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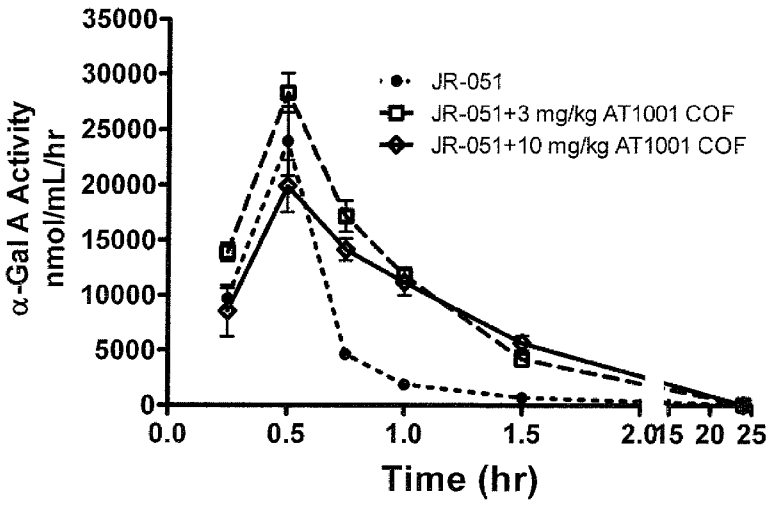
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(54) Title: ALPHA-GALACTOSIDASE A AND 1-DEOXYGALACTONOJIRIMYCIN CO-FORMULATION

Figure 1.



(57) Abstract: The present application provides for compositions comprising α-galactosidase A in combination with an active site-specific chaperone for the α-galactosidase A, and methods for treating Fabry disease in a subject in need thereof, that includes a method of administering to the subject such compositions. The present application also provides methods for increasing the in vitro and in vivo stability of an α-galactosidase A enzyme formulation.

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ALPHA-GALACTOSIDASE A AND 1-DEOXYGALACTONOJIRIMYCIN CO-FORMULATION

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 61/672,566 filed on July 17, 2012, which is incorporated by reference herein in its entirety.

1. INTRODUCTION

The present application relates to compositions comprising a co-formulation of an α-galactosidase A enzyme and 1-deoxygalactonojirimycin, and to methods of treating, preventing, and/or ameliorating Fabry Disease by administering such co-formulations to a subject in need of treatment. The present application also relates to compositions and medicaments which can be used in the treatment of Fabry Disease.

2. BACKGROUND

Fabry disease is a progressive, X-linked inborn error of glycospingolipid metabolism caused by a deficiency in the lysosomal enzyme α-galactosidase A (α-Gal A) as a result of mutations in the α-Gal A gene (GLA). Despite being an X-linked disorder, females can express varying degrees of clinical manifestations. Fabry is a rare disease with incidence estimated between 1 in 40,000 males to 1 in 117,000 in the general population. Moreover, there are variants of later-onset phenotype of Fabry disease that can be under-diagnosed, as they do not present with classical signs and symptoms. This, and the study of newborn screening for Fabry disease, suggests that the actual incidence of Fabry disease can be higher than currently estimated.

Clinical manifestation of the disease can correlate with residual α-Gal A levels. Untreated, life expectancy in Fabry patients is reduced and death usually occurs in the fourth or fifth decade because of vascular disease affecting the kidneys, heart and/or central nervous system. The enzyme deficiency leads to intracellular accumulation of the substrate, globotriaosylceramide (GL-3) in the vascular endothelium and visceral tissues throughout the body. Gradual deterioration of renal

function and the development of azotemia, due to glycospingolipid deposition, usually occur in the third to fifth decades of life, but can occur as early as in the second decade. Renal lesions are found in both hemizygous (male) and heterozygous (female) patients.

Cardiac disease occurs in most males and many females. Early cardiac findings include left ventricular enlargement, valvular involvement and conduction abnormalities. Mitral insufficiency is the most frequent valvular lesion typically present in childhood or adolescence. Cerebrovascular manifestations result primarily from multifocal small-vessel involvement and can include thromboses, transient ischemic attacks, basilar artery ischemia and aneurysm, seizures, hemiplegia, hemianesthesia, aphasia, labyrinthine disorders, or cerebral hemorrhages. Average age of onset of cerebrovascular manifestations is 33.8 years. Personality change and psychotic behavior can manifest with increasing age.

The current approved treatment for Fabry disease is enzyme replacement therapy ("ERT"). Two α-Gal A products are currently available for the treatment of Fabry disease: agalsidase alfa (Replagal®, Shire Human Genetic Therapies) and agalsidase beta (Fabrazyme®; Genzyme Corporation). These two forms of ERT are intended to compensate for a patient's inadequate α-Gal A activity with a recombinant form of the enzyme, administered intravenously. While ERT is effective in many settings, the treatment also has limitations. ERT has not been demonstrated to decrease the risk of stroke, cardiac muscle responds slowly, and GL-3 elimination from some of the cell types of the kidneys is limited. Some patients develop immune reactions to ERT.

1-deoxygalactonojirimycin and its salt, 1-deoxygalactonojirimycin hydrochloride (also known by its United States Adopted Name (USAN), migalastat hydrochloride) acts as a pharmacological chaperone for mutant α-Gal A by selectively binding to the enzyme, thereby increasing its stability and helping the enzyme fold into its correct three-dimensional shape. This stabilization of α-Gal A allows the cell's quality control mechanisms to recognize the enzyme as properly folded so that trafficking of the enzyme to the lysosome is increased, allowing it to carry out its intended biological function, the metabolism of GL-3. As a result of restoring the proper trafficking of α-Gal A from the ER to the lysosome, migalastat hydrochloride also reduces the accumulation of misfolded protein in the ER, which can alleviate stress on cells and some inflammatory-like responses that can be contributing factors

in Fabry disease. Multiple in vitro and in vivo preclinical studies, as well as clinical studies, of migalastat hydrochloride have been conducted. Migalastat hydrochloride has been shown to increase the amount of intracellular α-Gal A protein and to enhance transport of mutant enzyme to the lysosome.

3. SUMMARY OF THE APPLICATION

The present application relates to compositions for the treatment of Fabry Disease comprising an α-galactosidase A (α-Gal A) enzyme (*e.g.*, a recombinant human α-Gal A (rhα-Gal A)) in combination with an Active Site-Specific Chaperone (ASSC) for the α-Gal A enzyme (*e.g.*, 1-deoxygalactonojirimycin (DGJ)).

The present application also relates to methods for the treatment of Fabry Disease, by administering to a subject in need of such treatment an α-Gal A enzyme, in combination with an Active Site-Specific Chaperone (ASSC) for the α-Gal A enzyme (*e.g.*, 1-deoxygalactonojirimycin (DGJ)).

The present application also provides a combined formulation of an α-Gal A enzyme and an Active Site-Specific Chaperone (ASSC) for the α-Gal A enzyme (*e.g.*, 1-deoxygalactonojirimycin (DGJ)) for use in the treatment of Fabry disease.

The present application also provides the use of an α-Gal A enzyme and an Active Site-Specific Chaperone (ASSC) for the α-Gal A enzyme (*e.g.*, 1-deoxygalactonojirimycin (DGJ)) in the preparation of a medicament for the treatment of Fabry disease, wherein the treatment comprises administering a composition comprising a co-formulation of the α-Gal A enzyme and the ASSC for the α-Gal A enzyme to a subject in need thereof.

In a particular embodiment, the 1-deoxygalactonojirimycin is 1-deoxygalactonojirimycin-hydrochloride (DGJ-HCl), also known as migalastat hydrochloride. In one embodiment, the α-Gal A enzyme is agalsidase alfa or agalsidase beta.

In certain embodiments, the α-Gal A enzyme is combined with DGJ to create a co-formulation prior to administration to a subject.

In certain embodiments, the co-formulation of an α-Gal A enzyme and an Active Site-Specific Chaperone (ASSC) for the α-Gal A enzyme (*e.g.*, 1-deoxygalactonojirimycin (DGJ)) is presented for parenteral administration. In one embodiment administration is by single or repeated intravenous bolus injections of the co-formulation. In one embodiment administration is by intravenous infusion of the co-formulation.

In certain embodiments, the α-Gal A enzyme is combined with DGJ at a concentration of between about 0.5 and about 20 μM. In certain embodiments, the α-Gal A enzyme is combined with DGJ at a concentration of about 1.2, 2, 4, 8, or 12 μM.

In certain embodiments, DGJ is combined with the α-Gal A enzyme at a concentration of between about 50 and about 20,000 μM. In certain embodiments, DGJ is combined with the α-Gal A enzyme at a concentration of about 449, 748, 1,495, 4,490, or 14,950 μM.

In certain embodiments, the compositions of the application comprise about 1.2 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the compositions of the application comprise about 1.2 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the compositions of the application comprise about 1.2 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the compositions of the application comprise about 1.2 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the compositions of the application comprise about 1.2 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the compositions of the application comprise about 2 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the compositions of the application comprise about 2 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the compositions of the application comprise about 2 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the compositions of the application comprise about 2 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the compositions of the application comprise about 2 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the compositions of the application comprise about 4 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the compositions of the application comprise about 4 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the compositions of the application comprise about 4 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the compositions of the application comprise about 4 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the compositions of the application comprise about 4 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the compositions of the application comprise about 8 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the compositions of the application comprise about 8 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the compositions of the application comprise about 8 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the compositions of the application comprise about 8 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the compositions of the application comprise about 8 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the compositions of the application comprise about 12 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the compositions of the application comprise about 12 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the compositions of the application comprise about 12 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the compositions of the application comprise about 12 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the compositions of the application comprise about 12 μM α-Gal A enzyme and about 14,950 μM DGJ.

The present invention also provides the use of a co-formulation of between about 0.5 and about 20 μM α-Gal A and between about 50 and about 20,000 μM DGJ or a pharmaceutically acceptable salt thereof in the preparation of a

medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject.

The present invention also provides the use of a co-formulation of about 1.2, 2, 4, 8, or 12 μM α-Gal A and about 449, 748, 1,495, 4,490, or 14,950 μM DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject.

The present invention also provides a co-formulation of between about 0.5 and about 20 μM α-Gal A and between about 50 and about 20,000 μM DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally.

The present invention also provides a co-formulation of about 1.2, 2, 4, 8, or 12 μM α-Gal A and about 449, 748, 1,495, 4,490, or 14,950 μM DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation with a molar ratio of DGJ to α-Gal A enzyme of between about 13,000:1 and about 50:1, or between about 10,000:1 and about 75:1, or between about 1,000:1 and about 100:1.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme of the co-formulation administered to the subject is between about 0.1 and about 5 mg/kg. In certain embodiments, the dosage of α-Gal A enzyme of the co-formulation administered to the subject is about 0.3, 0.5, 1, 2 or 3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of DGJ of the co-formulation administered to the subject is between about 0.1 and about 15 mg/kg. In certain embodiments, the dosage of DGJ of the co-formulation administered to the subject is about 0.1, 0.3, 0.5, 1, 3, or 10 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.3 mg/kg, and the dosage of DGJ administered to the subject is about 0.1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.3 mg/kg, and the dosage of DGJ administered to the subject is about 0.3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.3 mg/kg, and the dosage of DGJ administered to the subject is about 0.5 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.3 mg/kg, and the dosage of DGJ administered to the subject is about 1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.3 mg/kg, and the dosage of DGJ administered to the subject is about 3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.3 mg/kg, and the dosage of DGJ administered to the subject is about 10 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.5 mg/kg, and the dosage of DGJ administered to the subject is about 0.1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.5 mg/kg, and the dosage of DGJ administered to the subject is about 0.3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.5 mg/kg, and the dosage of DGJ administered to the subject is about 0.5 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A

enzyme administered to the subject is about 0.5 mg/kg, and the dosage of DGJ administered to the subject is about 1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.5 mg/kg, and the dosage of DGJ administered to the subject is about 3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 0.5 mg/kg, and the dosage of DGJ administered to the subject is about 10 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 1 mg/kg, and the dosage of DGJ administered to the subject is about 0.1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 1 mg/kg, and the dosage of DGJ administered to the subject is about 0.3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 1 mg/kg, and the dosage of DGJ administered to the subject is about 0.5 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 1 mg/kg, and the dosage of DGJ administered to the subject is about 1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 1 mg/kg, and the dosage of DGJ administered to the subject is about 3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 1 mg/kg, and the dosage of DGJ administered to the subject is about 10 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 2 mg/kg, and the dosage of DGJ administered to the subject is about 0.1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 2 mg/kg, and the dosage of DGJ administered to the subject is about 0.3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 2 mg/kg, and the dosage of DGJ administered to the subject is about 0.5 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 2 mg/kg, and the dosage of DGJ administered to the subject is about 1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 2 mg/kg, and the dosage of DGJ administered to the subject is about 3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 2 mg/kg, and the dosage of DGJ administered to the subject is about 10 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 3 mg/kg, and the dosage of DGJ administered to the subject is about 0.1 mg/kg. In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 3 mg/kg, and the dosage of DGJ administered to the subject is about 0.3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A

enzyme administered to the subject is about 3 mg/kg, and the dosage of DGJ administered to the subject is about 0.5 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 3 mg/kg, and the dosage of DGJ administered to the subject is about 1 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 3 mg/kg, and the dosage of DGJ administered to the subject is about 3 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme administered to the subject is about 3 mg/kg, and the dosage of DGJ administered to the subject is about 10 mg/kg.

The present invention also provides the use of a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject, and wherein the α-Gal A of the co-formulation is formulated for administration in an amount between about 0.1 and about 0.5 mg/kg, and the DGJ of the co-formulation is formulated for administration in an amount between about 0.1 and about 15 mg/kg.

The present invention also provides the use of a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject, and wherein the α-Gal A of the co-formulation is formulated for administration in an amount of about 0.3, 0.5, 1, 2 or 3 mg/kg, and the DGJ of the co-formulation is formulated for administration in an amount of about 0.1, 0.3, 0.5, 1, 3, or 10 mg/kg.

The present invention also provides a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally, and wherein the α-Gal A of the co-formulation is administered in an amount between about 0.1 and about 0.5 mg/kg, and the DGJ of the co-formulation is administered in an amount between about 0.1 and about 15 mg/kg.

The present invention also provides a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally, and wherein the α-Gal A of the co-formulation is administered in an amount of about 0.3, 0.5, 1, 2 or 3 mg/kg, and the DGJ of the co-formulation is administered in an amount of about 0.1, 0.3, 0.5, 1, 3, or 10 mg/kg.

According to one aspect of the application, methods of enhancing delivery of α-Gal A to tissues, for example heart, kidney or skin tissue, of an individual with Fabry disease are provided. The methods include administering α-Gal A in combination with an ASSC intravenously to the individual, wherein the α-Gal A and ASSC are present in a co-formulation. In some embodiments, the α-Gal A in combination with an ASSC is administered in a sufficient dose to result in a peak concentration of α-Gal A in plasma and/or tissues of the subject within about 24 hours after the administration of the dose. In certain embodiments, the α-Gal A in combination with an ASSC is administered in a sufficient dose to result in a peak concentration of α-Gal A in plasma and/or tissues of the subject within about 0.2 to about 50 hours, or about 40, 30, 20, 10, 5, 1, 0.5 or fewer hours after the administration of the dose. In some embodiments, the dose does not result in a toxic level of α-Gal A in the liver of the individual.

The present application further provides a method for increasing the stability of an α-Gal A enzyme in a proper conformation, *in vivo* and *in vitro*. In one embodiment, an α-Gal A enzyme (*e.g.*, a recombinant human α-Gal A (rhα-Gal A)) in combination with an ASSC for the α-Gal A enzyme (*e.g.*, 1-deoxygalactonojirimycin) is administered to an individual in need of such treatment. The α-Gal A enzyme is stabilized conformationally when combined with an ASSC and is well- suited to withstand, for example, thermal and pH challenges.

In certain embodiments, the co-formulation compositions of the present application are stable at a pH equal to or greater than about pH 5, 6 or 7.

In certain embodiments, the co-formulations of the application maintain physical and chemical stability over extended periods, and have a viscosity suitable for intravenous administration.

In various non-limiting embodiments, the ASSC for the α-Gal A enzyme is a small molecule inhibitor of the α-Gal A enzyme, including reversible competitive inhibitors of the α-Gal A enzyme.

In one particular non-limiting embodiment, the ASSC is 1-deoxygalactonojirimycin (DGJ), or a pharmaceutically acceptable salt, ester or prodrug of 1-deoxygalactonojirimycin. In one embodiment, the salt is hydrochloride salt (i.e., 1-deoxygalactonojirimycin-HCl).

In one particular non-limiting embodiment, a combined formulation of recombinant human α-Gal A (rhα-Gal A)) with 1-deoxygalactonojirimycin-HCl is provided for intravenous administration to a patient in need thereof at a dose of 0.3 mg/kg rhα-Gal A and 0.1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment, a combined formulation of recombinant human α-Gal A (rhα-Gal A)) with 1-deoxygalactonojirimycin-HCl is provided for intravenous administration to a patient in need thereof at a dose of 0.3 mg/kg rhα-Gal A and 0.3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.3 mg/kg rhα-Gal A and 0.5 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.3 mg/kg rhα-Gal A and 1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.3 mg/kg rhα-Gal A and 3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.3 mg/kg rhα-Gal A and 10 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.5 mg/kg rhα-Gal A and 0.1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.5 mg/kg rhα-Gal A and 0.3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.5 mg/kg rhα-Gal A and 0.5 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.5 mg/kg rhα-Gal A and 1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.5 mg/kg rhα-Gal A and 3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 0.5 mg/kg rhα-Gal A and 10 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 1 mg/kg rhα-Gal A and 0.1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 1 mg/kg rhα-Gal A and 0.3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 1 mg/kg rhα-Gal A and 0.5 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 1 mg/kg rhα-Gal A and 1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 1 mg/kg rhα-Gal A and 3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 1 mg/kg rhα-Gal A and 10 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 2 mg/kg rhα-Gal A and 0.1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 2 mg/kg rhα-Gal A and 0.3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 2 mg/kg rhα-Gal A and 0.5 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 2 mg/kg rhα-Gal A and 1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 2 mg/kg rhα-Gal A and 3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 2 mg/kg rhα-Gal A and 10 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 3 mg/kg rhα-Gal A and 0.1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 3 mg/kg rhα-Gal A and 0.3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 3 mg/kg rhα-Gal A and 0.5 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 3 mg/kg rhα-Gal A and 1 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 3 mg/kg rhα-Gal A and 3 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

In a further embodiment the co-formulation is provided for intravenous administration to a patient in need thereof at a dose of 3 mg/kg rhα-Gal A and 10 mg/kg 1-deoxygalactonojirimycin (as the HCl salt).

The present invention also provides the use of a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for intravenous administration to a subject, and wherein the α-Gal A of the co-

formulation is formulated for administration in an amount of about 0.3, 0.5, 1, 2 or 3 mg/kg, and the DGJ (as the HCl salt) of the co-formulation is formulated for administration in an amount of about 0.1, 0.3, 0.5, 1, 3, or 10 mg/kg.

The present invention also provides a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered intravenously, and wherein the α-Gal A of the co-formulation is administered in an amount of about 0.3, 0.5, 1, 2 or 3 mg/kg, and the DGJ (as the HCl salt) of the co-formulation is administered in an amount of about 0.1, 0.3, 0.5, 1, 3, or 10 mg/kg.

The present application also provides a kit for treating Fabry disease in a subject, the kit comprising from between about 0.5 and 20 μM of an α-Gal A enzyme, and from between about 50 and about 20,000 μM of 1-deoxygalactonojirimycin.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows plasma PK of α-Gal A (JR-051) in WT mice following IV infusion administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 1. (DGJ or 1-deoxygalactonojirimycin is also referred to as "AT1001"). The co-formulated composition is referred to as "COF" in the graph.

Figure 2 shows the level of plasma α-Gal A (JR-051) as measured by Western blot in WT mice following IV infusion administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 1. "+": 1 mg/kg JR-051; "-": no compound (either JR-051, or DGJ, or both).

Figure 3A-B shows tissue uptake of α-Gal A (JR-051) in (A) kidney and (B) heart tissue in WT mice following IV infusion administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 1.

Figure 4 shows plasma PK of α-Gal A (JR-051) in GLA KO mice following IV infusion administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 2. The co-formulated composition is referred to as "COF" in the graph.

Figure 5A-C shows GL-3 reduction (A) and (C) and tissue uptake (B) of α-Gal A (JR-051) in heart tissue of GLA KO mice following IV infusion

administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 2.

Figure 6 shows GL-3 reduction (% reduction) in heart tissue of GLA KO mice following IV infusion administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 2.

Figure 7A-C shows GL-3 reduction (A) and (C) and tissue uptake (B) of α-Gal A (JR-051) in kidney tissue and of GLA KO mice following IV infusion administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 2.

Figure 8 shows GL-3 reduction (% reduction) in kidney tissue of GLA KO mice following IV infusion administration of α-Gal A or a co-formulation of α-Gal A and DGJ-HCl, as described in Example 2.

Figure 9A-B shows the stability of α-Gal A formulated with or without DGJ in human whole blood *ex vivo*. Co-formulating α-Gal A (JR-051) with DGJ-HCL increases the stability of the enzyme in blood, and prevents loss of enzyme activity.

Figure 10A-H shows α-Gal A uptake (A-D) and GL-3 reduction (E-H) in Fabry patient derived fibroblast cells. The fibroblast cells expressed the R301Q (A, C, E and G) or C52S (B, D, F and H) mutations. Cells were cultured with α-Gal A (JR-051 or Fabrazyme) formulated alone or with 0.1 μM, 1.0 μM, 10 μM or 100 μM DGJ.

Figure 11A-C shows GL-3 % reduction in skin, heart and kidney following administration of α-Gal A alone or as a co-formulation with DGJ to GLA KO mice, as described in Example 3. Data normalized to GL-3 levels in untreated KO and WT mouse tissues; % reduction = 100% - % of untreated.

5. DETAILED DESCRIPTION

For clarity and not by way of limitation, this detailed description is divided into the following sub-portions:

(i) Definitions;

(ii) Enzyme Replacement Therapy;

(iii) Pharmaceutical Compositions;

(iv) Treatment of Fabry Disease with ERT and an ASSC;

(v) *In Vitro* Stability; and

(vi) *In Vivo* Stability.

5.1 Definitions

The terms used in this specification generally have their ordinary meanings in the art, within the context of this application 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 application and how to make and use them.

The terms "Fabry disease" refers to classical Fabry disease, late-onset Fabry disease, and hemizygous females having mutations in the gene encoding α-galactosidase A (α-Gal A). The term "Fabry disease," as used herein, further includes any condition in which a subject exhibits lower than normal endogenous α-Gal A activity.

"1-deoxygalactonojirimycin" (DGJ) refers to (2R,3S,4R,5S)-2-(hydroxymethyl) piperdine-3,4,5-triol. As used herein, reference to "1-deoxygalactonojirimycin" or "DGJ" or "AT1001" throughout includes both the free base and any pharmaceutically acceptable salt forms of the same. The hydrochloride salt of DGJ is known as migalastat hydrochloride.

The terms "α-galactosidase A" or "α-Gal A" refer to a protein with enzymatic activity comprising hydrolysis of terminal, non-reducing α-D-galactose residues in α-D-galactosides, including galactose oligosaccharides, galactomannans and galactolipids. In certain embodiments, α-Gal A comprises the enzyme described by IUBMB Enzyme Nomenclature EC 3.2.1.22 (*see. e.g.*, http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/22.html; Suzuki et al., α-Galactosidase from *Mortierella vinacea*. Crystallization and properties. J. Biol. Chem. 245 (1970) 781-786; and Wiederschain, G. and Beyer, E. [Interrelation of α-D-fucosidase and α-D-galactosidase activities in man and animals]. Dokl. Akad. Nauk S.S.S.R. 231 (1976) 486-488.). In certain embodiments, α-Gal A comprises a protein encoded by a nucleic acid comprising the human *GLA* gene, for example, the human

GLA gene defined by GenBank Accession No. NM_000169. In certain embodiments, α-Gal A comprises a protein comprising the amino acid sequence defined by GenBank Accession No. NP_000160.

In certain embodiments, α-Gal A may be obtained from a cell endogenously expressing the α-Gal A, or the α-Gal A may be a recombinant human α-Gal A (rhα-Gal A), as described herein. In one, non-limiting embodiment, the rhα-Gal A is a full length wild-type α-Gal A. In other non-limiting embodiments, the rhα-Gal A comprises a subset of the amino acid residues present in a wild-type α-Gal A, wherein the subset includes the amino acid residues of the wild-type α-Gal A that form the active site for substrate binding and/or substrate reduction. As such, the present invention contemplates an rhα-Gal A that is a fusion protein comprising the wild-type α-Gal A active site for substrate binding and/or substrate reduction, as well as other amino acid residues that may or may not be present in the wild type α-Gal A.

α-Gal A may be obtained from commercial sources or may be obtained by synthesis techniques known to a person of ordinary skill in the art. The wild-type enzyme can be purified from a recombinant cellular expression system (e.g., mammalian cells such as CHO cells, or insect cells-see generally U.S. Pat. No. 5,580,757 to Desnick et al.; U.S. Pat. Nos. 6,395,884 and 6,458,574 to Selden et al.; U.S. Pat. No. 6,461,609 to Calhoun et al.; U.S. Pat. No. 6,210,666 to Miyamura et al.; and U.S. Pat. No. 6,083,725 to Selden et al.), human placenta, or animal milk.

Other synthesis techniques for obtaining α-Gal A suitable for pharmaceutical use can be found, for example, in U.S. Patent No. 7,560,424 and U.S. Patent No. 7,396,811 to Lebowitz et al., U.S. Published Application Nos. 2009/0203575, 2009/0029467, 2008/0299640, 2008/0241118, 2006/0121018, and 2005/0244400 to Lebowitz et al., U.S. Patent Nos. 7,423,135, 6,534,300, and 6,537,785; International Published Application No. 2005/077093 and U.S. Published Application Nos. 2007/0280925, and 2004/0029779. These references are hereby incorporated by reference in their entirety.

In certain embodiments, the α-Gal A is Agalsidase alpha, produced by genetic engineering technology in a human cell line. Agalsidase alpha is available as Replagal®, from Shire Plc. (Dublin, Ireland).

In certain embodiments, the α-Gal A is Agalsidase beta, produced by recombinant DNA technology in a Chinese hamster ovary (CHO) cell line.

Agalsidase beta is available as Fabrazyme®, from Genzyme Corporation (Cambridge, MA).

In certain embodiments, the α-Gal A is a recombinant human α-Gal A produced in CHO sells transformed with an expression vector encoding the human α-Gal A gene (JCR Pharmaceuticals Co. Ltd, (Japan)), identifed as JR-051.

In addition to proteins that comprise an amino acid sequence that is identical to the human α-Gal A proteins described herein, this disclosure also encompasses α-Gal A proteins that are "substantially similar" thereto. Proteins described herein as being "substantially similar" to a reference protein include proteins that retain certain structural and functional features of the native proteins yet differ from the native amino acid sequence at one or more amino acid positions (*i.e.*, by amino acid substitutions).

Proteins altered from the native sequence can be prepared by substituting amino acid residues within a native protein and selecting proteins with the desired activity. For example, amino acid residues of an α-Gal A protein can be systematically substituted with other residues and the substituted proteins can then be tested in standard assays for evaluating the effects of such substitutions on the ability of the protein to hydrolyze a terminal, non-reducing α-D-galactose residues in α-D-galactosides, including galactose oligosaccharides, galactomannans and galactolipids; and/or on the ability to treat or prevent Fabry disease.

In some embodiments, to retain functional activity, conservative amino acid substitutions are made. As used herein, the language a "conservative amino acid substitution" is intended to include a substitution in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), β-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Other substitutions involve replacement of an amino acid residue with another residue having a small side chain, such as alanine or glycine. Amino acid substituted peptides can be prepared by standard techniques, such as, for example, automated chemical synthesis.

In one embodiment, an α-Gal A protein of the present disclosure is at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, homologous to the amino acid sequence of an α-Gal A protein described herein or known in the art.

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

For example, the percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases, for example, to identify related sequences. Such searches can be performed, for example, using the XBLAST program (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized, for example, as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) are useful. See the National Center for Biotechnology Information (NCBI) website.

The term "AUC" represents a mathematical calculation to evaluate the body's total exposure over time to a given drug. In a graph plotting how concentration in the blood after dosing, the drug concentration variable lies on the y-axis and time lies on the x-axis. The area between a drug concentration curve and the

x-axis for a designated time interval is the AUC. AUCs are used as a guide for dosing schedules and to compare different drugs' availability in the body.

The term "Cmax" represents the maximum plasma concentration achieved after dosing.

According to the application, a "subject" or "patient" is a human or non-human animal. In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a human. Although the animal subject is preferably a human, the compounds and compositions of the application have application in veterinary medicine as well, *e.g.*, for the treatment of domesticated species such as canine, feline, and various other pets; farm animal species such as bovine, equine, ovine, caprine, porcine, etc.; wild animals, *e.g.*, in the wild or in a zoological garden; and avian species, such as chickens, turkeys, quail, songbirds, etc.

The term "enzyme replacement therapy" or "ERT" refers to refers to the introduction of a non-native, purified enzyme into an individual having a deficiency in such enzyme. The administered enzyme can be obtained from natural sources or by recombinant expression. 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 protein insufficiency. The introduced enzyme can be a purified, recombinant enzyme produced *in vitro*, or enzyme purified from isolated tissue or fluid, such as, *e.g.*, placenta or animal milk, or from plants.

The term "adjuvant" or "adjuvant therapy" refers to any additional substance, treatment, or procedure used for increasing the efficacy, safety, or otherwise facilitating or enhancing the performance of a primary substance, treatment, or procedure.

The term "co-formulation" refers to a composition comprising an enzyme, such as an enzyme used for ERT, for example, an α-Gal A enzyme (*e.g.*, a human recombinant α-Gal A enzyme (rhα-Gal A)), that is formulated together with an ASSC for the enzyme. In certain embodiments the ASSC is 1-deoxygalactonojirimycin (DGJ), or a pharmaceutically acceptable salt, ester or prodrug of 1-deoxygalactonojirimycin. In one embodiment, the salt is hydrochloride salt (i.e. 1-deoxygalactonojirimycin-HCl). In certain embodiments, treating a subject with the co-formulation comprises administering the co-formulation to the subject

such that the α-Gal A enzyme and ASSC are administered concurrently at the same time as part of the co-formulation.

The term "combination therapy" refers to any therapy wherein the results are enhanced as compared to the effect of each therapy when it is performed individually. The individual therapies in a combination therapy can be administered concurrently or consecutively.

Enhancement can include any improvement of the effect of the various therapies that may result in an advantageous result as compared to the results achieved by the therapies when performed alone. Enhanced effect and determination of enhanced effect can be measured by various parameters such as, but not limited to: temporal parameters (*e.g.*, length of treatment, recovery time, long-term effect of the treatment or reversibility of treatment); biological parameters (*e.g.*, cell number, cell volume, cell composition, tissue volume, tissue size, tissue composition); spatial parameters (*e.g.*, tissue strength, tissue size or tissue accessibility) and physiological parameters (*e.g.*, body contouring, pain, discomfort, recovery time or visible marks). Enhanced effect can include a synergistic enhancement, wherein the enhanced effect is more than the additive effects of each therapy when performed by itself. Enhanced effect can also include an additive enhancement, wherein the enhanced effect is substantially equal to the additive effect of each therapy when performed by itself. Enhanced effect can also include less than a synergistic effect, wherein the enhanced effect is lower than the additive effect of each therapy when performed by itself, but still better than the effect of each therapy when performed by itself.

The term "stabilize a proper conformation" refers to the ability of a compound or peptide or other molecule to associate with a wild-type protein, or to a mutant protein that can perform its wild-type function *in vitro* and *in vivo*, in such a way that the structure of the wild-type or mutant protein can be maintained as its native or proper form. This effect can manifest itself practically through one or more of (i) increased shelf-life of the protein; (ii) higher activity per unit/amount of protein; or (iii) greater *in vivo* efficacy. It may be observed experimentally through increased yield from the ER during expression; greater resistance to unfolding due to temperature increases (*e.g.*, as determined in thermal stability assays), or the present of chaotropic agents, and by similar means.

As used herein, the term "active site" refers to the region of a protein that has some specific biological activity. For example, it can be a site that binds a

substrate or other binding partner and contributes the amino acid residues that directly participate in the making and breaking of chemical bonds. Active sites in this application can encompass catalytic sites of enzymes, antigen biding sites of antibodies, ligand binding domains of receptors, binding domains of regulators, or receptor binding domains of secreted proteins. The active sites can also encompass transactivation, protein-protein interaction, or DNA binding domains of transcription factors and regulators.

As used herein, the term "active site-specific chaperone" refers to any molecule including a protein, peptide, nucleic acid, carbohydrate, etc. that specifically interacts reversibly with an active site of a protein and enhances formation of a stable molecular conformation. As used herein, "active site-specific chaperone" does not include endogenous general chaperones present in the ER of cells such as Bip, calnexin or calreticulin, or general, non-specific chemical chaperones such as deuterated water, DMSO, or TMAO.

The term "purified" as used herein refers to material that has been isolated under conditions that reduce or eliminate the presence of unrelated materials, i.e., contaminants, including native materials from which the material is obtained. For example, a purified protein is preferably substantially free of other proteins or nucleic acids with which it is associated in a cell; a purified nucleic acid molecule is preferably substantially free of proteins or other unrelated nucleic acid molecules with which it can be found within a cell. As used herein, the term "substantially free" is used operationally, in the context of analytical testing of the material. Preferably, purified material substantially free of contaminants is at least 95% pure; more preferably, at least 97% pure, and more preferably still at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, and other methods known in the art. In a specific embodiment, purified means that the level of contaminants is below a level acceptable to regulatory authorities for safe administration to a human or non-human animal.

As used herein, the terms "mutant" and "mutation" mean any detectable change in genetic material, *e.g.*, DNA, or any process, mechanism or result of such a change. This includes gene mutations, in which the structure (*e.g.*, DNA sequence) of a gene is altered, any gene or DNA arising from any mutation process, and any expression product (*e.g.*, RNA, protein or enzyme) expressed by a modified gene or DNA sequence.

As used herein the term "mutant protein" refers to proteins translated from genes containing genetic mutations that result in altered protein sequences. In a specific embodiment, such mutations result in the inability of the protein to achieve its native conformation under the conditions normally present in the ER. The failure to achieve this conformation results in these proteins being degraded, rather than being transported through their normal pathway in the protein transport system to their proper location within the cell. Other mutations can result in decreased activity or more rapid turnover.

As used herein the term "wild-type gene" refers to a nucleic acid sequences which encodes a protein capable of having normal biological functional activity in vivo. The wild-type nucleic acid sequence may contain nucleotide changes that differ from the known, published sequence, as long as the changes result in amino acid substitutions having little or no effect on the biological activity. The term wild-type may also include nucleic acid sequences engineered to encode a protein capable of increased or enhanced activity relative to the endogenous or native protein.

As used herein, the term "wild-type protein" refers to any protein encoded by a wild-type gene that is capable of having functional biological activity when expressed or introduced in vivo. The term "normal wild-type activity" refers to the normal physiological function of a protein in a cell. Such functionality can be tested by any means known to establish functionality of a protein.

The term "genetically modified" refers to cells that express a particular gene product following introduction of a nucleic acid comprising a coding sequence which encodes the gene product, along with regulatory elements that control expression of the coding sequence. Introduction of the nucleic acid can be accomplished by any method known in the art including gene targeting and homologous recombination. As used herein, the term also includes cells that have been engineered to express or overexpress an endogenous gene or gene product not normally expressed by such cell, *e.g.*, by gene activation technology.

The phrase "pharmaceutically acceptable", whether used in connection with the pharmaceutical compositions of the application, refers to molecular entities and compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a human. Preferably, 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. Pharmacopeia or other generally

recognized pharmacopeia for use in animals, and more particularly in humans. The term "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.

The terms "therapeutically effective dose" and "effective amount" refer to the amount of the compound that is sufficient to result in a therapeutic response. In embodiments where an ASSC and α-Gal A are administered together in a co-formulation, the terms "therapeutically effective dose" and "effective amount" may refer to the amount of the co-formulation that is sufficient to result in a therapeutic response. A therapeutic response can be any response that a user (*e.g.*, a clinician) will recognize as an effective response to the therapy. Thus, a therapeutic response will generally be an amelioration of one or more symptoms or sign of a disease or disorder.

Non-limiting examples of improvements in surrogate markers for Fabry disease include increases in α-GAL levels or activity in cells (*e.g.*, fibroblasts) and tissue; reductions in of GL-3 accumulation as measured by the change in kidney interstitial capillary biopsies using histology; decreased urine GL-3 levels; assessment of renal function (including glomerular filtration rate (GFR) and 24-hour urine protein; 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 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 (Fuller et al., *Clinical Chemistry*. 2005; 51: 688-694); 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).

It should be noted that a concentration of the ASSC that is inhibitory during in vitro production, transportation, or storage of the purified therapeutic protein can still constitute an "effective amount" for purposes of this application because of dilution (and consequent shift in binding due to the change in equilibrium), bioavailability and metabolism of the ASSC upon administration in vivo.

5.2 Enzyme Replacement Therapy

The current approved treatment for Fabry disease is enzyme replacement therapy. Two products are currently available for the treatment of Fabry disease: agalsidase alfa (Replagal®, Shire Human Genetic Therapies) and agalsidase beta (Fabrazyme®; Genzyme Corporation), marketed globally. These two forms of ERT are intended to compensate for a patient's inadequate α-Gal A activity with a recombinant form of the enzyme, administered intravenously. ERT has been demonstrated to reduce GL-3 deposition in capillary endothelium of the kidney and certain other cell types. While ERT is effective in many settings, the treatment also has limitations. ERT has not been demonstrated to decrease the risk of stroke, cardiac muscle responds slowly, and GL-3 elimination from some of the cell types of the kidneys is limited. Some patients develop immune reactions to ERT.

The recommended dosage of agalsidase alfa is 0.2 mg/kg body weight infused every 2 weeks as an intravenous infusion

The recommended dosage of agalsidase beta is 1 mg/kg body weight infused every 2 weeks as an intravenous infusion.

5.3 Pharmaceutical Compositions

According to the present application, 1-deoxygalactonojirimycin (DGJ) can be administered as the free base or as a pharmacologically acceptable salt form, including 1-deoxygalactonojirimycin hydrochloride (also known as, migalastat hydrochloride). It can be administered in a form suitable for parenteral administration, including *e.g.*, in a sterile aqueous solution for intravenous administration. The compounds and compositions of the application can be

formulated as pharmaceutical compositions by admixture with a pharmaceutically acceptable carrier or excipient.

In certain embodiments, DGJ and α-Gal A are formulated together in a single composition (*i.e.*, co-formulated together). Such a composition enhances stability of α-Gal A both during storage (*i.e.*, *in vitro*) and *in vivo* after administration to a subject, thereby increasing circulating half-life, tissue uptake, and resulting in increased therapeutic efficacy of α-Gal A (*e.g.*, increasing the reduction of tissue GL-3 levels). The co-formulation is preferably suitable for intravenous administration.

The present application features liquid pharmaceutical co-formulations (*e.g.*, formulations comprising α-Gal A and DGJ) having improved properties as compared to art-recognized formulations.

In certain embodiments, co-formulations of the application include an α-Gal A enzyme and DGJ that are suitable for intravenous administration.

In certain embodiments, the compositions of the present application comprise a co-formulation of an α-Gal A enzyme and an ASSC for the α-Gal A enzyme. In certain embodiments, the ASSC is 1-deoxygalactonojirimycin. In certain embodiments, the ASSC is 1-deoxygalactonojirimycin-HCl.

In certain embodiments, the co-formulation composition comprises α-Gal A at a concentration of between about 0.05 and about 100 μM, or between about 0.1 and about 75 μM, or between about 0.2 and about 50 μM, or between about 0.3 and about 40 μM, or between about 0.4 and about 30 μM, or between about 0.5 and about 20 μM, or between about 0.6 and about 15 μM, or between about 0.7 and about 10 μM, or between about 0.8 and about 9 μM, or between about 0.9 and about 8 μM, or between about 1 and about 7 μM, or between about 2 and about 6 μM, or between about 3 and about 5 μM. Concentrations and ranges intermediate to the above recited concentrations are also intended to be part of this application.

In certain embodiments, the co-formulation composition comprises α-Gal A at a concentration of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15 μM.

In certain embodiments, the co-formulation composition comprises α-Gal A at a concentration of between about 0.0025 and about 5 mg/ml, or between about 0.005 and about 4.5 mg/ml, or between about 0.025 and about 4 mg/ml, or between about 0.05 and about 3.5 mg/ml, or between about 0.25 and about 3 mg/ml,

or between about 0.5 and about 2.5 mg/ml, or between about 0.75 and about 2 mg/ml, or between about 1 and about 1.5 mg/ml. Concentrations and ranges intermediate to the above recited concentrations are also intended to be part of this application.

In certain embodiments, the co-formulation composition comprises DGJ at a concentration of between about 10 and about 25,000 μM, or between about 50 and about 20,000 μM, or between about 100 and about 15,000 μM, or between about 150 and about 10,000 μM, or between about 200 and about 5,000 μM, or between about 250 and about 1,500 μM, or between about 300 and about 1,000 μM, or between about 350 and about 550 μM, or between about 400 and about 500 μM. Concentrations and ranges intermediate to the above recited concentrations are also intended to be part of this application.

In certain embodiments, the co-formulation composition comprises DGJ at a concentration of between about 0.002 and about 5 mg/ml, or between about 0.005 and about 4.5 mg/ml, or between about 0.02 and about 4 mg/ml, or between about 0.05 and about 3.5 mg/ml, or between about 0.2 and about 3 mg/ml, or between about 0.5, and about 2.5 mg/ml, or between about 1 and about 2 mg/ml. Concentrations and ranges intermediate to the above recited concentrations are also intended to be part of this application.

In certain embodiments, the co-formulation composition comprises DGJ at a concentration of about 50; 100; 150; 200; 250; 300; 350; 400; 450; 500; 550; 600; 650; 700; 750; 800; 850; 900; 950; 1,000; 2,000; 3,000; 4,000; 5,000; 6,000; 7,000; 8,000; 9,000; 10,000; 11,000; 12,000; 13,000; 14,000; 15,000; 16,000; 17,000; 18,000; 19,000 or 20,000 μM.

In certain embodiments, the co-formulation composition comprises DGJ at a concentration of about 449, 748, 1,495, 4,490, or 14,950 μM.

In certain embodiments, the co-formulation composition of the application comprises α-Gal A enzyme and DGJ, wherein the α-Gal A is present at a concentration selected from the group consisting of 1.2, 2, 4, 8 and 12 μM; and wherein the DGJ is present at a concentration selected from the group consisting of 449, 748, 1,495, 4,490 and 14,950 μM.

In certain embodiments, the co-formulation composition of the application comprises about 1.2 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 1.2 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 1.2 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 1.2 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 1.2 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 2 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 2 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 2 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 2 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 2 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 4 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 4 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 4 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 4 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 4 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 8 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 8 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 8 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 8 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 8 μM α-Gal A enzyme and about 14,950 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 12 μM α-Gal A enzyme and about 449 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 12 μM α-Gal A enzyme and about 748 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 12 μM α-Gal A enzyme and about 1,495 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 12 μM α-Gal A enzyme and about 4,490 μM DGJ.

In certain embodiments, the co-formulation composition of the application comprises about 12 μM α-Gal A enzyme and about 14,950 μM DGJ.

The present invention also provides the use of a co-formulation of between about 0.5 and about 20 μM α-Gal A and between about 50 and 20,000 μM DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject.

The present invention also provides the use of a co-formulation of about 1.2, 2, 4, 8, or 12 μM α-Gal A and about 449, 748, 1,495, 4,490, or 14,950 μM DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject.

The present invention also provides the use of a co-formulation of between about 0.0025 and about 5 mg/ml, or between about 0.005 and about 4.5 mg/ml, or between about 0.025 and about 4 mg/ml, or between about 0.05 and about 3.5 mg/ml, or between about 0.25 and about 3 mg/ml, or between about 0.5 and about 2.5 mg/ml, or between about 0.75 and about 2 mg/ml, or between about 1 and about 1.5 mg/ml α-Gal A; and between about 0.002 and about 5 mg/ml, or between about 0.005 and about 4.5 mg/ml, or between about 0.02 and about 4 mg/ml, or between about 0.05 and about 3.5 mg/ml, or between about 0.2 and about 3 mg/ml, or between about 0.5, and about 2.5 mg/ml, or between about 1 and about 2 mg/ml DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject.

The present invention also provides the use of a co-formulation of between about 0.05 and about 100 μM, or between about 0.1 and about 75 μM, or between about 0.2 and about 50 μM, or between about 0.3 and about 40 μM, or between about 0.4 and about 30 μM, or between about 0.5 and about 20 μM, or between about 0.6 and about 15 μM, or between about 0.7 and about 10 μM, or between about 0.8 and about 9 μM, or between about 0.9 and about 8 μM, or between about 1 and about 7 μM, or between about 2 and about 6 μM, or between about 3 and about 5 μM α-Gal A; and between about 10 and about 25,000 μM, or between about 50 and about 20,000 μM, or between about 100 and about 15,000 μM, or between about 150 and about 10,000 μM, or between about 200 and about 5,000 μM, or between about 250 and about 1,500 μM, or between about 300 and about 1,000 μM, or between about 350 and about 550 μM, or between about 400 and about 500 μM DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject.

The present invention also provides the use of a co-formulation of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15 μM α-Gal A; and about 50; 100; 150; 200; 250; 300; 350; 400; 450; 500; 550; 600; 650; 700; 750; 800; 850; 900; 950; 1,000; 2,000; 3,000; 4,000; 5,000; 6,000; 7,000; 8,000; 9,000; 10,000; 11,000; 12,000; 13,000; 14,000; 15,000; 16,000; 17,000; 18,000; 19,000 or 20,000 μM DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject.

The present invention also provides a co-formulation of between about 0.5 and about 20 μM α-Gal A and between about 50 and about 20,000 μM DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally.

The present invention also provides a co-formulation of about 1.2, 2, 4, 8, or 12 μM α-Gal A and about 449, 748, 1,495, 4,490, or 14,950 μM DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally.

The present invention also provides a co-formulation of between about 0.0025 and about 5 mg/ml, or between about 0.005 and about 4.5 mg/ml, or between

about 0.025 and about 4 mg/ml, or between about 0.05 and about 3.5 mg/ml, or between about 0.25 and about 3 mg/ml, or between about 0.5 and about 2.5 mg/ml, or between about 0.75 and about 2 mg/ml, or between about 1 and about 1.5 mg/ml α-Gal A; and between about 0.002 and about 5 mg/ml, or between about 0.005 and about 4.5 mg/ml, or between about 0.02 and about 4 mg/ml, or between about 0.05 and about 3.5 mg/ml, or between about 0.2 and about 3 mg/ml, or between about 0.5, and about 2.5 mg/ml, or between about 1 and about 2 mg/ml DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally.

The present invention also provides a co-formulation of between about 0.05 and about 100 μM, or between about 0.1 and about 75 μM, or between about 0.2 and about 50 μM, or between about 0.3 and about 40 μM, or between about 0.4 and about 30 μM, or between about 0.5 and about 20 μM, or between about 0.6 and about 15 μM, or between about 0.7 and about 10 μM, or between about 0.8 and about 9 μM, or between about 0.9 and about 8 μM, or between about 1 and about 7 μM, or between about 2 and about 6 μM, or between about 3 and about 5 μM α-Gal A; and between about 10 and about 25,000 μM, or between about 50 and about 20,000 μM, or between about 100 and about 15,000 μM, or between about 150 and about 10,000 μM, or between about 200 and about 5,000 μM, or between about 250 and about 1,500 μM, or between about 300 and about 1,000 μM, or between about 350 and about 550 μM, or between about 400 and about 500 μM DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally.

The present invention also provides a co-formulation of about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15 μM α-Gal A; and about 50; 100; 150; 200; 250; 300; 350; 400; 450; 500; 550; 600; 650; 700; 750; 800; 850; 900; 950; 1,000; 2,000; 3,000; 4,000; 5,000; 6,000; 7,000; 8,000; 9,000; 10,000; 11,000; 12,000; 13,000; 14,000; 15,000; 16,000; 17,000; 18,000; 19,000 or 20,000 μM DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation with a molar ratio of DGJ to α-Gal A enzyme of between about 10:1 and about 20,000:1; or between about 20:1 and about 15,000:1; or between

about 50:1 and about 10,000:1; or between about 100:1 and about 5,000:1; or between about 150:1 and about 1,000:1; or between about 200:1 and about 500:1; or between about 250:1 and about 450:1; or between about 300:1 and about 400:1.

The pharmaceutical co-formulations suitable for intravenous administration use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In certain embodiments, the form is sterile and fluid to the extent that easy syringability exists. In certain embodiments, it is stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms such as bacteria and fungi. In certain embodiments, the composition comprises a carrier such as 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. The preventions 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 preferable 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. Sterile injectable solutions can be prepared by incorporating the α-Gal A and ASSC (*e.g.*, DGJ) in the required amounts 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.

In certain embodiments, the co-formulation can contain an excipient. Pharmaceutically acceptable excipients which can be included in the co-formulation

are buffers such as citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer, amino acids, urea, alcohols, ascorbic acid, 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 co-formulations include citrate; acetate; bicarbonate; and phosphate buffers.

The co-formulation also can contain a non-ionic detergent. Preferred non-ionic detergents include Polysorbate 20, Polysorbate 80, Triton X-100, Triton X-114, Nonidet P-40, Octyl α-glucoside, Octyl β-glucoside, Brij 35, Pluronic, and Tween 20.

For lyophilization of protein and chaperone preparations, the protein concentration can be 0.1-10 mg/mL. Bulking agents, such as glycine, mannitol, albumin, and dextran, can be added to the lyophilization mixture. In addition, possible cryoprotectants, such as disaccharides, amino acids, and PEG, can be added to the lyophilization mixture. Any of the buffers, excipients, and detergents listed above, can also be added.

In certain embodiments, the co-formulation composition of the application is formulated for intravenous administration. Administration of the above-described parenteral co-formulations can be by periodic injections of a bolus of the preparation, or can be administered by intravenous administration from a reservoir which is external (*e.g.*, an i.v. bag).

5.4 Treatment of Fabry Disease with ERT and an ASSC

In accordance with the application, there are provided methods of using α-Gal A (*e.g.*, rhα-Gal A) in combination with an ASSC for the α-Gal A (*e.g.*, DGJ). One embodiment of the present application provides for combination therapy of α-Gal A (*e.g.* rhα-Gal A ERT) and an ASSC, wherein the α-Gal A and ASSC are co-formulated together and administered to a subject concurrently as a co-formulation. In certain embodiments, the ASSC 1-deoxygalactonojirimycin is co-formulated with α-Gal A as a pharmaceutical composition.

In certain embodiments, the route of administration is intravenous. Administration can be by periodic injections of a bolus of the preparation, or as a

sustained release dosage form over long periods of time, such as by intravenous administration, for example, from a reservoir which is external (*e.g.*, an IV bag).

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of α-Gal A enzyme of the co-formulation administered to the subject is between about 0.05 and about 10 mg/kg, or between about 0.1 and about 5 mg/kg, or between about 0.2 and about 4 mg/kg, or between about 0.3 and about 3 mg/kg, or between about 0.4 and about 2 mg/kg, or between about 0.5 and about 1.5 mg/kg, or between about 0.5 and about 1 mg/kg. Concentrations and ranges intermediate to the above recited concentrations are also intended to be part of this application.

In certain embodiments, the dosage of α-Gal A enzyme of the co-formulation administered to the subject is about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mg/kg.

In certain embodiments, the α-Gal A enzyme and DGJ are combined to create a co-formulation for administration to a subject, wherein the dosage of DGJ of the co-formulation administered to the subject is between about 0.05 and 20 mg/kg, or between about 0.1 and about 15 mg/kg, or between about 0.2 and about 10 mg/kg, or between about 0.3 and about 10 mg/kg, or between about 0.4 and about 9 mg/kg, or between about 0.5 and about 8 mg/kg, or between about 0.6 and about 7 mg/kg, or between about 0.7 and about 6 mg/kg, or between about 0.8 and about 5 mg/kg, or between about 0.9 and about 4 mg/kg, or between about 1 and about 3 mg/kg, or between about 1.5 and about 2 mg/kg. Concentrations and ranges intermediate to the above recited concentrations are also intended to be part of this application.

In certain embodiments, the dosage of DGJ of the co-formulation administered to the subject is about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg.

In certain embodiments, the co-formulation composition of the application comprises α-Gal A enzyme and DGJ, wherein the dosage of α-Gal A of the co-formulation administered to a subject is at a concentration selected from the group consisting of 0.3, 0.5, 1, 2 and 3 mg/kg; and wherein the dosage of DGJ of the co-formulation administered to the subject is at a concentration selected from the group consisting of 0.1, 0.3, 0.5, 1, 3, and 10 mg/kg.

The present invention also provides the use of a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof in the preparation of a

medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject, and wherein the α-Gal A of the co-formulation is formulated for administration in an amount between about 0.1 and about 0.5 mg/kg, and the DGJ of the co-formulation is formulated for administration in an amount between about 0.1 and about 15 mg/kg.

The present invention also provides the use of a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject, and wherein the α-Gal A of the co-formulation is formulated for administration in an amount of about 0.3, 0.5, 1, 2 or 3 mg/kg, and the DGJ of the co-formulation is formulated for administration in an amount of about 0.1, 0.3, 0.5, 1, 3, or 10 mg/kg.

The present invention also provides the use of a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject, and wherein the α-Gal A of the co-formulation is formulated for administration in an amount between about 0.05 and about 10 mg/kg, or between about 0.1 and about 5 mg/kg, or between about 0.2 and about 4 mg/kg, or between about 0.3 and about 3 mg/kg, or between about 0.4 and about 2 mg/kg, or between about 0.5 and about 1.5 mg/kg, or between about 0.5 and about 1 mg/kg; and the DGJ of the co-formulation is formulated for administration in an amount between about 0.05 and 20 mg/kg, or between about 0.1 and about 15 mg/kg, or between about 0.2 and about 10 mg/kg, or between about 0.3 and about 10 mg/kg, or between about 0.4 and about 9 mg/kg, or between about 0.5 and about 8 mg/kg, or between about 0.6 and about 7 mg/kg, or between about 0.7 and about 6 mg/kg, or between about 0.8 and about 5 mg/kg, or between about 0.9 and about 4 mg/kg, or between about 1 and about 3 mg/kg, or between about 1.5 and about 2 mg/kg.

The present invention also provides the use of a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof in the preparation of a medicament for the treatment of Fabry disease, wherein the medicament is formulated for parenteral administration to a subject, and wherein the α-Gal A of the co-formulation is formulated for administration in an amount of about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mg/kg; and the DGJ of the co-formulation is

formulated for administration in an amount of about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg.

The present invention also provides a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally, and wherein the α-Gal A of the co-formulation is administered in an amount between about 0.1 and about 0.5 mg/kg, and the DGJ of the co-formulation is administered in an amount between about 0.1 and about 15 mg/kg.

The present invention also provides a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally, and wherein the α-Gal A of the co-formulation is administered in an amount of about 0.3, 0.5, 1, 2 or 3 mg/kg, and the DGJ of the co-formulation is administered in an amount of about 0.1, 0.3, 0.5, 1, 3, or 10 mg/kg.

The present invention also provides a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally, and wherein the α-Gal A of the co-formulation is administered in an amount between about 0.05 and about 10 mg/kg, or between about 0.1 and about 5 mg/kg, or between about 0.2 and about 4 mg/kg, or between about 0.3 and about 3 mg/kg, or between about 0.4 and about 2 mg/kg, or between about 0.5 and about 1.5 mg/kg, or between about 0.5 and about 1 mg/kg; and the DGJ of the co-formulation is administered in an amount between about 0.05 and 20 mg/kg, or between about 0.1 and about 15 mg/kg, or between about 0.2 and about 10 mg/kg, or between about 0.3 and about 10 mg/kg, or between about 0.4 and about 9 mg/kg, or between about 0.5 and about 8 mg/kg, or between about 0.6 and about 7 mg/kg, or between about 0.7 and about 6 mg/kg, or between about 0.8 and about 5 mg/kg, or between about 0.9 and about 4 mg/kg, or between about 1 and about 3 mg/kg, or between about 1.5 and about 2 mg/kg.

The present invention also provides a co-formulation of α-Gal A and DGJ or a pharmaceutically acceptable salt thereof for use in the treatment of Fabry disease, wherein the co-formulation is administered parenterally, and wherein the α-Gal A of the co-formulation is administered in an amount of about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mg/kg; and the DGJ of the co-formulation is

administered in an amount of about 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg.

In one non-limiting embodiment, DGJ and recombinant human α-Gal A administered as a co-formulation show surprising efficacy on plasma enzyme activity and enzyme uptake into tissue. In wild-type mice, the plasma half-life of recombinant human α-Gal A (rhα-Gal A) increased 5-fold following a single intravenous injection of a co-formulation of α-Gal A and DGJ, and plasma AUC was increased 4-fold compared to intravenous administration of α-Gal A alone. Additionally, in wild-type mice, the tissue uptake of α-Gal A into kidney and heart tissue was increased up to 1.5-fold following a single intravenous injection of a co-formulation of α-Gal A and DGJ compared to intravenous administration of α-Gal A alone.

In one non-limiting embodiment, DGJ and recombinant human α-Gal A administered as a co-formulation show surprising efficacy on plasma enzyme activity, on enzyme uptake into tissue, and GL-3 reduction in tissue. In mice deficient for endogenous α-Gal A expression (*i.e.*, GLA knockout mice) the plasma half-life of recombinant human α-Gal A (rhα-Gal A) increased up to 3-fold at 0.5 hr, and up to 6-fold at 1 hr following a single intravenous injection of a co-formulation of α-Gal A and DGJ. Additionally, there was a greater GL-3 reduction, and increase in α-Gal A in heart and kidney tissue of the mice after intravenous administration compared to treatment with α-Gal A alone.

In certain non-limiting embodiments, the co-formulation of α-Gal A and DGJ can be administered intravenously to a subject in an amount effective to achieve a plasma AUC concentration of between about 0.5 and 10-fold, or between about 1 and about 8-fold, or between about 1.5 and about 6-fold, or between about 2 and about 5.5-fold, or between about 2.5 and about 5-fold, or between about 3 and about 4.5-fold of the plasma AUC concentration achieved when α-Gal A is administered to a subject in the same dosage as the co-formulation, but in the absence of DGJ.

In certain non-limiting embodiments, the co-formulation of α-Gal A and DGJ can be administered intravenously to a subject in an amount effective to achieve a level of α-Gal A tissue uptake of between about 0.5 and 10-fold, or between about 1 and about 8-fold, or between about 1.5 and about 6-fold, or between about 2 and about 5.5-fold, or between about 2.5 and about 5-fold, or between about 3

and about 4.5-fold of the level of α-Gal A tissue uptake achieved when α-Gal A is administered to a subject in the same dosage as the co-formulation, but in the absence of DGJ.

Delivery of the co-formulation can be continuous over a pre-selected administration period ranging from several hours, one to several weeks, one to several months, or up to one or more years. In certain embodiments, the dosage form is one that is adapted for delivery of α-Gal A over an extended period of time. Such delivery devices may be adapted for administration of α-Gal A for several hours (*e.g.*, 2 hours, 12 hours, or 24 hours to 48 hours or more), to several days (*e.g.*, 2 to 5 days or more, from about 100 days or more), to several months or years. In some of these embodiments, the device is adapted for delivery for a period ranging from about 1 month to about 12 months or more. The α-Gal A delivery device can be one that is adapted to administer α-Gal A to an individual for a period of, *e.g.*, from about 2 hours to about 72 hours, from about 4 hours to about 36 hours, from about 12 hours to about 24 hours; from about 2 days to about 30 days, from about 5 days to about 20 days, from about 7 days to about 100 days or more, from about 10 days to about 50 days; from about 1 week to about 4 weeks; from about 1 month to about 24 months or more, from about 2 months to about 12 months, from about 3 months to about 9 months; or other ranges of time, including incremental ranges, within these ranges, as needed.

In certain embodiments, the methods of the application include administering to an individual, for example, by intravenous administration, a dose of α-Gal A present in a co-formulation with DGJ, wherein the dose is administered once per day, once every two days, once every three days, once every four days, once every five days, or once every six days. In certain embodiments, the dose does not result in a toxic level of α-Gal A in the liver of the individual. In some embodiments, the co-formulation of α-Gal A and DGJ is administered in a sufficient dose to result in a peak concentration of α-Gal A in tissues of the subject, within about 24 hours after the administration of the dose. In certain embodiments, the co-formulation is administered in a sufficient dose to result in a peak concentration of α-Gal A in tissues of the subject within between about 0.2 to about 50 hours, or between about 0.2 to about 24 hours, or between about 0.2 to about 5 hours, or between about 0.2 to about 1 hour, or between about 0.2 to about 0.5 hour, or about 40, 30, 20, 10, 5, 1, 0.5 or fewer hours after the administration of the dose. Ranges intermediate to the above

recited ranges are also intended to be part of this application. In some embodiments, the co-formulation is administered as a single-dose. In some embodiments, the co-formulation is administered as a multi-dose.

5.5 In Vitro Stability

Ensuring the stability of a replacement protein formulation during its shelf life is a major challenge. For example, vials of recombinant enzyme are often for single use only and unused product should be discarded. Additionally, recombinant enzyme often must often be reconstituted, diluted, and administered by a health care professional, and that administration should be without delay. Recombinant enzymes must often be stored at low temperatures, for example, 2 to 8°C, and the product only stable for a limited amount of time, for example, up to 24 hours.

When an ASSC and α-Gal A are present in the same composition, the co-formulated compositions of the application provide more stable compositions. In addition to stabilizing the administered protein *in vivo*, the ASSC reversibly binds to and stabilizes the conformation of the α-Gal A *in vitro*, thereby preventing aggregation and degradation, and extending the shelf-life of the co-formulation. Analysis of the ASSC/replacement protein interaction can be evaluated using techniques well-known in the art, such as, for example, differential scanning calorimetry, or circular dichroism.

For example, where an aqueous injectable co-formulation of the composition is supplied in a stoppered vial suitable for withdrawal of the contents using a needle and syringe, the presence of an ASSC inhibits aggregation of the α-Gal A. The vial could be for either single use or multiple uses. In another embodiment, the co-formulation is in a dry or lyophilized state, which would require reconstitution with a standard or a supplied, physiological diluent to a liquid state. In this instance, the presence of an ASSC stabilizes the α-Gal A during and post-reconstitution to prevent aggregation. In the embodiment where the co-formulation is a liquid for intravenous administration, such as in a sterile bag for connection to an intravenous administration line or catheter, the presence of an ASSC confers the same benefit.

In addition to stabilizing the replacement protein to be administered, the presence of an ASSC enables the α-Gal A co-formulation to be stored at a neutral pH of about 7.0-7.5. This confers a benefit to proteins that normally must be stored at a lower pH to preserve stability. For example, lysosomal enzymes, such as α-Gal A,

typically retain a stable conformation at a low pH (*e.g.*, 5.0 or lower). However, extended storage of the replacement enzyme at a low pH may expedite degradation of the enzyme and/or co-formulation. In certain embodiments, the co-formulations of the present application remain stable at a pH of between about 5 and about 9, or between about 6 and about 8, or between about 6.5 and about 7.5, or between about 7 and about 7.5.

As described above, the liquid co-formulation of the application has advantageous stability and storage properties. Stability of the liquid co-formulation is not dependent on the form of storage, and includes, but is not limited to, co-formulations which are frozen, lyophilized, spray-dried, or co-formulations in which the active ingredient is suspended. Stability can be measured at a selected temperature for a selected time period. In one aspect of the application, the protein in the liquid co-formulations is stable in a liquid form for at least about 1 week; at least about 2 weeks; at least about 3 weeks; at least about 1 month; at least about 2 months; at least about 3 months; at least about 4 months, at least about 5 months; at least about 6 months; at least about 12 months; at least about 18 months. Values and ranges intermediate to the above recited time periods are also intended to be part of this application, *e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or about 24 months. In addition, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

In certain embodiments, the co-formulation is stable at room temperature (about 30 °C) or at about 37 °C, or at about 40 °C, or at about 45 °C for at least about 1 month and/or stable at about 2-8 °C for at least about 1 year, or more preferably stable at about 2-8 °C for at least about 2 years. In certain embodiments, the co-formulation is stable at a temperature of between about 35 and about 90 °C, or between about 40 and about 85 °C, or between about 45 and about 80 °C, or between about 50 and about 75 °C, or between about 55 and about 70 °C, or between about 60 and about 70 °C. Furthermore, the co-formulation is preferably stable following freezing (to, *e.g.*, -80 °C) and thawing of the co-formulation, hereinafter referred to as a "freeze/thaw cycle."

Stability of a protein (*e.g.*, protein stability and/or reduction in contamination) in a liquid co-formulation can also be defined as the percentage of monomer, aggregate, or fragment, or combinations thereof, of the protein in the co-formulation. In certain embodiments, a protein "retains its physical stability" in a co-

formulation if it shows substantially no signs of aggregation, precipitation and/or denaturation upon visual examination of color and/or clarity, or as measured by UV light scattering or by size exclusion chromatography, non-denaturing PAGE, or other methods for determining size, etc. In one aspect of the application, a stable liquid co-formulation is a co-formulation having less than about 10%, or less than about 5%, or less than about 5%, or less than about 4%, or less than about 3%, or less than about 2%, or less than about 1% of the protein being present as aggregate in the co-formulation.

In one embodiment, the physical stability of a liquid co-formulation is determined by determining turbidity of the co-formulation following a stir stress assay, *e.g.*, 24 hour or 48-hour stir-stress assay. For example, a stir stress assay can be performed by placing a suitable volume of a liquid co-formulation in a beaker with a magnetic stirrer, *e.g.*, (multipoint HP, 550 rpm), removing aliquots at any suitable time, *e.g.*, at T0-T48 (hrs), and performing suitable assays as desired on the aliquots. Samples of a co-formulation under the same conditions but without stirring serve as control.

Turbidity measurements can be performed using a laboratory turbidity measurement system from Hach (Germany) and are reported as nephelometric units (NTU).

Stability of the composition (*e.g.*, protein stability and/or reduction in contamination) can also be measured, *e.g.*, by measuring protein degradation or contaminant growth or presence. Protein degradation can be determined, *e.g.*, by reverse phase HPLC, non-denaturing PAGE, ion-exchange chromatography, peptide mapping, or similar methods.

The stability of α-Gal A in the presence of an ASSC, at a concentration described herein, can be measured, *e.g.*, as a percent aggregation or degradation, at a predetermined time and compared with one or more standards. For example, a suitable standard is a composition similar to the test conditions except that the α-Gal A is not contacted with an ASSC. The stabilities of the α-Gal A at a concentration are compared. Suitability can be shown by the α-Gal A at a particular concentration in combination with an ASSC having comparable or better stability than in the absence of the ASSC.

5.6 In Vivo Stability

As described above for the in vitro co-formulations, the presence of an ASSC for the α-Gal A has the benefit of prolonging in plasma the half-life of the exogenous α-Gal A, thereby maintaining effective replacement protein levels over longer time periods, resulting in increased exposure of clinically affected tissues to the α-Gal A and, thus, increased uptake of protein into the tissues. This confers such beneficial effects to the patient as enhanced relief, reduction in the frequency, and/or reduction in the amount administered. This will also reduce the cost of treatment.

In addition to stabilizing wild-type replacement α-Gal A, the ASSC will also stabilize and enhance expression of endogenous mutant α-Gal A that are deficient as a result of mutations that prevent proper folding and processing in the ER, as in conformational disorders such as Fabry Disease.

The compositions and methods of the present application are not to be limited in scope by the specific embodiments described herein and the Examples that follow. Indeed, various modifications of the compositions and methods described by the present application, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and the accompanying Examples and Figures. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLES

EXAMPLE 1: Pharmacokinetics and Tissue Uptake of rhα-Gal A and DGJ-HCl Administered Intravenously to Wild-Type Mice

The pharmacokinetics (PK) and tissue uptake of a co-formulation of recombinant human α-Gal A (rhα-Gal A) and 1-deoxygalactonojirimycin-HCl (DGJ-HCl) were determined following a single intravenous (IV) infusion administration to wild-type (WT) mice. (1-deoxygalactonojirimycin is also referred to as "AT1001", recombinant human α-Gal A is also referred to as "JR051").

Eight-week old male WT C57BL/6 mice (n=5) received either rhα-Gal A 1 mg/kg alone (via jugular vein IV infusion) or co-formulated with 3 or 10 mg/kg

of DGJ-HCl (free base equivalents of 3.66 and 12.2 mg/kg DGJ-HCl, respectively). Table 1 describes the molar ratios of these compounds:

Table 1

JR-051 (mg/kg)	AT1001 (mg/kg)	JR-051 (μM)	AT1001 (μM)	Ratios in co-formulation mixture (AT1001:JR-051)
1	3	4	4490	1122:1
1	10	4	14950	3740:1

Plasma was collected at 0, 15, 30, 45, 60, and 90 min, and 24 hrs post-JR-051. Skin, heart, and kidney were collected 24 hrs post-JR-051. JR-051 plasma levels and tissue uptake were assessed by activity and Western blotting (*see* Methods section below). A comparison was made between JR-051 administered alone and JR-051 co-formulated with AT1001 groups.

Results

Plasma: Plasma PK of α-Gal A (JR-051) in WT mice following IV infusion administration is shown in Figures 1 and 2. Co-formulation of DGJ with α-Gal A improves α-Gal A PK (as measured by activity and Western blot) in WT mice. Overall, co-formulating DGJ with α-Gal A increases α-Gal A AUC and half-life up to 4- and 5-fold, respectively, as shown in Table 2, below. The effect of DGJ on plasma α-Gal A levels is dose-dependent.

Table 2. Summary of half-life and AUC of α-Gal A ± DGJ

Group	**$T_{1/2}$ (hr)**	**AUC (nmol/mL)**
JR-051 alone	0.11	17960
JR-051 + 3 mg/kg DGJ COF	0.37	67307
JR-051 + 10 mg/kg DGJ COF	0.56	79799

Heart and Kidney Tissue: Co-formulating DGJ with α-Gal A improves α-Gal A uptake into kidney as well as in heart tissue (as measured by activity and Western blot) in wild-type mice, as shown in Figure 3. Overall, co-formulating DGJ with α-Gal A increases α-Gal A uptake up to 1.5-fold greater than seen with

administration of α-Gal A alone. In skin, there was no significant effect seen with either α-Gal A or co-formulated α-Gal A + DGJ (data not shown).

EXAMPLE 2: Pharmacokinetics, Tissue Uptake and Substrate Reduction of rhα-Gal A and DGJ-HCl Administered Intravenously to GLA Knockout Mice

The pharmacokinetics (PK), tissue uptake and substrate reduction in disease-relevant tissue of a co-formulation of recombinant human α-Gal A (rhα-Gal A) and 1-deoxygalactonojirimycin-HCl (DGJ-HCl) were determined following a single intravenous (IV) infusion administration to GLA knockout mice (GLA KO) (*i.e.*, mice deficient for endogenous α-Gal A expression).

Twelve-week old male GLA KO mice (n=6) received either JR-051: 0.3, 1, or 3 mg/kg alone via jugular vein IV infusion or co-formulated with 1, 3, and 10 mg/kg DGJ (free base equivalents of 1.22, 3.66, and 12.2 mg/kg DGJ-HCl, respectively). Plasma was collected 0.5 and 1 hr after start of infusion to measure JR-051 levels. Enzyme uptake and GL-3 levels were measured in heart, and kidney 7 days post-JR-051 administration (*see* Methods section below). Table 3 describes the molar ratios of these compounds:

Table 3

JR-051 (mg/kg)	AT1001 (mg/kg)	JR-051 (μM)	AT1001 (μM)	Ratios in co-formulation mixture (AT1001:JR-051)
0.3	1	1.2	1495	1246: 1
0.3	3	1.2	4490	3742:1
0.3	10	1.2	14950	12460:1
1	1	4	1495	374:1
1	3	4	4490	1122:1
1	10	4	14950	3740:1
3	1	12	1495	125:1
3	3	12	4490	374:1
3	10	12	14950	1250:1

Results

Plasma: Plasma levels of JR-051 in GLA KO mice following IV infusion administration are shown in Figure 4. There is a dose-dependent increase in

the circulating levels of α-Gal A alone in GLA KO mice. Co-formulating DGJ with α-Gal A further increases the circulating levels of α-Gal A. Overall, DGJ co-formulation increases plasma levels of α-Gal A up to 3-fold at 0.5 hr. and up to 6-fold at 1 hr, from start of infusion.

Heart Tissue: Tissue uptake and reduction in GL-3 in heart tissue of GLA KO mice is shown in Figures 5 and 6. There is a dose-dependent GL-3 reduction and α-Gal A increase seen with α-Gal A administration alone in heart. Overall, the co-formulation of DGJ and α-Gal A lead to greater GL-3 reduction and α-Gal A activity than seen with administration of α-Gal A alone. Though the effect of co-formulating α-Gal A and DGJ on α-Gal A activity in heart tissue seems to be dose-dependent with regard to DGJ, the dose-dependent effect was not obvious for GL-3 reduction. GL-3 reduction in heart indicates that when DGJ is co-formulated with lower doses of α-Gal A (*e.g.*, 0.3 or 1 mg/kg), the potency of α-Gal A is increased, achieving effects similar to those of higher doses of α-Gal A alone (3 mg/kg).

Kidney Tissue: Tissue uptake and reduction in GL-3 in kidney tissue of GLA KO mice is shown in Figures 7 and 8. There is a dose-dependent GL-3 reduction and α-Gal A increase seen with α-Gal A administration alone in kidney. Overall, the co-formulation of DGJ and α-Gal A lead to greater GL-3 reduction and α-Gal A activity than seen with administration of α-Gal A alone. Though the effect of co-formulating α-Gal A and DGJ on α-Gal A activity in kidney tissue seems to be dose-dependent with regard to DGJ, the dose-dependent effect was not obvious for GL-3 reduction. GL-3 reduction in kidney indicates that when DGJ is co-formulated with lower doses of α-Gal A (e.g., 1 mg/kg), the potency of α-Gal A is increased, achieving effects similar to those of higher doses of α-Gal A alone (3 mg/kg).

EXAMPLE 3: Pharmacokinetics, Tissue Uptake and Substrate Reduction of rhα-Gal A and DGJ-HCl Administered Intravenously to GLA Knockout Mice

Repeat administration of co-formulated product was tested in fourteen-week old GLA KO mice. The mice received four biweekly (once every two weeks) IV bolus injections of 0.5, 1 or 3 mg/kg α-Gal A (JR-051) alone, or co-formulated with 0.3, 1, 3 or 10 mg/kg of DGJ (AT1001). Table 4 describes the molar ratios of these compounds. Plasma and tissue levels of α-Gal A, and tissue levels of GL-3 were measured.

Table 4.

JR-051 (mg/kg)	AT1001 (mg/kg)	JR-051 (μM)	AT1001 (μM)	Ratios in co-formulation mixture (AT1001:JR-051)
0.5	0.3	2	449	224: 1
0.5	1	2	1495	747:1
0.5	3	2	4490	2245:1
0.5	10	2	14950	7475:1
1	0.3	4	449	112:1
1	1	4	1495	374:1
1	3	4	4490	1122:1
1	10	4	14950	3740:1
3	0.3	12	449	37:1
3	1	12	1495	125:1
3	3	12	4490	374:1
3	10	12	14950	1246:1

Results

α-Gal A Plasma Levels

Administration of the DGJ co-formulation showed trends for increased α-Gal A levels. Plasma collected for 1-hour time point analysis of α-Gal A levels was collected over a 1-2 hour window. Plasma collected for 2-hour time point analysis of α-Gal A levels was collected over a 3-4.5 hour window.

α-Gal Tissue Uptake

Overall, there was an increase in α-Gal A uptake into disease-relevant tissues (skin, heart, and kidney) with the DGJ co-formulation. Co-formulation with DGJ at 3 and 10 mg/kg resulted in greater tissue uptake at every dose level of α-Gal A. In heart and kidney, when DGJ was administered in combination with lower doses of α-Gal A, the potency of α-Gal A increased, achieving effects similar to those of higher α-Gal A doses alone. Although α-Gal A alone showed dose-dependent tissue uptake, a clear dose-dependent effect of DGJ was not detected in the samples tested in this experiment.

GL-3 Reduction in Tissue

As shown in Tables 5-7 below, and Figure 11A-C, a dose-dependent GL-3 reduction was observed in skin, heart, and kidney with repeat administration of α-Gal A alone. DGJ co-formulation with all doses of α-Gal A resulted in significantly greater GL-3 reduction in all tissues tested. While a clear dose-dependent effect for DGJ was not detected in the samples tested in this example, higher doses of DGJ (3 and 10 mg/kg) showed greater GL-3 reductions. When lower doses of α-Gal A (e.g., 0.5 or 1 mg/kg) were co-formulated with DGJ (3 or 10 mg/kg), the potency was similar or greater than that achieved with higher doses of α-Gal A alone.

Table 5. GL-3 % reduction in skin: data normalized to GL-3 levels in untreated KO and WT mouse tissues; % reduction = 100% - % of untreated

Skin		α-Gal A (mg/kg)		
		0.5	1	3
DGJ (mg/kg)	0	85.7	90.7	97.4
	0.3	92.5#	95.5	97.7
	1	90.0	95.9	98.7#
	3	95.4#	97.0#	98.7#
	10	94.6#	97.6#	99.2#

p< 0.05 in 2-sided t-test; #: Vs. respective JR-051 alone)

Table 6. GL-3 % reduction in heart: data normalized to GL-3 levels in untreated KO and WT mouse tissues; % reduction = 100% - % of untreated

Heart		α-Gal A (mg/kg)		
		0.5	1	3
DGJ (mg/kg)	0	72.1	82.7	93.7
	0.3	82.7#	87.9	93.5
	1	77.1	88.0	98.3#
	3	94.7#	98.5#	98.6#
	10	94.7#	97.0#	98.6#

p< 0.05 in 2-sided t-test; #: Vs. respective JR-051 alone)

Table 7. GL-3 % reduction in kidney: data normalized to GL-3 levels in untreated KO and WT mouse tissues; % reduction = 100% - % of untreated

kidney		α-Gal A (mg/kg)		
		0.5	1	3
DGJ (mg/kg)	0	42.7	54.9	69.5
	0.3	48.5	59.1	64.8
	1	40.1	58.3	78.8#
	3	66.3#	79.4#	76.0
	10	58.5#	64.5	77.1

$p < 0.05$ in 2-sided t-test; #: Vs. respective JR-051 alone)

Alternative Dosing Regimens

In an alternative regime repeat administration of co-formulated product will be tested in GLA KO mice. The mice will receive either 0.5, 1.0, or 2 mg/kg α-Gal A (JR-051) alone, or co-formulated with 0.3, 1, 3, and 10 mg/kg of DGJ. These compounds will be administered via IV bolus tail vein injection every other week for a total of 4 injections (total duration of study = 8 weeks). Table 8 describes the molar ratios of these compounds.

Table 8.

JR-051 (mg/kg)	AT1001 (mg/kg)	JR-051 (μM)	AT1001 (μM)	Ratios in co-formulation mixture (AT1001:JR-051)
0.5	0.3	2	449	224: 1
0.5	1	2	1495	747:1
0.5	3	2	4490	2245:1
0.5	10	2	14950	7475:1
1	0.3	4	449	112:1
1	1	4	1495	374:1
1	3	4	4490	1122:1
1	10	4	14950	3740:1
2	0.3	8	449	56:1
2	1	8	1495	187:1

2	3	8	4490	560:1
2	10	8	14950	1870:1

In an alternative regime repeat administration of co-formulated product can be tested in GLA KO mice. The mice will receive either 0.3, 1.0, or 3 mg/kg α-Gal A (JR-051) alone, or co-formulated with 0.5, 1, 3, and 10 mg/kg of DGJ. These compounds will be administered via IV bolus tail vein injection every other week for a total of 4 injections (total duration of study = 8 weeks). Table 9 describes the molar ratios of these compounds.

Table 9.

JR-051 (mg/kg)	AT1001 (mg/kg)	JR-051 (μM)	AT1001 (μM)	Ratios in co-formulation mixture (AT1001:JR-051)
0.3	0.5	1.2	748	623: 1
0.3	1	1.2	1495	1246:1
0.3	3	1.2	4490	3742:1
0.3	10	1.2	14950	12458:1
1	0.5	4	748	187:1
1	1	4	1495	374:1
1	3	4	4490	1122:1
1	10	4	14950	3740:1
3	0.5	12	748	62:1
3	1	12	1495	124:1
3	3	12	4490	374:1
3	10	12	14950	1246:1

Methods for Examples 1-3

Measurement of Plasma α-Gal A Activity

For plasma α-Gal A activity measurement, samples were diluted ~400-fold with Lysis Buffer (1% Triton X-100, 150 mM NaCl, 25 mM Bis-Tris, pH 6.5) prior to assay. Diluted plasma (20 μL) was added to 50 μL Assay Buffer (27 mM sodium citrate, 46 mM sodium phosphate dibasic, 6 mM 4-methylumbeliferryl-α-D-

galactopyranoside (4-MUG), 90 mM *N*-acetyl-D-galactosamine, pH 4.6) and incubated for 1 hour at 37 °C. Reactions were stopped by addition of 70 µL 0.4 M glycine, pH 10.8. Fluorescence at 460 nm was read on a Victor^3 plate reader (Perkin Elmer, Waltham, MA) after excitation at 355 nm. Raw fluorescence counts were background subtracted (defined by Assay Buffer only). A 4-MU standard curve ranging from 7 nM to 15 µM was run each day for conversion of fluorescence counts to absolute α-Gal A activity, expressed as nanomoles of released 4-MU per milliliter of plasma per hour (nmol/mL/hr).

Measurement of Plasma α-Gal A Protein by Western Blot

Diluted plasma (1:50) in Lysis Buffer (~10 µg total protein) was subjected to SDS-PAGE on 4 - 12% polyacrylamide gels (Invitrogen, Grand Island, NY), transferred to nitrocellulose membranes (Invitrogen), and immunoblotted with rabbit anti-human α-Gal A primary antibody (1:2000 dilution; from Shire Pharmaceuticals, Cambridge, MA). Protein bands were detected using peroxidase-conjugated goat anti-rabbit secondary antibody (Jackson Immunosearch Labs, West Grove, PA) in combination with enhanced chemiluminescence (Pierce, Rockford, IL). Blots were scanned on an Image Station 4000R (Kodak, Rochester, NY).

Measurement of Tissue α-Gal A Activity (uptake) and Western Blot

Tissue lysates from mutant mice were prepared by homogenization of ~50 mg tissue for 3 to 5 seconds on ice with a micro-homogenizer (Pro Scientific, Thorofare, NJ) in 200 µL Lysis Buffer (0.1% Triton X-100, 27 mM sodium citrate, 46 mM sodium phosphate dibasic, pH 4.6). Lysates (20 µL) were added to 50 µL Assay Buffer (27 mM sodium citrate, 46 mM sodium phosphate dibasic, 6 mM 4-methylumbeliferryl-α-D-galactopyranoside (4-MUG), 90 mM *N*-acetyl-D-galactosamine, pH 4.6) and incubated for 1 hour at 37 °C. Reactions were stopped by the addition of 70 µL 0.4 M glycine, pH 10.8. Fluorescence at 460 nm was read on a Victor^3 plate reader (Perkin Elmer, Waltham, MA) after excitation at 355 nm. Raw fluorescence counts were background subtracted (defined by Assay Buffer only). A BCA Protein Assay (Pierce, Rockford, IL) was used according to the manufacturer's instructions to determine total protein concentration in tissue lysates. A 4-MU standard curve ranging from 1.3 nM to 30 mM was run each day for conversion of fluorescence counts to absolute α-Gal A activity, expressed as nanomoles of released

4-MU per milligram of total protein per hour (nmol/mg protein/hr). For tissue Westerns exactly same procedure was followed as described above for plasma except that 50 μg tissue lysate was used.

Measurement of Tissue GL-3 Levels

Mouse tissues were homogenized in water (1:16.7 tissue:water) using a Fast Prep homogenizer (Pro Scientific, Thorofare, NJ). Acetone:methanol (50:50) was added at 5x the volume of the homogenate. Samples were vortexed, re-homogenized in the Fast Prep system, and centrifuged at 10600g for 10 minutes at room temperature. Each supernatant (200 μL of the upper organic portion) was prepared for solid phase extraction by addition of 200 μL dimethyl sulfoxide (DMSO), 50 μL lactosyl ceramide internal standard (from bovine buttermilk; Matreya, LLC, Pleasant Gap, PA) at a final concentration of 1.4 μg/mL, and 600 μL of water:methanol (13:87), followed by vortexing. Solid phase extraction was conducted on a pre-conditioned Bond Elut 40 μm, 100 mg C-18 column (Varian Inc, Palo Alto, CA) by washing with 67:23:10 methanol:acetone:water and elution with 1 mL of 9:1 acetone:methanol into silanized glass culture tubes containing 200 μL 0.2 mM sodium acetate in DMSO. Total GL-3 levels were determined from 20 μL of each sample extract by liquid chromatography - tandem mass spectroscopy (LC-MS/MS). LC was conducted using an acetone:methanol:acetonitrile (ACN) with sodium acetate binary mobile phase system (mobile phase A: 40:60 methanol:water with 0.1 mM sodium acetate; mobile phase B: 75:15:10 acetone:methanol:ACN with 0.1 mM sodium acetate) with a flow rate of 0.5 mL/min on a C18 column (Aqua 3 μm 100 x 3.0 mm, 125A, Phenomenex, Torrance, CA). The final GL-3 elution condition was 90% mobile phase B. MS/MS analysis was carried out under positive ion mode (ESI+) and twelve different isoforms of GL-3 [C16:0, C18:0, C20:0, C22:1, C22:0, C23:0, C22:0(2OH), C24:2, C24:1, C24:0, C24:1(2OH), C24:0(2OH)] as well as internal standard were identified in each sample. The following transitions were monitored: m/z 1046.70→m/z 884.7 for C16:0; m/z 1074.8→m/z 912.8 for C18:0; m/z 1102.8→m/z 940.8 for C20:0; m/z 1128.8→m/z 966.8 for C22:1; m/z 1130.9→m/z 968.8 for C22:0; m/z 1144.9→m/z 982.8 for C23:0; m/z 1146.9→m/z 984.8 for C22:0(2OH); m/z 1154.9→m/z 992.8 for C24:2; m/z 1156.9→m/z 994.8 for C24:1; m/z 1158.9→m/z 996.9 for C24:0; m/z 1172.9→m/z 1010.8 for C24:1(2OH); m/z 1174.9→m/z 1012.8 for C24:0(2OH); and m/z 982.9→m/z 820.8 for the lactosyl

ceramide internal standard. For quantitation, the area counts for each isoform were determined and then summed to obtain the total GL-3 area counts. The ratio of the total GL-3 area counts to that of the internal standard was determined and used to calculate the final concentration of GL-3 in each sample based on a linear least squares fit equation applied to an 11-point calibration curve prepared in DMSO. Total GL-3 measurements were normalized to the wet tissue weight of each sample.

EXAMPLE 4: Co-formulating DGJ with α-Gal A Increases α-Gal A Stability in Blood Ex Vivo

The effect of co-formulating α-Gal A with DGJ on α-Gal A stability in blood ex vivo was examined. Time-dependent loss of α-Gal A activity in whole blood at 37 °C was determined. 5 nM and 50 nM α-Gal A was formulated with or without 10 μM DGJ. As shown in Figure 9A-B, the half-life of α-Gal A in the whole blood samples when formulated without DGJ was about 4-5 hours. DGJ stabilized the enzyme and prevented enzyme inactivation.

EXAMPLE 5: Co-formulating DGJ with α-Gal A Increases Enzyme Uptake and GL-3 Reduction in Fabry Patient Derived Cells

The effect of co-formulating α-Gal A with DGJ on α-Gal A uptake and GL-3 reduction in R301Q and C52S fibroblast cells derived from Fabry patients was determined. 0.5 nM α-Gal A was tested alone or co-formulated with 0.1, 1.0, 10 or 100 μM DGJ. As shown in Figure 10A and C, in R301Q cells, DGJ increased the uptake of JR051 up to 3 –fold compared to up to 4.6 –fold with Fabrazyme®. As shown in Figure 10B and D, in C52S cells, DGJ increased uptake of both enzymes up to 4.2 –fold. The graph bars represent the average of compiled data from 4 separate experiments.

With regard to reducing GL-3 levels, As shown in Figure 10E-H, in both cell lines, JR051 at 0.5 nM results in similar reduction in GL-3 levels as Fabrazyme® (~70-80% of baseline). Further reduction after co-incubation with DGJ is also similar between the two enzymes (~40-50% of baseline). The graph bars represent the average of compiled data from 4 separate experiments.

* * *

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims. In addition, ranges and concentrations intermediate to any ranges and concentrations recited herein are also intended to be part of this application.

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

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a co-formulation of

between about 0.5 and about 20 μM α-galactosidase A; and

between about 50 and about 20,000 μM 1-deoxygalactonojirimycin, or a pharmaceutically acceptable salt thereof,

wherein the pharmaceutical composition is formulated for parenteral administration to a subject.

2. The pharmaceutical composition of claim 1, wherein the α-galactosidase A is a recombinant human wild-type α-galactosidase A.

3. The pharmaceutical composition of claim 1, wherein the 1-deoxygalactonojirimycin is a hydrochloride salt of 1-deoxygalactonojirimycin.

4. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is formulated for intravenous administration.

5. The pharmaceutical composition of claim 1, wherein the α-galactosidase A is present at a concentration selected from the group consisting of about 1.2, 2, 4, 8, and 12 μM; and the 1-deoxygalactonojirimycin is present at a concentration selected from the group consisting of about 449, 1,495, 4,490, and 14,950 μM.

6. The pharmaceutical composition of claim 1, wherein the 1-deoxygalactonojirimycin and α-galactosidase A are present in a molar ratio of 1-deoxygalactonojirimycin to α-galactosidase A of between about 13,000:1 and about 50:1.

7. A method of treating Fabry disease in a subject comprising administering to the subject a pharmaceutical composition comprising a co-formulation of

between about 0.5 and about 20 μM α-galactosidase A; and

between about 50 and about 20,000 μM 1-deoxygalactonojirimycin, or a pharmaceutically acceptable salt thereof,

wherein the pharmaceutical composition is administered to the subject parenterally.

8. The method of claim 7, wherein the pharmaceutical composition is administered intravenously.

9. The method of claim 7, wherein the pharmaceutical composition is administered as a dosage, wherein the dosage of α-galactosidase A is between about 0.1 and about 5 mg/kg, and the dosage of 1-deoxygalactonojirimycin is between about 0.1 and about 15 mg/kg.

10. The method of claim 7, wherein the dosage of α-galactosidase A is selected from the group consisting of about 0.3, 0.5, 1, 2, and 3 mg/kg.

11. The method of claim 7, wherein the dosage of 1-deoxygalactonojirimycin is selected from the group consisting of about 0.1, 0.3, 1, 3, and 10 mg/kg.

12. The method of claim 7, wherein the dosage of α-galactosidase A is 1 mg/kg, and wherein the dosage of 1-deoxygalactonojirimycin is 3 mg/kg.

13. The method of claim 7, wherein the pharmaceutical composition is administered in a dose sufficient to achieve a peak plasma concentration of α-galactosidase A at a time point between about 0.2 and about 5 hours after the administration of the dose.

14. The method of claim 7, wherein the pharmaceutical composition is administered once per day, once every two days, once every three days, once every four days, once every five days, once every six days, or once every seven days.

15. The method of claim 7, wherein the subject is a mammal.

16. The method of claim 7, wherein the subject is a human.

Figure 1.

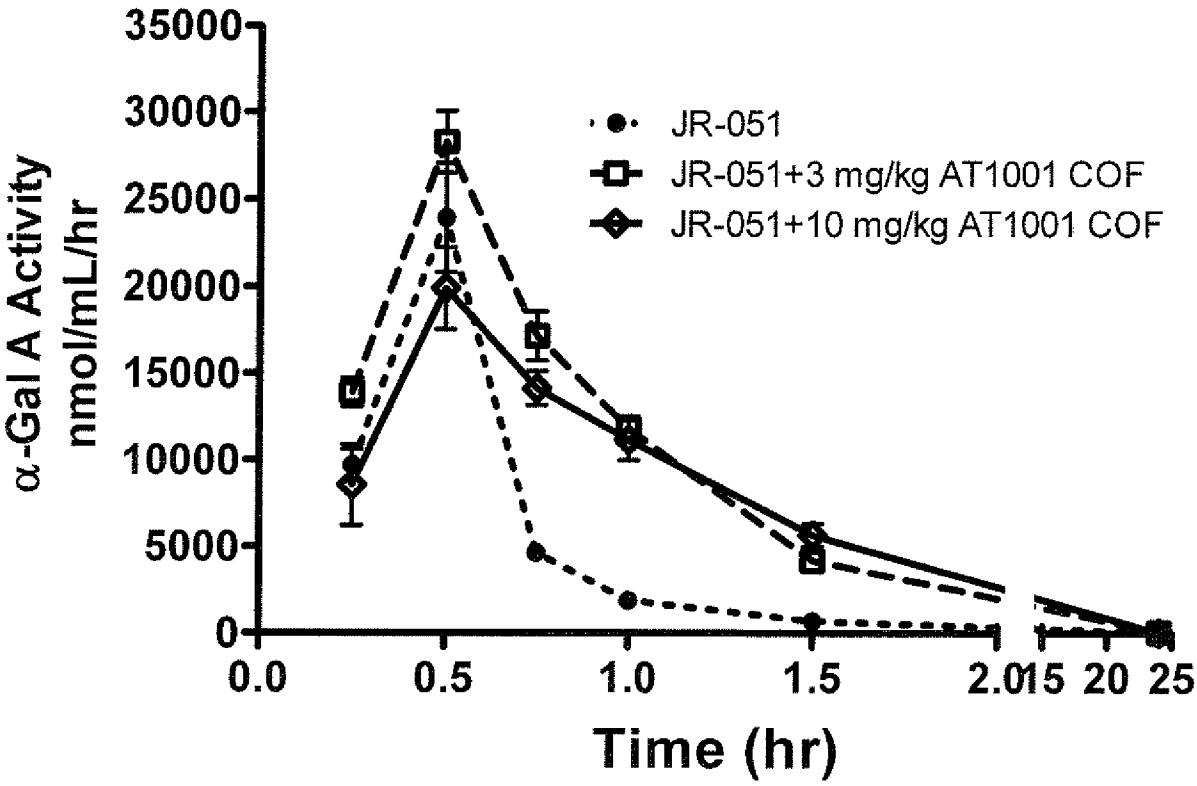

35000
30000
25000
20000
15000
10000
5000
0
α-Gal A Activity
nmol/mL/hr
JR-051
JR-051+3 mg/kg AT1001 COF
JR-051+10 mg/kg AT1001 COF
0.0
0.5
1.0
1.5
2.0
15
20
25
Time (hr)

Figure 2

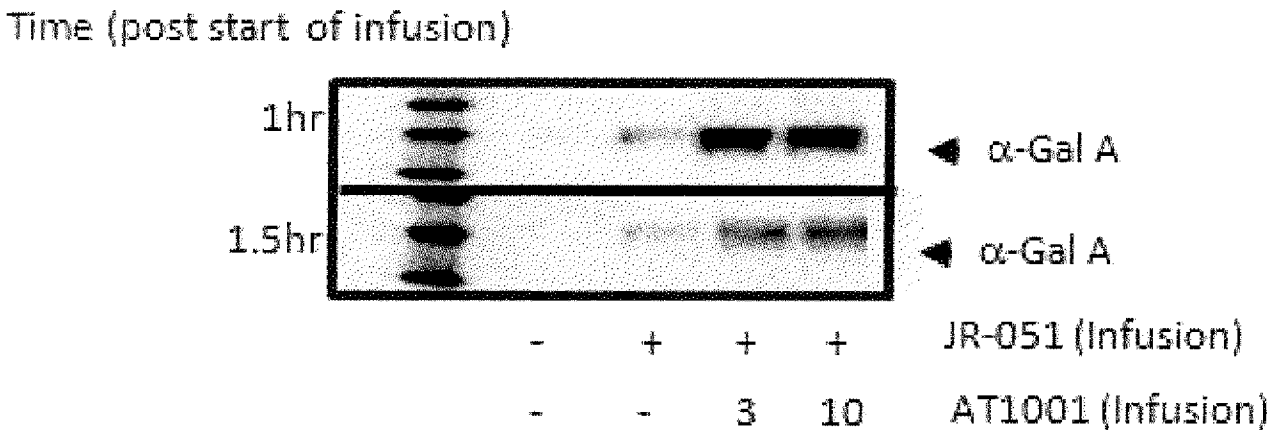
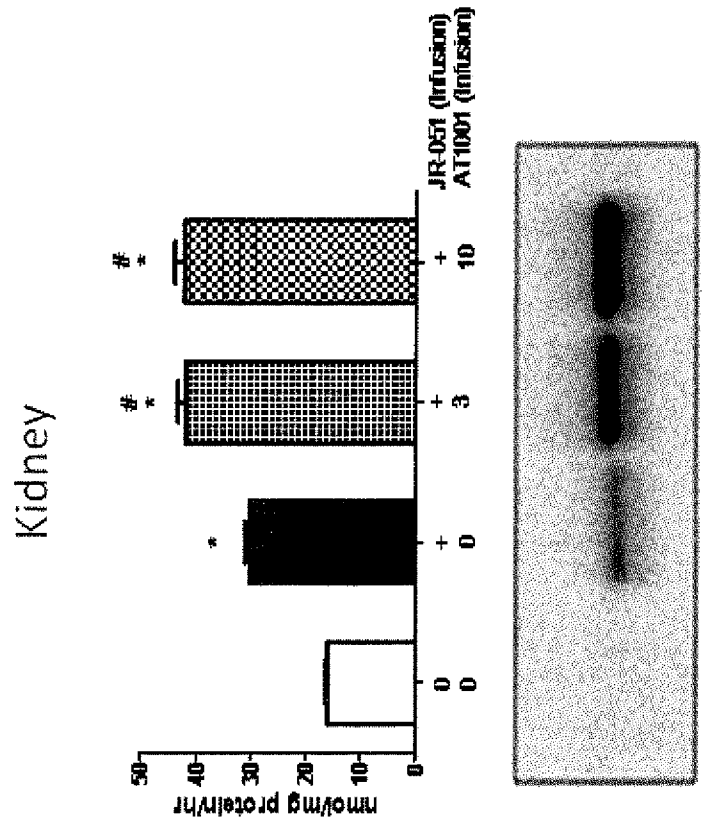
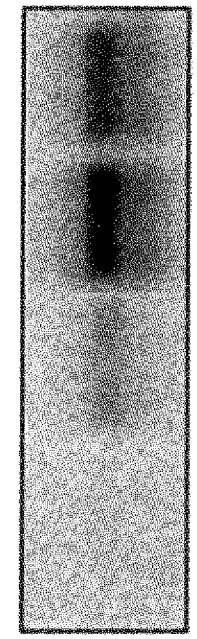

Time (post start of infusion)
1hr
1.5hr
α-Gal A
α-Gal A
- + + + JR-051 (Infusion)
- - 3 10 AT1001 (Infusion)

Figure 3

A.





α-Gal A

B.

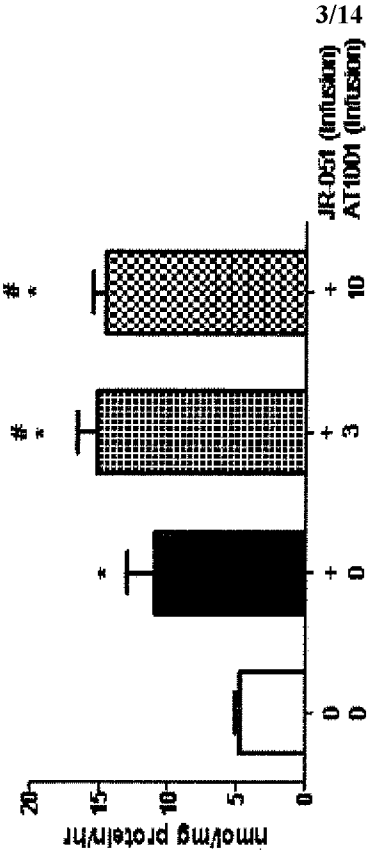
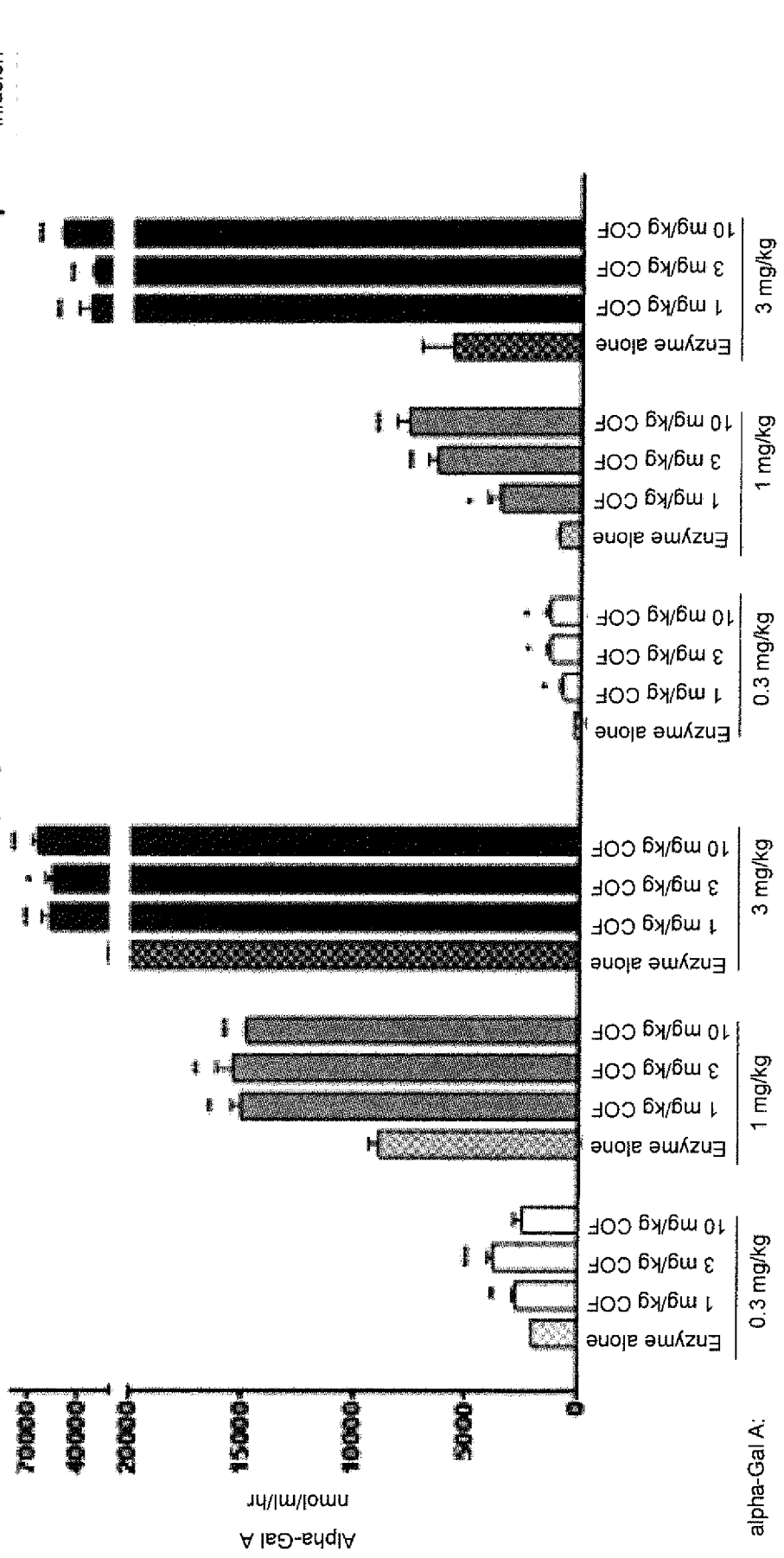


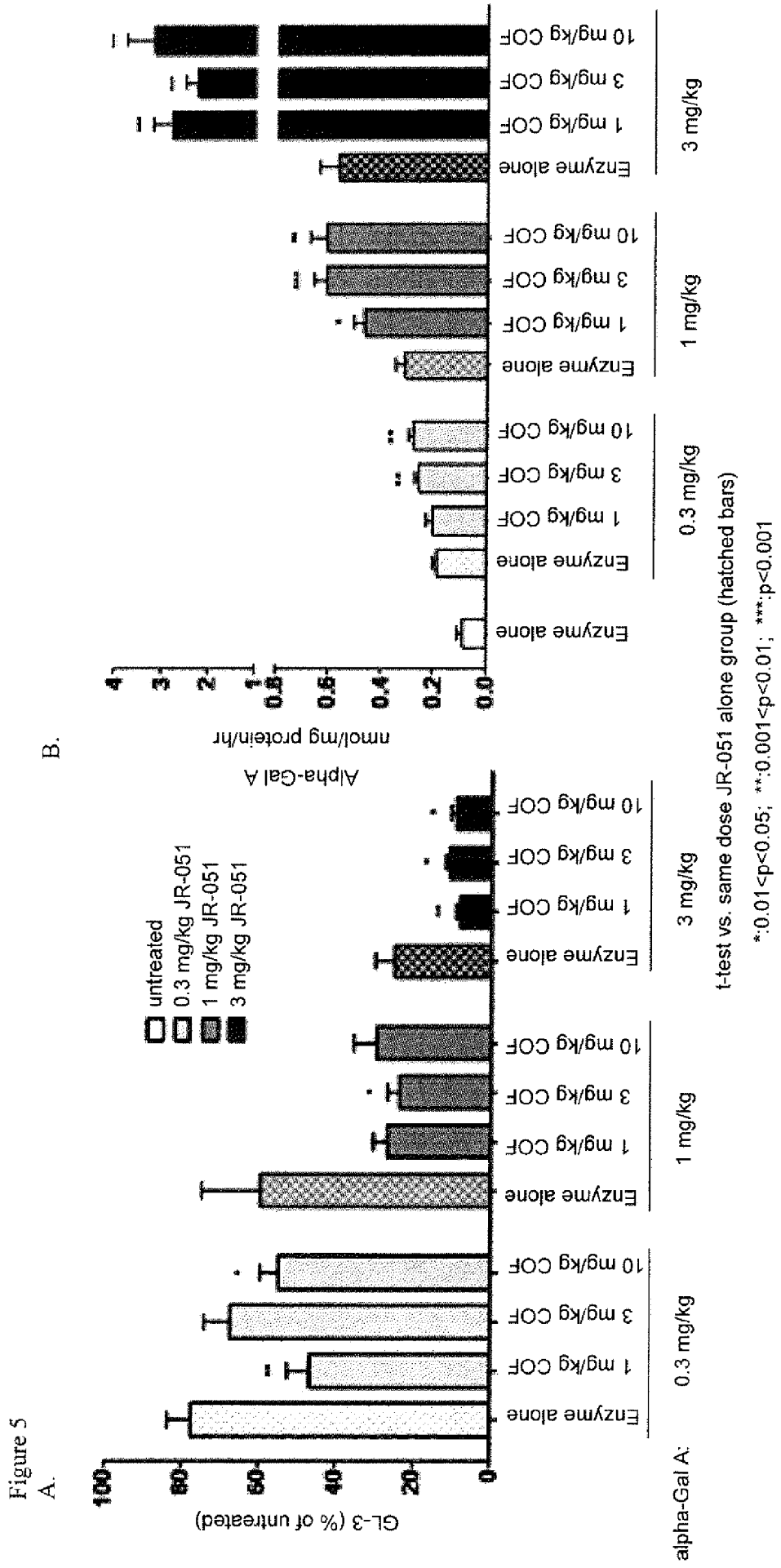
Figure 4



t-test vs. same dose JR-051 alone group (hatched bars)

*0.01<p<0.05; **0.001<p<0.01; ***p<0.001

Figure 5


A.
GL-3 (% of untreated)
untreated
0.3 mg/kg JR-051
1 mg/kg JR-051
3 mg/kg JR-051
Enzyme alone
1 mg/kg COF
3 mg/kg COF
10 mg/kg COF
alpha-Gal A: 0.3 mg/kg 1 mg/kg 3 mg/kg
B.
Alpha-Gal A
nmol/mg protein/hr
0.3 mg/kg 1 mg/kg 3 mg/kg
t-test vs. same dose JR-051 alone group (hatched bars)
*:0.01<p<0.05; **:0.001<p<0.01; ***:p<0.001

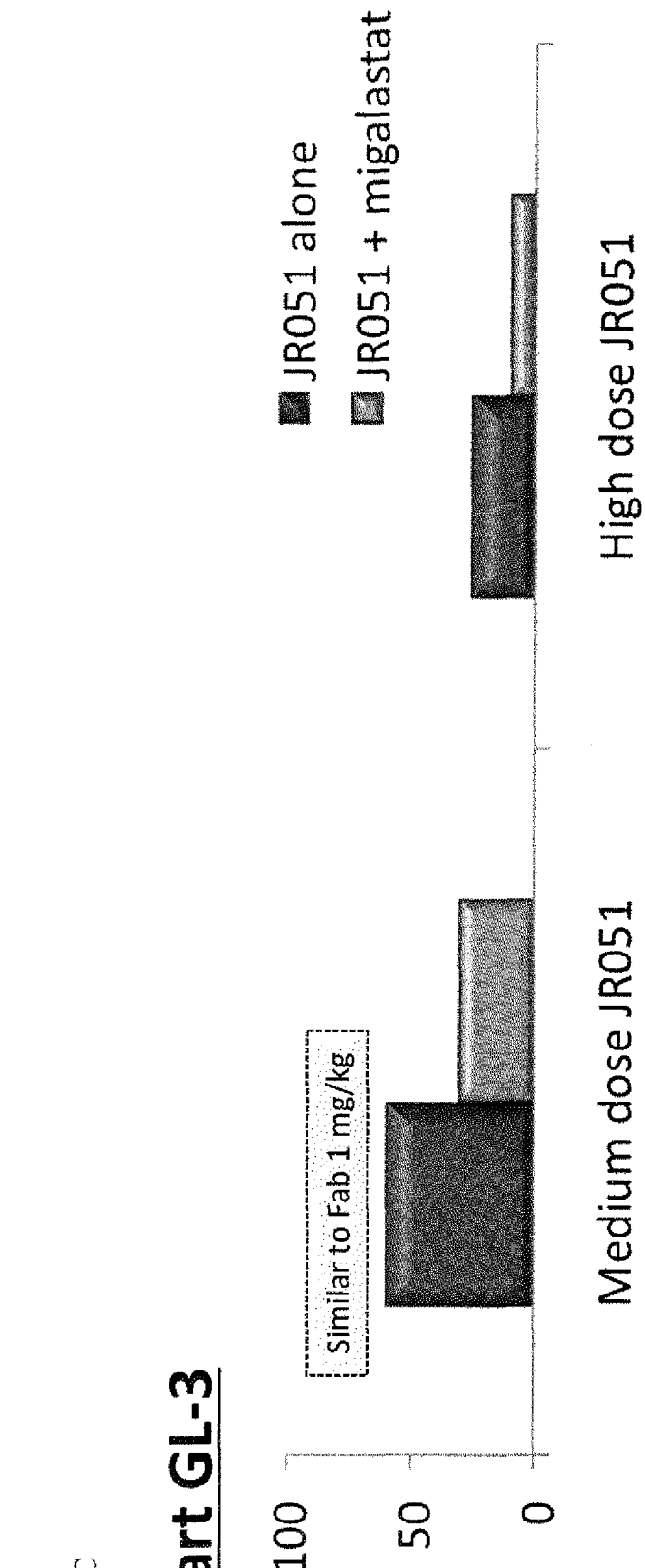

Heart GL-3
100
50
0
Similar to Fab 1 mg/kg
JR051 alone
JR051 + migalastat
Medium dose JR051
High dose JR051

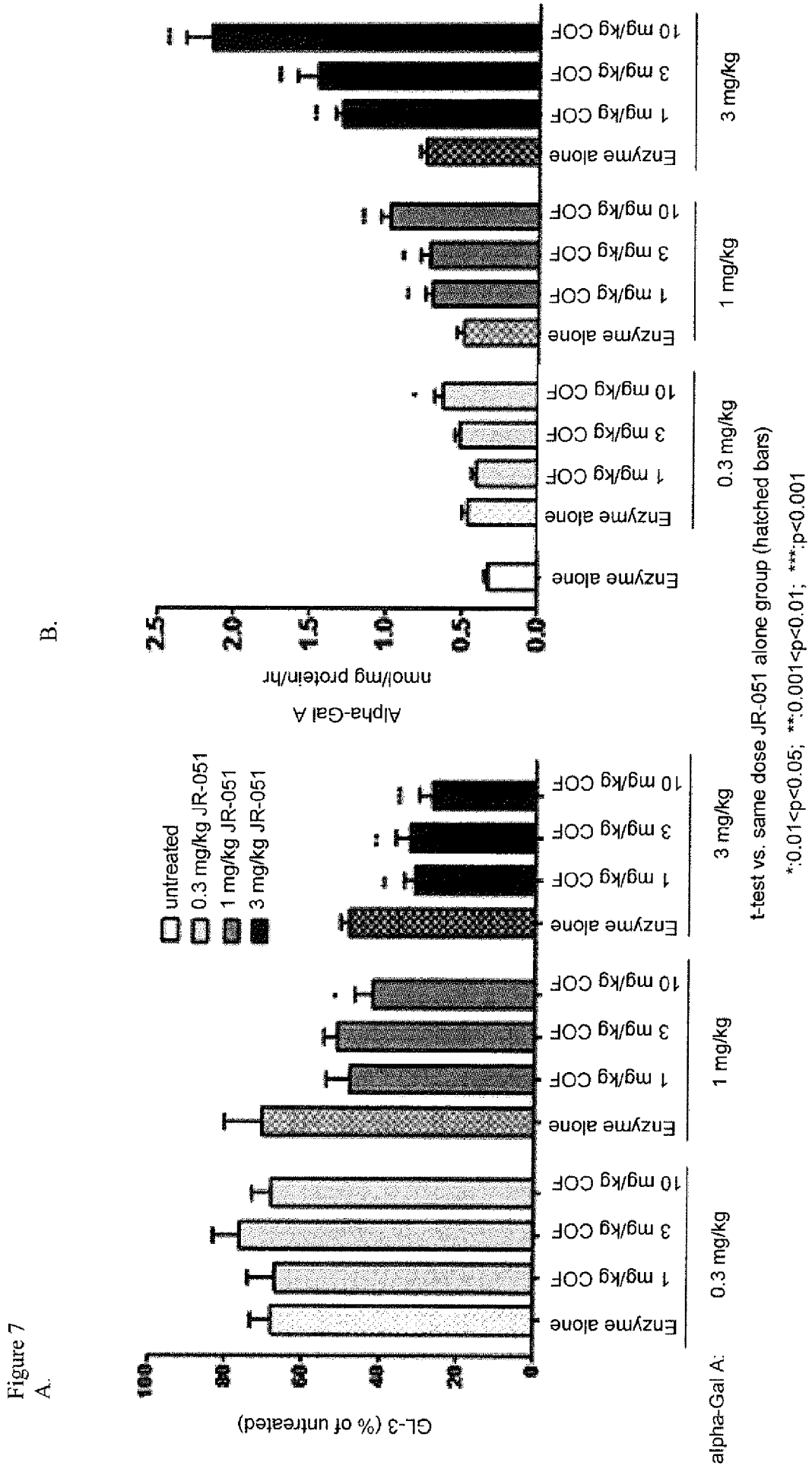
Figure 5C

Figure 6

Treatment group	0.3 mg/kg JR-051	1 mg/kg JR-051	3 mg/kg JR-051
Enzyme alone	22.3 →	40.3 →	74.9
+ 1 mg/kg AT1001	63.3**	73.2	92.0**
+ 3 mg/kg AT1001	32.7	76.4*	89.1*
+ 10 mg/kg AT1001	45.0*	70.3	90.7*

Data normalized to GL-3 levels in untreated KO mouse heart (100%; 242 µg/g tissue) and wild-type (0%; 5 µg/g tissue)
(% reduction = 100% - % of untreated)

Figure 7


A.
GL-3 (% of untreated)
100
80
60
40
20
0
untreated
0.3 mg/kg JR-051
1 mg/kg JR-051
3 mg/kg JR-051
Enzyme alone
1 mg/kg COF
3 mg/kg COF
10 mg/kg COF
alpha-Gal A: 0.3 mg/kg
1 mg/kg
3 mg/kg
B.
Alpha-Gal A
nmol/mg protein/hr
2.5
2.0
1.5
1.0
0.5
0.0
0.3 mg/kg
1 mg/kg
3 mg/kg
t-test vs. same dose JR-051 alone group (hatched bars)
*:0.01<p<0.05; **:0.001<p<0.01; ***:p<0.001

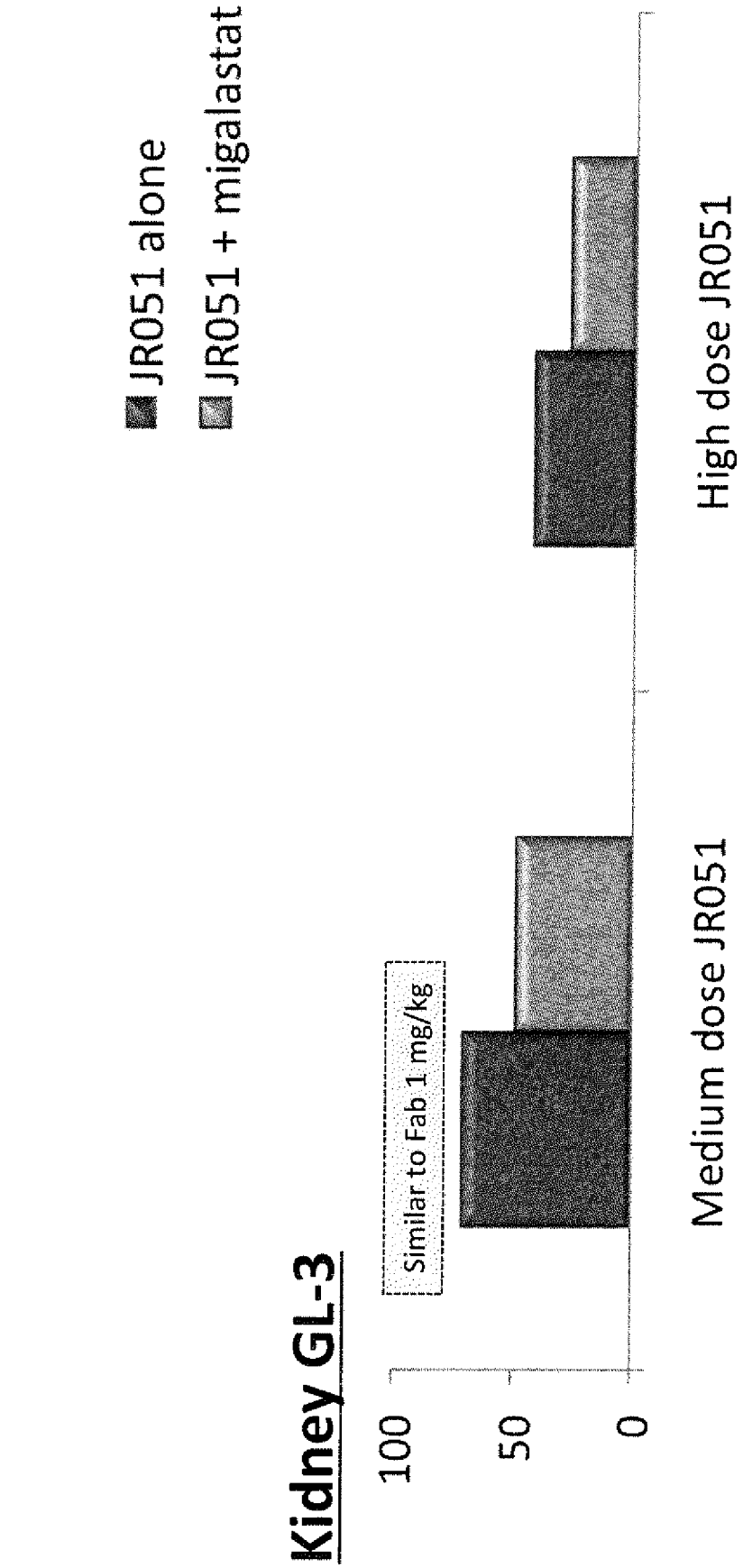

JR051 alone
JR051 + migalastat
Kidney GL-3
Similar to Fab 1 mg/kg
100
50
0
Medium dose JR051
High dose JR051

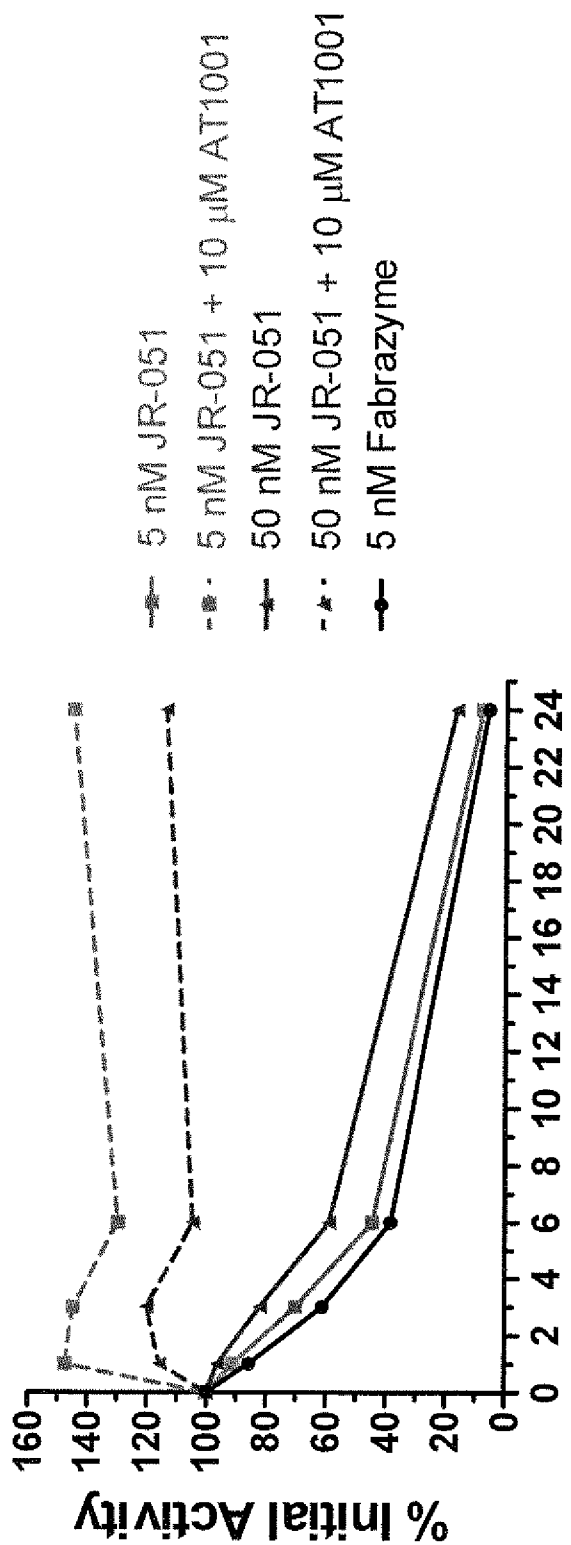
Figure 7C

Figure 8

Treatment group (PO; COF)	0.3 mg/kg JR-051	1 mg/kg JR-051	3 mg/kg JR-051
Enzyme alone	32.0	29.4	51.6
+ 1 mg/kg AT1001	33.0	52.0	68.8**
+ 3 mg/kg AT1001	23.8	48.7	67.3**
+ 10 mg/kg AT1001	31.9	58.0*	73.0***

Data normalized to GL-3 levels in untreated KO mouse kidney (100%; 521 µg/g tissue) and wild-type (0%; 75 µg/g tissue)
(% reduction = 100% - % of untreated)

Figure 9
A.

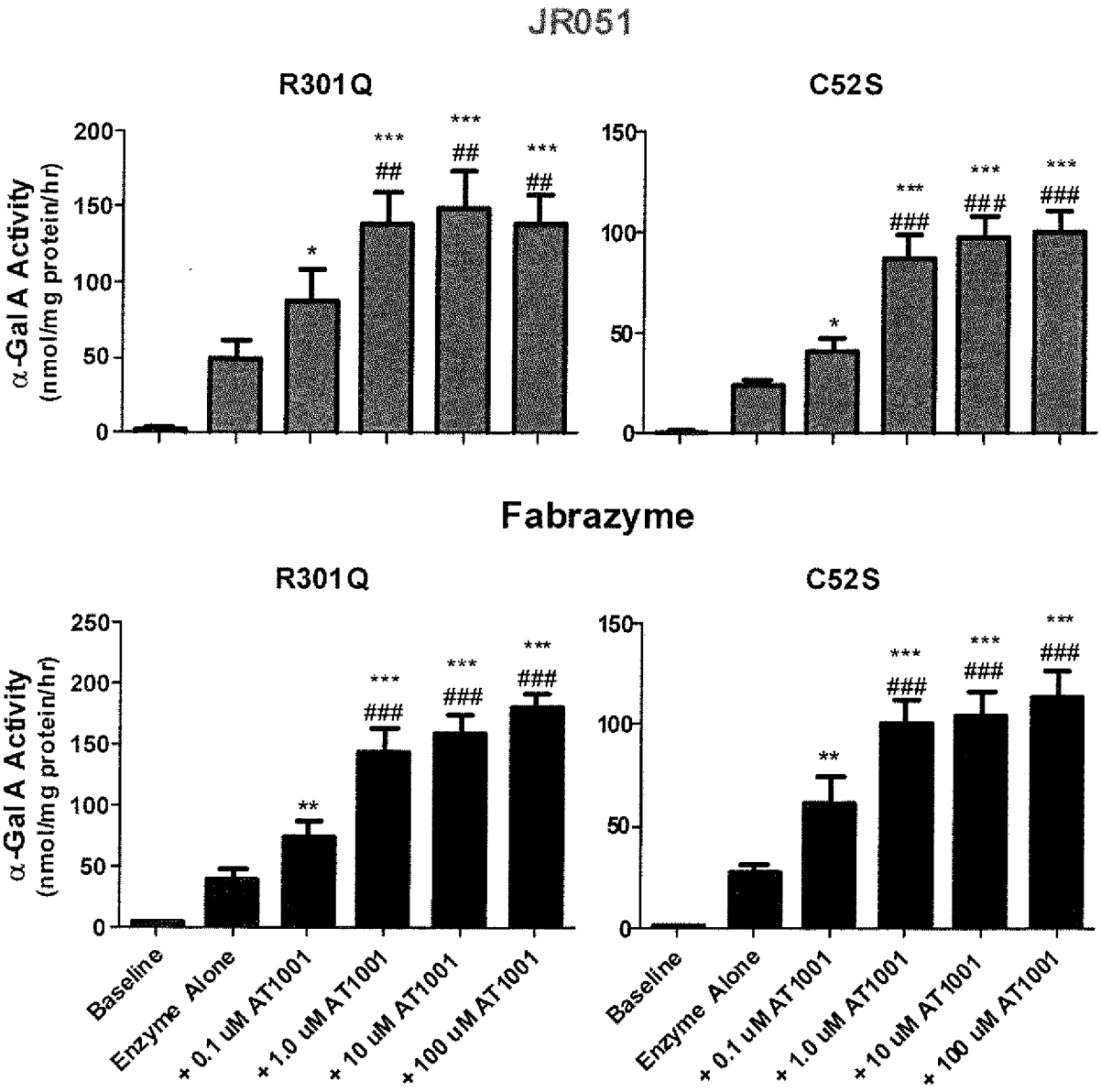


B.

Time (hr)	5 nM JR051	5 nM JR051 10 µM AT1001	50 nM JR051	50 nM JR051 10 µM AT1001	5 nM Fabrazyme
0	100.00	100.00	100.00	100.00	100.00
1	91.12	147.52	96.36	115.84	85.74
3	70.26	144.80	82.09	120.06	61.24
6	44.66	129.85	59.01	104.71	38.53
24	7.93	145.09	16.73	114.23	5.97

Figure 10

A. B.

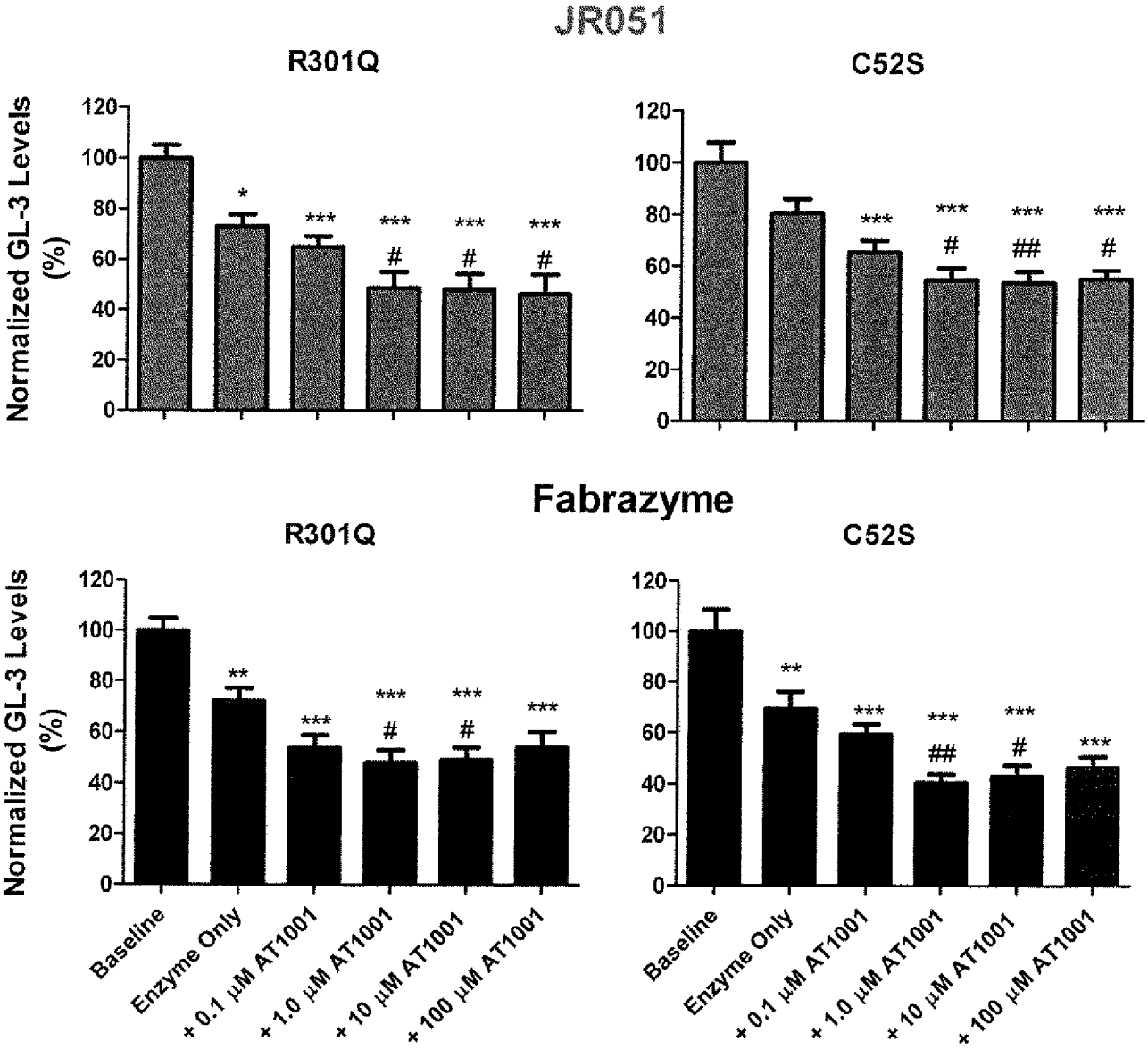


*p value <0.05, **p value <0.01, *** p value <0.001 compared to baseline
#p value <0.05, ##p value <0.01, ###p value <0.001 compared to enzyme alone

C. D.

Figure 10

E. F.



*p value <0.05, *** p value <0.001 compared to baseline; #p value <0.05, ##p value <0.01 compared to enzyme alone

G. H.

Figure 11

A.

Skin		JR-051 (mg/kg)		
		0.5	1	3
AT1001 (mg/kg)	0	85.7	90.7	97.4
	0.3	92.5 #	95.5	97.7
	1	90.0	95.9	98.7 #
	3	95.4 #	97.0 #	98.7 #
	10	94.6 #	97.6 #	99.2 #

B.

HEART		JR-051 (mg/kg)		
		0.5	1	3
AT1001 (mg/kg)	0	72.1	82.7	93.7
	0.3	82.7 #	87.9	93.5
	1	77.1	88.0	98.3 #
	3	94.7 #	98.5 #	98.6 #
	10	94.7 #	97.0 #	98.6 #

C.

KIDNEY		JR-051 (mg/kg)		
		0.5	1	3
AT1001 (mg/kg)	0	42.7	54.9	69.5
	0.3	48.5	59.1	64.8
	1	40.1	58.3	78.8 #
	3	66.3 #	79.4 #	76.0
	10	58.5 #	64.5	77.1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/50721

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/43; C07H 21/04; C07K 1/00; C12N 9/40 (2013.01)
USPC - 424/94.1; 514/328; 536/23.1, 23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 38/43, 31/445; C07H 21/04; C07K 1/00; C12N 9/40, 15/00; C12P 19/34, 21/06; A61P 3/00 (2013.01)
USPC - 424/94.1; 514/328; 435/69.1, 91.1, 208, 320.1; 530/350; 536/23.1, 23.2

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); IP.com; Google Scholar; PubMed; galactosidase A, 1-deoxygalactononirimycin, parenteral, intravenous, injection, pharmaceutical, formulation, composition, Fabry disease, pharmacologic chaperone

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/0077806 A1 (SELDEN, RF et al.) April 24, 2003; paragraphs [0145], [0151]	1-16
A	US 7,910,545 B2 (MEEKER, D et al.) March 22, 2011; column 13, lines 40-46; column 18, lines 52-56; column 28, lines 38-44	1-6
A	US 2011/0143419 A1 (DO, HV) June 16, 2011; paragaphs [0053], [0068]	1-6
A	US 2006/0153829 A1 (FAN, JQ) July 13, 2006; paragraphs [0087], [0117]	1-6
A	EP 1 171 128 B1 (DWEK, RA et al.) June 18, 2003; paragraph [0007]	7-16
A	US 2010/0113517 A1 (PALLING, D) May 6, 2010; paragraphs [0018], [0064]	7-16
A	US 7,750,050 B2 (SCHUCHMAN, EH et al.) July 6, 2010; column 2, lines 40-44; column 11, lines 36-45; column 14, lines 4-17	7-16
A	US 7,833,742 B2 (TRECO, DA et al.) November 16, 2010; column 2, lines 37-48; column 25, lines 23-34	1-16
A	US 7,446,098 B2 (FAN, JQ) November 4, 2008; column 11, lines 8-55; column 21, lines 20-30	7-16

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

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2013 (12.12.2013)	17 DEC 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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