remained 5- to 6 fold higher than those with normal renal function.

Table 4

SubjID	Renal Function Group	BSA-Adj eGFR$_{Cockcroft-Gault}$ (mL/min/1.73m^2)	AUC_{0-48h}	R_{ac48h}	AUC_{0-72h}	R_{ac72h}	AUC_{0-96h}	R_{ac96h}	AUC_{0-168h}	R_{ac168h}	AUC_{PPK}	R_{acPPK}	AUC_{ss}	$AUC_{0-\infty}$
10-104	Moderate (≥30-≤35)	35.3	31920	1.12	34056	1.05	34978	1.02	35487	1.00	9515	3.75	35716	36051
9-102	Moderate (≥30-≤35)	35.0	35320	1.17	38364	1.08	39608	1.05	41252	1.00	9515	4.36	41475	40653
5-306	Moderate (≥30-≤35)	32.2	17507	1.12	18670	1.05	19211	1.02	19460	1.00	9515	2.06	19560	19723
25-419	Severe (<30)	18.4	59178	1.42	69646	1.20	75485	1.11	81332	1.03	9515	8.81	83805	83312
26-111	Severe (<30)	17.0	44124	1.21	48296	1.10	50597	1.05	52861	1.00	9515	5.59	53212	54470
27-331	Severe (<30)	20.6	37409	1.28	41912	1.14	44571	1.07	47263	1.01	9515	5.02	47769	48890
28-113	Severe (<30)	15.8	41687	1.54	50314	1.28	55234	1.16	61478	1.05	9515	6.74	64157	62034
29-213	Severe (<30)	21.9	45790	1.29	52219	1.13	55599	1.07	58554	1.01	9515	6.23	59238	59638
30-214	Severe (<30)	29.3	56331	1.17	61017	1.08	63406	1.04	65284	1.00	9515	6.91	65750	65916
31-215	Severe (<30)	14.4	23732	1.45	27882	1.24	30325	1.14	33445	1.03	9515	3.62	34459	36227
32-114	Severe (<30)	24.4	39012	1.26	43768	1.12	46504	1.06	48776	1.01	9515	5.17	49167	49905
Geometric Mean		**22.9**	**37256**	**1.27**	**41756**	**1.13**	**44156**	**1.07**	**46541**	**1.01**	**9515**	**4.96**	**47238**	**47570**
CV%		**33.8**	**33.4**	**11.1**	**34.8**	**6.7**	**35.7**	**4.3**	**36.8**	**1.7**	**0.0**	**37.5**	**37.5**	**36.4**

NCA SD Data

Sim3-C Model

811 Pop PK model

Table 5

SubjID	Renal Function Group	BSA-Adj eGFR$_{Cockcroft-Gault}$ (mL/min/1.73m^2)	AUC_{ss} (ng*hr/mL)	$C_{max,SD}$ (ng/mL)	QOD $C_{min,SD}$ (ng/mL)	QOD $C_{max,SS}$ (ng/mL)	QOD $C_{min,SS}$ (ng/mL)	Q3D $C_{min,SD}$ (ng/mL)	Q3D $C_{max,SS}$ (ng/mL)	Q3D $C_{min,SS}$ (ng/mL)	Q4D $C_{min,SD}$ (ng/mL)	Q4D $C_{max,SS}$ (ng/mL)	Q4D $C_{min,SS}$ (ng/mL)	Q7D $C_{min,SD}$ (ng/mL)	Q7D $C_{max,SS}$ (ng/mL)	Q7D $C_{min,SS}$ (ng/mL)
9-102	Moderate (>=30-<60)	35.0	41475	1899	223	2122	260	86.0	1976	89.5	33.0	1927	33.3	1.88	1887	1.83
10-104	Moderate (>=30-<60)	35.3	35716	1967	155	2111	169	48.0	2008	48.9	14.9	1979	14.8	0.446	1967	0.431
5-306	Moderate (>=30-<60)	32.2	19560	882	87.8	971	109	39.1	916	42.8	17.6	89.5	18.1	1.60	883	1.57
25-419	Severe (<30)	18.4	83805	2331	562	3086	876	344	2719	443	211	2545	243	48.7	2375	49.9
26-111	Severe (<30)	17.0	53212	2329	294	2646	368	134	2454	147	61.0	2381	62.9	5.79	2333	5.68
27-331	Severe (<30)	20.6	47769	1953	297	2306	395	150	2105	171	75.6	2022	79.9	9.74	1950	9.63
28-113	Severe (<30)	15.8	64157	1364	436	1923	709	267	1665	364	173	1540	209	48.0	1404	49.9
29-213	Severe (<30)	21.9	59238	1965	379	2404	518	200	2162	232	105	2059	113	15.5	1978	15.4
30-214	Severe (<30)	29.3	65750	3667	315	3999	381	134	3787	143	56.5	3714	57.5	4.31	3639	4.22
31-215	Severe (<30)	14.4	34459	900	229	1201	358	140	1056	181	86.1	986	99.5	20.1	918	20.5
32-114	Severe (<30)	24.4	49167	1729	312	2073	405	152	1873	170	73.8	1795	76.9	8.51	1733	8.39
Geometric Mean		**22.9**	**47238**	**1770**	**270**	**2114**	**358**	**129**	**1929**	**149**	**62.3**	**1501**	**66.5**	**7.01**	**1780**	**6.97**
CV%		**33.8**	**37.5**	**43.2**	**48.4**	**39.2**	**62.1**	**69.6**	**40.5**	**83.1**	**98.6**	**60.5**	**110.7**	**250.4**	**42.5**	**260.9**

Table 6

Group	AUC$_{0-\tau}$ (ng*hr/mL) Geometric mean (log SD) [%CV] (range)	C_{max} (ng/mL) Geometric mean (log SD) [%CV] (range)	C_{trough} (ng/mL) Geometric mean (log SD) [%CV] (range)	C_{48} (ng/mL) Geometric mean (log SD) [%CV] (range)
Normal qod	9117 (0.3) [33.0%] (5480-15382)	1372 (0.34) [34.9%] (783-2595)	5.8 (0.64) [71.6%] (2.1-16.5)	5.8 (0.64) [71.6%] (2.1-16.5)
>40-60 mL/min/1.73m^2 qod	18099 (0.3) [33.7%] (10581-30566)	1694 (0.33) [34.2%] (978-2953)	31.2 (0.64) [70.8%] (10.9-85)	31.2 (0.64) [70.8%] (10.9-85.0)
>35-40 mL/min/1.73m^2 q4d	23270 (0.3) [33.6%] (13618-39906)	1757 (0.34) [35.4%] (998-3125)	10 (0.67) [75.6%] (3.2-30.4)	51.0 (0.55) [59.3%] (20.2-123.4)
>35-40 mL/min/1.73m^2 q1w	23077 (0.3) [31.9%] (8252-61784)	1745 (0.34) [34.5%] (640-4776)	1.05 (0.76) [86.4%] (0.1-10.9)	47.9 (0.51) [55.1%] (9.8-205.4)
>=30-35 mL/min/1.73m^2 q4d	26936 (0.3) [33.7%] (15631-45737)	1820 (0.34) [35.4%] (1044-3195)	14.9 (0.67) [74.9%] (4.9-42.9)	73.7 (0.53) [56.6%] (29.6-166.2)
>=30-35 mL/min/1.73m^2 q1w	26376 (0.3) [32.1%] (8723-73640)	1781 (0.34) [34.5%] (581-4832)	1.63 (0.76) [88.2%] (0.14-20.4)	68.0 (0.49) [52.5%] (14.1-305.1)
>=20-29 mL/min/1.73m^2 q4d	34599 (0.3) [35.5%] (19741-61742)	1905 (0.35) [35.6%] (1084-3420)	30.3 (0.70) [79.1%] (9.5-97.3)	134.7 (0.53) [56.6%] (55.3-318.1)
>=20-29 mL/min/1.73m^2 q1w	34115 (0.3) [33.7%] (19948-59335)	1867 (0.33) [34.1%] (1098-3239)	3.9 (0.82) [98.1%] (1-15)	124.2 (0.49) [52.1%] (54.0-271.4)
<20 mL/min/1.73m^2 q4d	55850 (0.4) [38.3%] (30143-101549)	2105 (0.35) [35.7%] (1189-3709)	107.9 (0.69) [78.5%] (33-320.4)	350.2 (0.49) [52.2%] (151.3-752.8)
<20 mL/min/1.73m^2 q1w	55150 (0.4) [37.1%] (30510-99679)	2002 (0.33) [34.3%] (1152-3505)	19.7 (0.88) [108%] (4.4-80.2)	312.5 (0.45) [47.4%] (143.5-628.0)
<20 mL/min/1.73m^2 q2w	55567 (0.36) [37.4%] (30271-99289)	1987 (0.34) [35.2%] (1132-3468)	0.54 (1.38) [240%] (0.05-4.71)	307.1 (0.43) [45.4%] (145-598)

Table 7

Group	Time above Most Sensitive IC_{50} (hr) Geometric mean (log SD) [%CV] (range)	Time above Least Sensitive IC_{50} (hr) Geometric mean (log SD) [%CV] (range)
Normal qod	12.6 (0.17) [17.4%] (9.6-16.8)	11.6 (0.17) [17.3%] (8.9-15.5)
>40-60 mL/min/1.73m^2 qod	24.9 (0.23) [23.7%] (17.4-37.5)	22.5 (0.23) [23.1%] (15.8-33.3)
>35-40 mL/min/1.73m^2 q4d	31.4 (0.22) [22.2%] (22.5-46.3)	28.2 (0.21) [21.3%] (20.5-40.8)
>35-40 mL/min/1.73m^2 q1w	30.7 (0.20) [19.9%] (18.2-65.6)	27.6 (0.19) [19.1%] (16.7-56.7)
>=30-35 mL/min/1.73m^2 q4d	36.9 (0.23) [23.7%] (25.8-55.3)	33.0 (0.23) [22.9%] (23.4-48.3)
>=30-35 mL/min/1.73m^2 q1w	35.6 (0.21) [21.4%] (20.5-83.9)	31.9 (0.20) [20.6%] (18.2-74.0)
>=20-29 mL/min/1.73m^2 q4d	49.7 (0.28) [28.3%] (32.4-82.9)	44.2 (0.28) [28.1%] (29.1-73.5)
>=20-29 mL/min/1.73m^2 q1w	47.6(0.26) [26.0%] (32.3-74.2)	42.3 (0.25) [25.5%] (29.0-65.3)
<20 mL/min/1.73m^2 q4d	80.4 (0.22) [21.9%] (51.6-95.9)	74.3 (0.25) [25.8%] (45.8-95.9)
<20 mL/min/1.73m^2 q1w	82.6 (0.31) [31.3%] (50.0-137.9)	73.5 (0.31) [31.9%] (44.4-123.9)
<20 mL/min/1.73m^2 q2w	82.1 (0.30) [30.6%] (50.3-132.8)	72.9 (0.30) [31.0%] (44.5-119.5)

[00172] Example 3: Pharmacokinetics of Migalastat in Fabry Patients with Renal Impairment

[00173] The computer modeling above provides scenarios for plasma migalastat exposure, but it does not account for renal impairment in Fabry patients. That is, the data does not include the pharmacodynamic component (plasma lyso-Gb3). Thus, two Fabry patients with renal impairment were evaluated. One patient (P1) had moderate renal impairment, while the other patient (P2) had severe renal impairment. Table 8 below shows plasma migalastat concentration for P1 compared with a phase 3 study by Amicus Therapeutics, Inc. (the FACETS study, Clinical Trial NCT00925301) and moderately impaired subjects from the renal impairment study of Example 1. There are two sets of migalastat concentration measurements taken 6 months apart, and the patient had been previously treated with migalastat. Table 9 shows similar information for P2, except compared with severely impaired patients from the renal impairment study of Example 1. The FACETS study was carried out in Fabry patients with amenable mutations where population PK was performed from sparse blood sampling. The comparison with the results from the FACETS study allows for comparison of PK in the Fabry population with mostly normal, but some mild and a few moderately impaired Fabry patients. None had severe renal impairment because these patients were excluded from the study.

Table 8

Hour Nominal	Time (hr)	Migalastat Conc (ng/mL)	Migalastat Conc 6 months later (ng/mL)	Comparison to FACETS PPK	Comparison to Example 1 Moderate Impairment
0	Pre-dose	19.9	36.4	8.70	64.5 (105.6%)
3	3 Hrs Post	1620	2160	1180 (31.0%)	1868 (29.7%)
24	24 Hrs Post	168	211	-	239 (85.1%)
48	48 Hrs Post	41.8	62.4	8.70	64.5 (105.6%)

Table 9

Hour Nominal	Time Text	Occasion	Migalastat Concentration (ng/mL)	Comparison to FACETS PPK	Comparison to Example 1 Severe Impairment
2	2h	1	564	-	1549 (59.3%)
48	48h	1	322	8.70	334 (38.2%)
24	24h	2	569	-	770 (26.5%)
48	48h	2	260	8.70	334 (38.2%)

[00174] As seen from Table 8, C_{48h} concentration, although increased by 49% over 6 months, remains similar to Example 1 non-Fabry subjects with moderate renal impairment. C_{max} has increased by 33% over 6 months, but remains similar to Example 1. C_{24h} is similar to Example 1 for moderate renal impairment. $eGFR_{MDRD}$ remains within range for moderate impairment as well (32 mL/min).

[00175] The percentages in parentheses are coefficients of variation, which are relatively high, corresponding to variability in the time 0h or time 48h concentrations. This result is likely due to the fact that half of the subjects from Example 1 with moderate renal impairment had low concentrations and half of them high concentrations.

[00176] The concentrations at 48 hours are higher than at 0 hours for P1 (third and fourth columns), but for a person with moderate impairment from Example 1, the concentration at 48 hours is the same as at 0 hours. This is because separate blood samples were taken at times 0 and 48 in P1. However, repeat dose modeling simulation outputs from single dose data were used in Example 1, therefore the values are one in the same.

[00177] Similar trends can be seen from Table 9. Accordingly, Tables 8 and 9 confirm similar pharmacokinetics of migalastat in Fabry and non-Fabry patients having similar renal impairment.

[00178] FIG. 5 shows the Fabry patients' plasma migalastat trough concentrations (C_{48h}) versus the renal impairment study of Example 1. FIG. 6 shows the mean (SD) renal impairment study exposures versus Fabry patient estimated AUCs. As seen from the figure, P1 and P2 followed the general trend of the renal impairment study results in non-Fabry patients.

[00179] Table 10 below shows the Lyso-Gb3/eGFR for P1.

Table 10:

Visits	Lyso-Gb3 (nM/L)	eGFR (MDRD), IDMS Traceable
18 Month Visit	11.1	42
24 Month Visit	13.1	37
30 Month Visit	10.8	Unavailable
34-Month Visit	9.3	32

[00180] Despite continued decline in renal function to eGFR of 32 mL/min/1.73 m^2, plasma lyso-Gb3 has not shown clinically relevant changes from previous visits, and plasma migalastat concentrations remain similar to those observed in non-Fabry patients with moderate renal impairment.

[00181] This study demonstrates that the renal impairment and pharmacokinetic trends in Fabry patients correlates with the trends of non-Fabry patients. Thus, the computer modeling can be relied upon to select an appropriate dosing regimen (*i.e.*, every 2, 4 or 7 days).

[00182] Example 4: Additional Simulations on Renal Impairment Subjects

[00183] This example provides additional computer simulations of migalastat dosing of the renal impairment subjects of Example 1.

[00184] FIGS. 7A-D show simulated median and observed migalastat concentration versus time in normal, severe, mild and moderate renal impairment subjects, respectively. Table 11 below shows the data:

Table 11:

Renal Function Group (CL_{CR} range mL/min), N	**C_{max} [a] (ng/ml)**	**$AUC_{0-\infty}$ [a] (hr*ng/ml)**	**AUC Ratio**	**$t_{1/2}$ [c] (hr)**
Normal (>=90), 8	2270 (37.6)	12808 (31.3)	-	6.2 (1.6)
Mild (>=60-<90), 8	2278 (22.5)	15359 (25.2)	1.2	8.0 (2.8)
Moderate (>=30-<60), 8	2058 (47.1)	23897 (38.9)	1.9	23.0 (13.3)
Severe (<30), 4	2122 (29.1)	61208 (23.1)	4.8	32.5 (2.4)

[a] Geometric mean (CV%)

[c] Mean (SD)

[00185] FIGS. 8A-D show C_{max}, AUC, C_{min} and C_{48h}, respectively, for normal, mild, moderate and severe renal impairment subjects.

[00186] FIGS. 9A-D show the steady state prediction for QOD. The dashed line is the mean value from the QT study. FIGS. 10A-D show C_{max}, AUC, C_{min} and C_{48h}, respectively for the same simulation.

[00187] FIGS. 11A-B compare migalastat concentration after administration of 100 mg migalastat over 96 hours in a patient with moderate renal impairment to administration of 150 mg migalastat over 48 hours in a patient with normal kidney function. FIGS. 12A-D compare the C_{max}, AUC, C_{min} and C_{48h}, respectively, for the same simulation.

[00188] Example 5: Proposed Study for Evaluation of Safety, Pharmacokinetics and Pharmacodynamics of Migalastat HCl in Fabry Patients with Amenable Mutations and Severe Renal Impairment

[00189] A study is proposed to evaluate the safety, pharmacokinetics and pharmacodynamics of migalastat HCl in Fabry subjects with amenable mutations and severe renal impairment (i.e., eGFR < 30 mL/min/1.73 m^2). Instead of lowering the dosage (i.e., less than 150 mg) a dose of 150 mg of migalastat HCl was maintained but administered less frequently. Subjects with $eGFR_{MDRD}$ greater than or equal to 10 and less than 20 will receive the dose every 7 days (Q7D). Subjects with $eGFR_{MDRD}$ greater than or equal to 20 and less than 30 will receive the dose every 4 days (Q4D). If a subject receiving the Q4D dose has a renal function which declines below 20 mL/min/1.73 m^2, the subject's dosing regimen is changed to Q7D. Any subject who begins dialysis treatment or undergoes renal transplantation will be discontinued from the study.

[00190] All subjects entering in this study will undergo screening (Visit 1) to confirm enrollment eligibility. Subjects who meet eligibility criteria will have a Baseline Visit (Visit 2) within 30 days of screening, including PK assessments. On-study visits will be scheduled every 3 months for a total of 12 months. Based on the PK/PD results from each site visit, as needed a follow-up visit or phone contact will be scheduled 1 month later. If PK/PD results indicate that a change in dose regimen is warranted, the subject will be advised to adjust the duration between doses and laboratory assessments will be done either locally or at the site.

[00191] Safety Analysis

[00192] There will be continuous monitoring of safety data and specific stopping criteria will be established for discontinuation of subjects who show evidence of declining renal

function. Subjects with an eGFR < 10 mL/min/1.73 m^2 on 2 consecutive visits will be discontinued from migalastat and withdrawn from the study.

[00193] Pharmacokinetic Sampling

[00194] Full PK blood sampling will be conducted at Visit 2 according to each subject's starting migalastat regimen. Subjects starting at a Q4D regimen will have PK assessments conducted predose and at 1, 2, 3, 4, 6, 8, 12, 24, 48, and 96 hours postdose. Subjects starting at a Q7D regimen will have PK assessments conducted predose and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, and 168 hours postdose. At subsequent visits, subjects will undergo sparse sampling at 24, 48, and 96 hours postdose for subjects on Q4D regimen and at 24, 48, 96, and 168 hours for subjects on Q7D regimen.

[00195] At Visit 2, spot urine collections will be taken within 1 hour before dosing followed by a postdose total urine collection for the duration of each dosing interval at 0 to 4 hours, 4 to 8 hours, 8 to 12 hours, 12 to 24 hours, 24 to 48 hours, 48 to 72 hours, and 72 to 96 hours for subjects on a Q4D regimen. Collection intervals will be the same for subjects on a Q7D regimen with the addition of collections at 96 to 120 hours, 120 to 144 hours, and 144 to 168 hours.

[00196] For subjects with a dose regimen change, full PK blood and urine collections as detailed above will be done at the visit following the regimen change.

[00197] Dose Regimen Modifications

[00198] This protocol allows dose regimen changes on a subject-specific basis. Starting dose for each subject will be migalastat HCl 150 mg at a regimen based on eGFR, as noted above. For subjects who begin the study on a Q4D regimen, a decrease in eGFR to < 20 mL/min/1.73 m^2 at 2 consecutive visits (including follow-up visits) automatically will trigger a switch to the Q7D regimen.

[00199] Plasma lyso-Gb3 will be monitored at each visit. If a subject has an increase in plasma lyso-Gb3 relative to their previous measurement, a confirmatory retest will take place. If the confirmatory value is also higher than their previous measurement, the average of the 2 values will be the subject's new reference value. If the retest is not higher than the subject's previous measurement, there will be no new reference value at that visit.

[00200] Throughout the study, stopping criteria will be applied on a per-subject basis. Subjects who have $eGFR_{MDRD}$ < 10 mL/min/1.73 m^2 for 2 consecutive visits or who undergo a

dialysis or renal transplant will be discontinued from treatment. Subjects may also be discontinued from treatment at the discretion of the investigator and a medical monitor.

[00201] Duration of Study Treatment

[00202] Following a screening period of up to 30 days, enrolled subjects will receive migalastat treatment for 12 months. At the end of 12 months, subjects may be eligible to enroll in a separate open-label extension study.

[00203] Criteria for Evaluation

[00204] Safety: Safety parameters include physical examinations, vital signs (blood pressure, heart rate, respiratory rate, and body temperature), 12-lead electrocardiograms, clinical laboratory parameters (serum chemistry, hematology, and urinalysis), eGFR, and adverse events.

[00205] PK: The following PK parameters will be calculated, if available, based on the plasma concentrations of migalastat: maximum observed concentration (C_{max}), concentration at the end of a dosing interval at steady state (C_{trough}), average plasma migalastat concentration over the dosing interval (C_{avg}), time to maximum concentration (t_{max}), apparent terminal elimination half-life ($t_{1/2}$), area under the concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) and extrapolated to infinity ($AUC_{0-\infty}$), and plasma clearance (CL/F).

[00206] The following PK parameters will be calculated based on urine migalastat concentrations: total amount excreted over the dosing interval (Ae_{0-t}), fraction of the dose recovered in urine over the dosing interval (Fe), and renal clearance (CLr).

[00207] PD: PD parameters include plasma lyso-Gb3, $eGFR_{MDRD}$, and $eGFR_{CKD-EPI}$.

[00208] Statistical Methods

[00209] Plasma migalastat concentrations from serial PK blood and urine sampling will be determined by noncompartmental analysis using Phoenix®-WinNonlin® software, version 7.0 or higher. Plasma migalastat sparse PK blood sampling will be analyzed by a Population PK model. The Population PK model will assess and validate severe renal impairment dose regimen simulations, and will be provided as a separate report. PK/PD modeling may be explored.

[00210] Continuous PD and safety data will be summarized using descriptive statistics (number, mean, median, minimum, and maximum). Categorical variables will be presented by number (%).

[00211] Example 6: Pharmacokinetics of Migalastat HCl in a Fabry Patient with Severe Renal Impairment

[00212] A patient (P3) was enrolled in a previous migalastat study, but discontinued in May 2016 as a result of sever renal impairment (i.e. eGFR <30 mL/min/1.73m²). Beginning in May 2017, P3 was dosed with migalastat HCl Q4D. PK data was collected every three months. Table 12 shows P3's PK data compared to patients dosed QOD with varying levels of renal function.

Table 12:

QOD Regimen PK in AT1001-015, -011 vs Q4D Regimen in Severe RI Patient 2004-5029									
Renal Function Group (CL_{cr} range mL/min), N	C_{max}[a] (ng/mL)	t_{max}[b] (hr)	C_{48h}[c] (ng/mL)	C_{96h}[c] (ng/mL)	AUC_{0-t}[a] (hr*ng/mL)	$AUC_{0-\infty}$[a] (hr*ng/mL)	$t_{½}$[c] (hr)	CL/F[c] (L/hr)	F_{rel}
Normal (≥90), 8	2100 (26.1)	2.5 (1.5 - 3.0)	5.70 (18.9)	BLQ	12299 (25.0)	12389 (24.8)	6.4 (29.7)	12.5 (30.4)	-
Mild (≥60-<90), 8	2191 (27.5)	2.5 (1.5 - 4.0)	9.34 (62.1)	BLQ	14374 (31.1)	14520 (30.8)	7.7 (39.0)	10.7 (29.0)	1.2
Moderate (≥30-<60), 8	1868 (29.7)	3.0 (1.5 - 4.0)	64.5 (105.6)	16.7 (65.3)	22122 (41.4)	22455 (41.2)	22.2 (64.0)	7.16 (39.1)	1.8
Severe (≥15-<30), 8	2078 (43.3)	4.0 (3.0 - 8.0)	334 (37.7)	105 (37.4)	53098 (25.3)	56174 (24.2)	32.3 (22.9)	2.74 (25.2)	4.5
011 PPK (≥30-≥90), 62	1180 (32.9)	3.0 (2.0 - 4.5)	7.54 (84.3)	-	9033 (35.1)	-	20.6 (19.2)	17.1 (54.8)	-
2004-5029 (21), 5/17	2250	2.0	77.4	18.4	31870	32101	13.2	4.67	2.6
2004-5029 (19), 8/17	N/A	N/A	130	61.3	N/A	N/A	N/A	N/A	N/A
2004-5029 (15), 11/17	N/A	N/A	401	86.2	N/A	N/A	N/A	N/A	N/A

[a] Geometric mean (CV%)
[b] Median (min - max)
[c] Mean (CV%)
N/A = not available

[00213] Table 13 shows the plasma concentration of migalastat for P3 after dosing at various time points.

Table 13:

	Plasma Migalastat Conc (ng/mL)					
Time (h)	Mar '11	Feb '15	Aug '15	May '17	Aug '17	Nov '17
0	38.1	19.9	36.4	BLQ	-	-
3	1560	1620	2160	2110	-	-
4	1570	-	-	1970	-	-
8	802	-	-	1380	-	-
24	-	168	211	358	-	-
48	-	41.8	62.4	77.4	130	401
96	-	-	-	18.4	61.3	86.2
eGFR(mL/min/1.73m^2)	59	N/A	32	21	19	15

[00214] Table 14 shows the Lyso-Gb3 and eGFR of P3 over time.

Table 14:

Visit Date	Migalastat Dosing	Lyso-Gb3 (nmol/L)	eGFR (MDRD),

	Frequency		IDMS Traceable
September 2010	QOD	27.30	54
March 2011	QOD	24.15	59
February 2014	QOD	11.10	42
August 2014	QOD	13.10	37
February 2015	QOD	10.80	---
June 2015	QOD	9.30	32
September 2015	QOD	7.36	33
March 2016	QOD	8.46	28
May 2017	Q4D	13.10	21
August 2017	Q4D	9.85	19
November 2017	Q4D	8.55	15
January 2018	Q7D	9.04	13
February 2018	Q7D	7.04	13

[00215] FIG. 15 shows the Lyso-Gb3 and eGFR of P3 over time.

[00216] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

[00217] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.