



- (51) International Patent Classification:
A61K 38/46 (2006.01) *A61K 38/47* (2006.01)
- (21) International Application Number:
PCT/US2013/028608
- (22) International Filing Date:
1 March 2013 (01.03.2013)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/606,089 2 March 2012 (02.03.2012) US
- (71) Applicant: SHIRE HUMAN GENETIC THERAPIES, INC. [US/US]; 300 Shire Way, Lexington, MA 02421 (US).
- (72) Inventors: CROMBEZ, Eric; 300 Shire Way, Boston, MA (US). BHIRANG, Kiran; 300 Shire Way, Demarest, NJ (US). COHN, Gabriel, Martin; 300 Shire Way, East Longmeadow, MA (US).
- (74) Agent: LAWRENCE, Laurie, Butler; Lando & Anastasi LLP, Riverfront Office Park, One Main Street, Suite 02142, Cambridge, MA 02142 (US).

- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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

(54) Title: COMPOSITIONS AND METHODS FOR TREATING TYPE III GAUCHER DISEASE

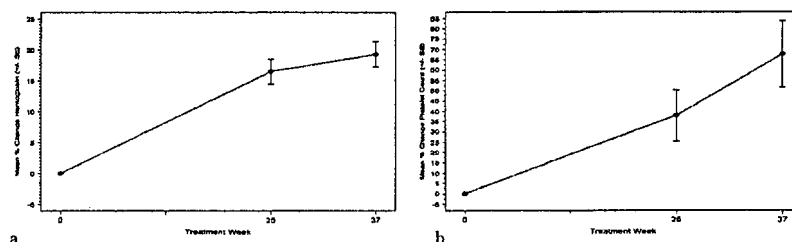


FIG. 1

(57) Abstract: Methods and compositions for treating Gaucher disease are described.

COMPOSITIONS AND METHODS FOR TREATING TYPE III GAUCHER DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/606,089, filed on March 2, 2012. The disclosure of the prior application is considered part of (and is incorporated by reference in) the disclosure of this application.

5

BACKGROUND

Gaucher disease is an autosomal recessive lysosomal storage disorder characterized by a deficiency in the lysosomal enzyme, glucocerebrosidase (GCB). GCB hydrolyzes the glycolipid glucocerebroside that is formed after degradation of glycosphingolipids in the membranes of white blood cells and red blood cells. The deficiency in this enzyme causes glucocerebroside to accumulate in large quantities in the lysosomes of phagocytic cells located in the liver, spleen and bone marrow of Gaucher patients. Accumulation of these molecules causes a range of clinical manifestations including splenomegaly, hepatomegaly, skeletal disorder, thrombocytopenia and anemia. (Beutler et al. Gaucher disease; In: The Metabolic and Molecular Bases of Inherited Disease (McGraw-Hill, Inc, New York, 1995) pp.2625-2639)

10
15

20

Treatments for patients suffering from this disease include administration of analgesics for relief of bone pain, blood and platelet transfusions and, in some cases, splenectomy. Joint replacement is sometimes necessary for patients who experience bone erosion.

25

Enzyme replacement therapy with GCB has been used as a treatment for Gaucher disease. Current treatment of patients with Gaucher disease includes administration of a carbohydrate remodeled GCB derived from human placenta or Chinese hamster ovary (CHO) cells transfected with a GCB expression construct and known as alglucerase or imiglucerase, respectively.

SUMMARY

The disclosure is based, in part, on the discovery that velaglucerase elicits less of an immune response (e.g., less production of antibody, e.g., less production of

neutralizing antibody) than imiglucerase upon administration to a subject (e.g., a subject with Type III Gaucher disease). The invention relates, *inter alia*, to compositions and methods for selecting a treatment for a subject with Type III Gaucher disease, selecting subjects for treatment with velaglucerase (e.g., alone or in combination with another therapy), methods for reducing injection site reaction in subjects undergoing treatment for Type III Gaucher disease, and methods of treating Type III Gaucher disease. The invention also relates, *inter alia*, to methods of determining antibody production, e.g., neutralizing antibody production, in a subject being treated for Type III Gaucher disease. The present disclosure is at least in part on the premise that a glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase, or uplyso), will be able to cross the blood-brain barrier and thus can be used to treat, or prevent, or prevent progression of a neurological parameter or a neurological symptom, e.g., a neurological parameter or a neurological symptom associated with Type III Gaucher disease.

In some aspects, the disclosure features a method of treating a subject with Type III Gaucher disease, the method comprising

administering a glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase, or uplyso) by intravenous infusion to the subject over a period of less than 2 hours, e.g., 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50 minutes or 45 minutes, to thereby treat the subject.

In some embodiments, the glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase, uplyso) is administered more than once and each additional dose of the glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase, or uplyso) is administered by intravenous administration over a period of less than 2 hours, e.g., 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50 minutes or 45 minutes), to the subject.

In some embodiments, the glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase, uplyso) is administered at a dose of 15 to 60 U/kg (e.g., 30 U/kg to 60 U/kg, e.g., 15 U/kg, 30 U/kg, 45U/kg, or 60 U/kg), at a dose equal to or below 22.5 U/kg, at a dose between 22.5 and 37.5 U/kg, at a dose between 37.5 and 52.5 U/kg, or at a dose equal to or above 52.5 U/kg. In some embodiments, glucocerebrosidase

enzyme replacement therapy (e.g., velaglucerase, imiglucerase, uplyso) is administered at a dose of 2.5 U/kg to 60 U/kg. In some embodiments, the glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase, uplyso) is administered every other week. In other embodiments, the glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase, uplyso) is administered every week. In some
5 embodiments, the velaglucerase is administered three times a week by intravenous infusion, e.g., at a dose of 2.5 U/kg.

 In some embodiments, the infusion of one or more dose (e.g., a dose described herein) occurs over 60 minutes.

10 In some embodiments, the glucocerebrosidase enzyme replacement therapy is velaglucerase and the method includes:

 reconstituting lyophilized velaglucerase with a pharmaceutically acceptable carrier such as Sterile Water for Injection (e.g., reconstituting a 200 unit vial with 2.2 mL of Sterile Water for Injection or a 400 unit vial with 4.3 mL Sterile Water for Injection),
15 thereby forming a solution, e.g., wherein the vial is not shaken after addition of the Sterile Water for Injection; optionally, inspecting the solution in the vials (and, e.g., optionally determining if the solution is discolored or if particulate matter is present, and optionally deciding not to use the solution if the solution is discolored or if particulate matter is present);

20 withdrawing a volume of solution to provide a pre-selected dose (e.g., a dose described herein such as 15 U/kg, 30 U/kg, 45 U/kg, or 60 U/kg);

 diluting the volume, e.g., in 100mL of 0.9% sodium chloride solution suitable for intravenous administration, thereby forming a diluted solution; optionally rocking the diluted solution gently, but do not shaking the diluted solution; and

25 administering the diluted solution to the subject by intravenous infusion, e.g., wherein the diluted solution is administered over one hour or at a rate of 1 U/kg/minute.

 In some embodiments, the glucocerebrosidase enzyme replacement therapy is administered at an infusion rate of 2 U/kg/minute, 1.5 U/kg/minute, 1 U/kg/minute, or 0.5 U/kg/minute.

30 In some embodiments, the administering comprises home therapy (e.g., in the subject's home, workplace, or other non-clinical (e.g., non-hospital) setting). In some

embodiments, the administering (e.g., via infusion) is by a health care professional (e.g., nurse or physician's assistant). For example, if the subject has not experienced an adverse event (AE) (e.g., a drug-related serious AE or an infusion-related AE, e.g., an event described herein), e.g., after one, two, or three administrations (e.g., via infusion) of the glucocerebrosidase enzyme replacement therapy, the subject is eligible to receive home therapy for subsequent administrations.

In another aspect, the disclosure provides a method for identifying a subject as suitable for (e.g., being a candidate for) treatment with glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase). The method includes evaluating (e.g., measuring, e.g., by a method described herein such as ELISA or radioimmunoprecipitation assay (RIP)), for the presence of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy (e.g., to the therapy currently being administered to the subject) (e.g., imiglucerase or uplyso) in a sample from the subject, e.g., and comparing the measured value of antibody to the therapy to a standard (e.g., a negative control). For example, if the subject has measured antibody values that are greater than the value measured for the negative control (e.g., a negative control in an ELISA), the subject is identified as having antibodies to the glucocerebrosidase enzyme replacement therapy. The subject is optionally identified as suitable for treatment with an alternative Type III Gaucher disease treatment (e.g., treatment with velaglucerase), e.g., if the measured value is greater than the value for the standard, e.g., by more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%.

In some embodiments, the sample is a blood or serum sample. In some embodiments, the sample has been modified. For example, the measured antibody values were obtained by contacting the sample with an analytical reagent, e.g., a labeled reagent (e.g., labeled glucocerebrosidase), and/or a substrate or cell, e.g., a substrate or cell that binds to an antibody to the glucocerebrosidase enzyme replacement therapy. In some embodiments, the measured antibody values were obtained from a sample that has been enriched for antibodies, e.g., a concentrated portion of a blood or serum sample. In some embodiments, the evaluation was obtained by a method described herein.

In some embodiments, the method includes measuring the measured antibody value, e.g., by a method described herein.

In another aspect, the disclosure provides a method for selecting a subject with Type III Gaucher disease for treatment with a glucocerebrosidase enzyme replacement therapy. Optionally, the method includes selecting a subject for such treatment on the basis that the subject has tested positive for the production of antibodies (e.g., neutralizing antibodies) to the treatment the subject is currently taking for Type III Gaucher disease. The method includes evaluating (e.g., measuring, e.g., by a method described herein such as ELISA or radioimmunoprecipitation assay (RIP)) or obtaining an evaluation of the presence of antibodies to a glucocerebrosidase enzyme replacement therapy (e.g., to the therapy currently being administered to the subject) (e.g., imiglucerase or uplyso) in a sample from the subject, e.g., and comparing the measured value of antibody to the therapy to a standard (e.g., a negative control). For example, if the subject has measured antibody values that are greater than the value measured for the negative control (e.g., negative control in an ELISA), the subject is identified as having antibodies to the glucocerebrosidase enzyme replacement therapy. The methods optionally include, selecting an alternative Type III Gaucher disease treatment for the subject (e.g., treatment with velaglucerase), e.g., if the measured value is greater than the value for the standard (e.g., negative control), e.g., by more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%.

In some embodiments, the sample is a blood or serum sample. In some embodiments, the sample has been modified. For example, the measured antibody values were obtained by contacting the sample with an analytical reagent, e.g., a labeled reagent (e.g., labeled glucocerebrosidase), and/or a substrate or cell, e.g., a substrate or cell that binds to an antibody to the glucocerebrosidase enzyme replacement therapy. In some embodiments, the measured antibody values were obtained from a sample that has been enriched for antibodies, e.g., a concentrated portion of a blood or serum sample. In some embodiments, the evaluation was obtained by a method described herein.

In some embodiments, the method includes measuring the antibody value, e.g., by a method described herein.

In some embodiments, the subject tested positive for the production of IgE antibodies to the treatment the subject is currently taking for Type III Gaucher disease (e.g., imiglucerase or uplyso).

In some embodiments, the subject tested positive for the production of IgM antibodies to the treatment the subject is currently taking for Type III Gaucher disease (e.g., imiglucerase or uplyso).

In some embodiments, the subject tested positive for the production of IgG and/or IgA antibodies to the treatment the subject is currently taking for Type III Gaucher disease (e.g., imiglucerase or uplyso).

In another aspect, the disclosure provides a method for treating a subject with Type III Gaucher disease. The method includes selecting a subject on the basis that the subject has tested positive for the production of antibodies (e.g., neutralizing antibodies) to the treatment the subject is currently taking for Type III Gaucher disease, e.g., by a method described herein, or on the basis that the subject is at risk for developing antibodies (e.g., neutralizing antibodies) to a treatment for Type III Gaucher disease (e.g., imiglucerase or uplyso) and administering velaglucerase to the subject.

In some embodiments, the subject tested positive for the production of IgE antibodies to the treatment the subject is currently taking for Type III Gaucher disease (e.g., imiglucerase or uplyso).

In some embodiments, the subject tested positive for the production of IgM antibodies to the treatment the subject is currently taking for Type III Gaucher disease (e.g., imiglucerase or uplyso).

In some embodiments, the subject tested positive for the production of IgG and/or IgA antibodies to the treatment the subject is currently taking for Type III Gaucher disease (e.g., imiglucerase or uplyso).

In some embodiments, velaglucerase is administered at a dose of 15 to 60 U/kg (e.g. 30 U/kg to 60 U/kg, e.g., 15 U/kg, 30 U/kg, 45U/kg, or 60 U/kg), at a dose equal to or below 22.5 U/kg, at a dose between 22.5 and 37.5 U/kg, at a dose between 37.5 and 52.5 U/kg, or at a dose equal to or above 52.5 U/kg. In some embodiments, velaglucerase is administered at a dose of 2.5 U/kg to 60 U/kg. In some embodiments,

the velaglucerase is administered every other week by intravenous infusion. In other embodiments, the velaglucerase is administered every week by intravenous infusion. In some embodiments, the velaglucerase is administered three times a week by intravenous infusion, e.g., at a dose of 2.5 U/kg.

5 In some embodiments, the infusion of the dose (e.g., a dose described herein) occurs over less than 2 hours, e.g., 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50 minutes or 45 minutes. In preferred embodiments, the infusion of the dose occurs over 60 minutes.

In some embodiments, the method includes:

10 reconstituting lyophilized velaglucerase with a pharmaceutically acceptable carrier such as a pharmaceutically acceptable carrier such as Sterile Water for Injection (e.g., reconstituting a 200 unit vial with 2.2 mL of Sterile Water for Injection or a 400 unit vial with 4.3 mL Sterile Water for Injection), thereby forming a solution, e.g., wherein the vial is not shaken after addition of the Sterile Water for Injection; optionally, inspecting
15 the solution in the vials (and, e.g., optionally determining if the solution is discolored or if particulate matter is present, and optionally deciding not to use the solution if the solution is discolored or if particulate matter is present);

withdrawing a volume of solution to provide a pre-selected dose (e.g., a dose described herein, e.g., 15 U/kg, 30 U/kg, 45U/kg, or 60 U/kg);

20 diluting the volume, e.g., in 100mL of 0.9% sodium chloride solution suitable for intravenous administration, thereby forming a diluted solution; optionally rocking the diluted solution gently, but do not shaking the diluted solution; and

administering the diluted solution to the subject by intravenous infusion.

In some embodiments, velaglucerase is administered at a rate of 2 U/kg/minute,
25 1.5 U/kg/minute, 1 U/kg/minute, or 0.5 U/kg/minute.

In some embodiments, the administering comprises home therapy (e.g., in the subject's home, workplace, or other non-clinical (e.g., non-hospital) setting). In some embodiments, the administering (e.g., via infusion) is by a health care professional (e.g., nurse or physician's assistant). For example, if the subject has not experienced an
30 adverse event (AE) (e.g., a drug-related serious AE or an infusion-related AE, e.g., an event described herein), e.g., after one, two, or three administrations (e.g., via infusion) of

velaglucerase, the subject is eligible to receive home therapy for subsequent administrations.

In another aspect, the disclosure provides a method for identifying a subject as suitable for (e.g., being a candidate for) treatment with glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase). The method includes evaluating (e.g., measuring) the subject for the presence of infusion site reaction (i.e., infusion-related adverse event) (e.g., during or within 12 hours of infusion of glucocerebrosidase enzyme replacement therapy) (e.g., to the therapy currently being administered to the subject, e.g., imiglucerase or uplyso), e.g., and comparing the measured level of the site reaction to a standard (e.g., a negative control). For example, if the subject has a measured value of site reaction to the treatment the subject is currently taking for Type III Gaucher disease that is greater than the value measured for the negative control (e.g., the site reaction observed after placebo infusion in the subject), the subject is identified as suitable for treatment with an alternative Type III Gaucher disease treatment (e.g., treatment with velaglucerase), e.g., if the measured value of site reaction to the treatment the subject is currently taking for Type III Gaucher disease is greater than the value for the standard, e.g., by more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%.

In some embodiments, the method includes determining the infusion site value, e.g., by a method described herein.

In another aspect, the disclosure provides a method for selecting a subject with Type III Gaucher disease for treatment with glucocerebrosidase enzyme replacement therapy. Optionally, the method includes selecting a subject for such treatment on the basis that the subject is in need of reduced infusion site reaction (e.g., reduced as compared to the reaction associated with or caused by the treatment the subject is currently taking for Type III Gaucher disease, e.g., imiglucerase or uplyso). The method includes evaluating (e.g., measuring) or obtaining an evaluation of the subject for the presence of infusion site reaction (i.e., infusion-related adverse event) (e.g., during or within 12 hours of infusion of glucocerebrosidase enzyme replacement therapy) (e.g., to

the therapy currently being administered to the subject, e.g., imiglucerase or uplyso), e.g., and comparing the measured level of the site reaction to a standard (e.g., a negative control). For example, if the subject has a measured value of site reaction to the treatment the subject is currently taking for Type III Gaucher disease that is greater than
5 (e.g., greater by more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%) the value measured for the negative control (e.g., the site reaction observed after placebo infusion in the subject), the subject is selected for an alternative Type III Gaucher disease treatment (e.g., treatment with velaglucerase), e.g., if the measured value of site reaction to the treatment the subject is
10 currently taking for Type III Gaucher disease is greater than the value for the standard (e.g., negative control), e.g., by more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%.

In some embodiments, the method includes measuring the antibody value, e.g., by a method described herein.

15 In another aspect, the disclosure provides a method for treating a subject with Type III Gaucher disease. The method includes selecting a subject for such treatment on the basis that the subject is in need of reduced infusion site reaction (e.g., reduced as compared to the reaction associated with or caused by the treatment the subject is
20 currently taking for Type III Gaucher disease, e.g., imiglucerase or uplyso), e.g., by a method described herein, and administering velaglucerase to the subject.

In some embodiments, velaglucerase is administered at a dose of 15 to 60 U/kg (e.g. 30 U/kg to 60 U/kg, e.g., 15 U/kg, 30 U/kg, 45U/kg, or 60 U/kg), at a dose equal to or below 22.5 U/kg, at a dose between 22.5 and 37.5 U/kg, at a dose between 37.5 and
25 52.5 U/kg, or at a dose equal to or above 52.5 U/kg. In some embodiments, velaglucerase is administered at a dose of 2.5 U/kg to 60 U/kg. In some embodiments, the velaglucerase is administered every other week by intravenous infusion. In other embodiments, the velaglucerase is administered every week by intravenous infusion. In some embodiments, the velaglucerase is administered three times a week by intravenous
30 infusion, e.g., at a dose of 2.5 U/kg.

In some embodiments, the infusion of the dose (e.g., a dose described herein) occurs over less than 2 hours, e.g., 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50 minutes or 45 minutes.

In some embodiments, the method includes:

5 reconstituting lyophilized velaglucerase with a pharmaceutically acceptable carrier, e.g., Sterile Water for Injection (e.g., reconstituting a 200 unit vial with 2.2 mL of Sterile Water for Injection or a 400 unit vial with 4.3 mL Sterile Water for Injection), thereby forming a solution, e.g., wherein the vial is not shaken after addition of the Sterile Water for Injection; optionally, inspecting the solution in the vials (and, e.g., optionally
10 determining if the solution is discolored or if particulate matter is present, and optionally deciding not to use the solution if the solution is discolored or if particulate matter is present);

withdrawing a volume of solution to provide a pre-selected dose (e.g., a dose described herein such as 15 U/kg, 30 U/kg, 45 U/kg, or 60 U/kg);

15 diluting the volume, e.g., in 100mL of 0.9% sodium chloride solution suitable for intravenous administration, thereby forming a diluted solution; optionally rocking the diluted solution gently, but do not shaking the diluted solution; and

administering the diluted solution to the subject by intravenous infusion.

In some embodiments, the velaglucerase is administered at a rate of 2.0
20 U/kg/minute, 1.5 U/kg/minute, 1.0 U/kg/minute, or 0.5 U/kg/minute.

In some embodiments, the administering comprises home therapy (e.g., in the subject's home, workplace, or other non-clinical (e.g., non-hospital) setting). In some embodiments, the administering (e.g., via infusion) is by a health care professional (e.g., nurse or physician's assistant). For example, if the subject has not experienced an
25 adverse event (AE) (e.g., a drug-related serious AE or an infusion-related AE, e.g., an event described herein), e.g., after one, two, or three administrations (e.g., via infusion) of velaglucerase, the subject is eligible to receive home therapy for subsequent administrations.

30 In some aspects, the disclosure features a method of selecting a subject with Type III Gaucher disease for treatment with velaglucerase, the method comprising

identifying a subject with Type III Gaucher disease that has received a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or uplyso) and has a platelet count less than a standard; and

selecting the subject for treatment with velaglucerase on the basis that the subject
5 has a platelet count less than the standard.

In some embodiments, the method includes evaluating (e.g., measuring platelet count or obtaining an evaluation of platelet count of a sample from the subject, and comparing the measured value of the platelet count to the standard (e.g., negative control).

10 In some embodiments, the sample is a blood or serum sample. In some embodiments, the sample has been modified. In some embodiments, the measured antibody values were obtained from a sample that has been enriched for platelets, e.g., a concentrated portion of a blood sample. In some embodiments, the evaluation was obtained by a method described herein.

15 In some embodiments, the method includes measuring the platelet count, e.g., by a method described herein.

In some embodiments, the methods further includes administering velaglucerase to the subject, e.g., at a dose and/or dosing schedule described herein.

20 In some embodiments, the velaglucerase is administered by intravenous infusion over a course of less than 2 hours, e.g., 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50 minutes or 45 minutes. In some embodiments, the infusion occurs at a rate of 2 U/kg/minute, 1.5 U/kg/minute, 1 U/kg per minute or 0.5 U/kg/minute.

25 In some embodiments, the standard is a platelet count below or equal to 80×10^3 platelets/mm³. In some embodiments, the standard is based on the percentage increase in mean platelet count after 6, 9, or 12 months of treatment with the glucocerebrosidase enzyme replacement therapy. For example, a subject in which the mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g.,
30 imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) is identified for treatment with velaglucerase. As another example, a subject in which the

mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) is
5 identified for treatment with velaglucerase.

In some aspects, the disclosure features a method of treating a subject with Type III Gaucher disease, the method comprising

selecting a subject with Type III Gaucher disease that has received a
10 glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or uplyso) and has a platelet count less than a standard, e.g., a subject identified by a method described herein; and

administering velaglucerase to the subject.

In some embodiments, velaglucerase is administered at a dose of 15 to 60 U/kg
15 (e.g. 30 U/kg to 60 U/kg, e.g., 15 U/kg, 30 U/kg, 45 U/kg, or 60 U/kg), at a dose equal to or below 22.5 U/kg, at a dose between 22.5 and 37.5 U/kg, at a dose between 37.5 and 52.5 U/kg, or at a dose equal to or above 52.5 U/kg. In some embodiments, velaglucerase is administered at a dose of 2.5 U/kg to 60 U/kg. In some embodiments, the velaglucerase is administered every other week by intravenous infusion. In other
20 embodiments, the velaglucerase is administered every week by intravenous infusion. In some embodiments, the velaglucerase is administered three times a week by intravenous infusion, e.g., at a dose of 2.5 U/kg.

In some embodiments, the infusion of the dose (e.g., a dose described herein) occurs over less than 2 hours, e.g., 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50
25 minutes or 45 minutes.

In some embodiments, the method includes:

reconstituting lyophilized velaglucerase with a pharmaceutically acceptable carrier such as Sterile Water for Injection (e.g., reconstituting a 200 unit vial with 2.2 mL of Sterile Water for Injection or a 400 unit vial with 4.3 mL Sterile Water for Injection),
30 thereby forming a solution, e.g., wherein the vial is not shaken after addition of the Sterile Water for Injection; optionally, inspecting the solution in the vials (and, e.g., optionally

determining if the solution is discolored or if particulate matter is present, and optionally deciding not to use the solution if the solution is discolored or if particulate matter is present);

withdrawing a volume of solution to provide a pre-selected dose (e.g., a dose
5 described herein such as 15 U/kg, 30 U/kg, 45 U/kg, or 60 U/kg);

diluting the volume, e.g., in 100mL of 0.9% sodium chloride solution suitable for intravenous administration, thereby forming a diluted solution; optionally rocking the diluted solution gently, but do not shaking the diluted solution; and

administering the diluted solution to the subject by intravenous infusion.

10 In some embodiments, the velaglucerase is administered at a rate of 2 U/kg/minute, 1.5 U/kg/minute, 1 U/kg/minute or 0.5 U/kg/minute.

In some embodiments, the administering comprises home therapy (e.g., in the subject's home, workplace, or other non-clinical (e.g., non-hospital) setting). In some
15 embodiments, the administering (e.g., via infusion) is by a health care professional (e.g., nurse or physician's assistant). For example, if the subject has not experienced an adverse event (AE) (e.g., a drug-related serious AE or an infusion-related AE, e.g., an event described herein), e.g., after one, two, or three administrations (e.g., via infusion) of velaglucerase, the subject is eligible to receive home therapy for subsequent
20 administrations.

In another aspect, the disclosure provides a method for evaluating a subject, e.g., a
25 subject to whom a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso) is currently being administered. The method includes evaluating (e.g., measuring) or obtaining an evaluation of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of the following parameters of the subject:

hemoglobin concentration, platelet count, liver volume (e.g., as a percentage of
30 total body weight), spleen volume (e.g., as a percentage of total body weight), infusion site reaction, a skeletal parameter, a neurological parameter, or presence of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy (e.g., the mean value of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of these parameters).

The skeletal parameter can be, e.g., bone mineral density (BMD) (e.g., as measured by a change (e.g., improvement) in Z score). BMD can be evaluated e.g., by dual energy X-ray absorptiometry (DEXA). Other skeletal parameters that can be evaluated include, e.g., T-score (e.g., to determine WHO classification of normal bone, osteopenia, and osteoporosis), growth of a subject (e.g., a pediatric subject); skeletal age of a subject (e.g., a pediatric subject); and bone marrow burden (BMB) (e.g., in an adult subject).

The neurological parameter can be e.g., an eye movement parameter (e.g., abnormal eye movements, abnormal vertical saccades, abnormal horizontal saccades, slow horizontal saccades, and supranuclear ophthalmoplegia). Methods of assessing eye movement parameters can include but are not limited to, e.g., general ophthalmologic assessment, and saccadic eye movement assessment (e.g., sclera search coil technique, video technique, and infrared technique). The neurological parameter can be a hearing parameter (e.g., hearing loss. Methods of assessing hearing parameters can include but are not limited to, general auditory assessment. The neurological parameter can be, e.g., abnormal brainstem auditory evoked potentials, cranial nerve palsies, seizures, progressive myoclonic seizures, tremors, EEG abnormalities, dementia, cognitive impairment, ataxia, loss of muscle coordination, and poor mobility. Methods of assessing neurological parameters can include but are not limited to, e.g., EEG, brainstem auditory evoked response (BAER), measurement of auditory evoked potentials (e.g., electrode caps), measurement of somatosensory evoked potentials (e.g., median nerve stimulation and stretch evoked methods), mental state assessment (e.g., the neuropsychological mental status exam), cranial nerve exam, motor skill assessment (e.g., Bruininks-Oseretsky test of motor proficiency, the school function assessment test, the Beery-Buktenica developmental test of visual-motor integration, the clinical observation of motor and postural skills (COMPS)), seizure assessment (e.g., neuroimaging (e.g., CT, MRI, diffusion weighted imaging), EEG, patient history, physical examination), manual dexterity and hand eye coordination testing, (e.g., Purdue Pegboard test), standardized IQ testing (e.g., Wechsler IQ scale), verbal performance full scale IQ test, Rey auditory verbal learning test, measures of attention (e.g., D2 test of attention), continuous performance test, trail making test, Benton visual retention test, measures of memory

(e.g., Wechsler Memory Scale-Revised, presentation of stimuli to later be recalled, California Verbal Learning Test, Rey Auditory Verbal Learning Test, selective reminding test, Benton Test of Visual Retention-Revised, Memory for Designs Test, Rey-O Complex Figure), assessment of cognitive function (e.g., Wechsler Similarities Subtest, Wisconsin card sorting test, category test, stroop test, neurological test batteries (e.g., the flexible battery, Halstead-Reitan neuropsychological battery, Luria-Nebraska neuropsychological battery), and psychometric exam. The evaluating can be performed, e.g., about every week, about every two weeks, about every three weeks, about every four weeks, about every two months, about every three months, about every four months, about every five months, about every six months, about every seven months, about every eight months, about every nine months, about every ten months, about every eleven months, or about every twelve months during the course of treatment. The evaluating can also be performed prior to commencing treatment (e.g., to establish a baseline value). The evaluating can include comparing the value of the parameter from the subject to a standard (e.g., a standard described herein, e.g., a negative control), and optionally determining if a difference exists between the value of the parameter from the subject and the value from the standard. The standard can be, e.g., a value of the parameter measured in a subject with Type III Gaucher disease being treated with a different therapy for Type III Gaucher disease (e.g., a treatment described herein), or a mean value for a cohort of such subjects (e.g., after the same length of therapy), or the baseline value for the subject (or a mean baseline value for a cohort of subjects with Type III Gaucher disease) prior to commencing therapy (e.g., prior to commencement of a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or velaglucerase)). For example, a skeletal parameter (e.g., bone mineral density) can be evaluated for long term changes, e.g., after 1, 2, 3, 4, or more years of glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso).

The evaluating can include determining if one or more of the following are present:

the difference between one or more of the hemoglobin concentration, platelet count, liver volume, spleen volume, a neurological parameter, or a skeletal parameter and that of a standard (for the given parameter) is greater than 5%, 10%, 15%, 20%, 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%.

Alternatively or in addition, the evaluating can include determining if an infusion site reaction is present (e.g., during or within 12 hours after infusion) and/or if antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or uplyso) are present.

In some embodiments, the evaluation can be of one or more of hemoglobin concentration, platelet count and determining if antibodies (e.g., neutralizing antibodies) to a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or uplyso) are present. In some embodiments, the evaluation for one or more of these parameters is performed on a sample from the subject, e.g., a blood or serum sample. In some embodiments, the sample has been modified. For example, the values were obtained by contacting the sample with an analytical reagent and/or a substrate or cell, e.g., a substrate or cell that binds to an antibody to the glucocerebrosidase enzyme replacement therapy. In some embodiments, the values were obtained from a sample that has been enriched for, e.g., hemoglobin, platelets and/or antibodies, e.g., a concentrated portion of a blood or serum sample. In some embodiments, the evaluation was obtained by a method described herein.

Based on the determination, a treatment decision can be made for the subject. For example, if a subject receiving a treatment for Type III Gaucher disease, such as a glucocerebrosidase enzyme replacement therapy, e.g., imiglucerase or uplyso, has a value for one or more of the parameters that differs from the value for a standard (e.g., a subject with Type III Gaucher disease who is receiving a different therapy (velaglucerase)), a decision can be made to transfer the subject currently receiving a glucocerebrosidase enzyme replacement treatment (e.g., imiglucerase treatment) to a different glucocerebrosidase enzyme replacement treatment (e.g., velaglucerase treatment). For example, if antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to imiglucerase are detected in a subject undergoing treatment with imiglucerase, the subject can be transferred to treatment with velaglucerase. As another example, if an infusion site reaction is detected during or within 12 hours of administration of imiglucerase to a subject undergoing treatment with imiglucerase, the

subject can be transferred to treatment with velaglucerase. As another example, if the mean platelet count in a subject undergoing treatment with imiglucerase is lower (e.g., 5%, 10%, %, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% lower) than the mean platelet count value obtained for a cohort of subjects with Type III Gaucher disease treated with velaglucerase for the same duration at the same dose and same frequency of treatment administration, the subject can be transferred to velaglucerase treatment.

In another aspect, the disclosure provides a method of selecting a treatment for administration to a subject with Type III Gaucher disease, the method comprising selecting a treatment on the basis that the treatment can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment. Optionally, the method can include providing the treatment to the subject, e.g., wherein providing includes administering the treatment or transferring the treatment to the subject's possession. In some embodiments, the treatment can be administered at a dose and/or dosing schedule described herein.

The method can include evaluating (e.g., measuring) or obtaining an evaluation of one or more of these parameters, e.g., by a method described herein.

In one aspect, the disclosure provides a method of selecting a treatment for administration to a subject in need of an increase in hemoglobin concentration, an increase in platelet level, a decrease in liver volume, a decrease in spleen volume, a decreased likelihood of injection site reaction, a change in a skeletal parameter (e.g., an

increase in bone mineral density), a change in a neurological parameter (e.g., improvement in a neurological parameter), and/or a decreased likelihood of production of antibodies to the treatment, the method comprising

selecting a treatment on the basis that the treatment can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), a change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment. Optionally, the method can include providing the treatment to the subject, e.g., wherein providing includes administering the treatment or transferring the treatment to the subject's possession. In some embodiments, the treatment can be administered at a dose and/or dosing schedule described herein.

The method can include evaluating (e.g., measuring) or obtaining an evaluation of one or more of these parameters, e.g., by a method described herein.

In another aspect, the disclosure provides a method for evaluating a subject, e.g., a subject who has been selected to receive treatment with a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso). The method includes evaluating (e.g., measuring) one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of the following parameters of the subject:

hemoglobin concentration, platelet count, liver volume (e.g., as a percentage of total body weight), spleen volume (e.g., as a percentage of total body weight), infusion site reaction, a skeletal parameter, a neurological parameter, or presence of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy (e.g., the mean value of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of these parameters).

The skeletal parameter can be, e.g., bone mineral density (BMD) (e.g., as measured by a change (e.g., improvement) in Z score). BMD can be evaluated e.g., by dual energy X-ray absorptiometry (DEXA). Other skeletal parameters that can be evaluated include, e.g., T-score (e.g., to determine WHO classification of normal bone, osteopenia, and osteoporosis), growth of a subject (e.g., a pediatric subject); skeletal age of a subject (e.g., a pediatric subject); and bone marrow burden (BMB) (e.g., in an adult subject).

The neurological parameter can be e.g., an eye movement parameter (e.g., abnormal eye movements, abnormal vertical saccades, abnormal horizontal saccades, slow horizontal saccades, and supranuclear ophthalmoplegia). Methods of assessing eye movement parameters can include but are not limited to, e.g., general ophthalmologic assessment, and saccadic eye movement assessment (e.g., sclera search coil technique, video technique, and infrared technique). The neurological parameter can be a hearing parameter (e.g., hearing loss. Methods of assessing hearing parameters can include but are not limited to, general auditory assessment. The neurological parameter can be, e.g., abnormal brainstem auditory evoked potentials, cranial nerve palsies, seizures, progressive myoclonic seizures, tremors, EEG abnormalities, dementia, cognitive impairment, ataxia, loss of muscle coordination, and poor mobility. Methods of assessing neurological parameters can include but are not limited to, e.g., EEG, brainstem auditory evoked response (BAER), measurement of auditory evoked potentials (e.g., electrode caps), measurement of somatosensory evoked potentials (e.g., median nerve stimulation and stretch evoked methods), mental state assessment (e.g., the neuropsychological mental status exam), cranial nerve exam, motor skill assessment (e.g., Bruininks-Oseretsky test of motor proficiency, the school function assessment test, the Beery-Buktenica developmental test of visual-motor integration, the clinical observation of motor and postural skills (COMPS)), seizure assessment (e.g., neuroimaging (e.g., CT, MRI, diffusion weighted imaging), EEG, patient history, physical examination), manual dexterity and hand eye coordination testing, (e.g., Purdue Pegboard test), standardized IQ testing (e.g., Wechsler IQ scale), verbal performance full scale IQ test, Rey auditory verbal learning test, measures of attention (e.g., D2 test of attention), continuous performance test, trail making test, Benton visual retention test, measures of memory

(e.g., Wechsler Memory Scale-Revised, presentation of stimuli to later be recalled, California Verbal Learning Test, Rey Auditory Verbal Learning Test, selective reminding test, Benton Test of Visual Retention-Revised, Memory for Designs Test, Rey-O Complex Figure), assessment of cognitive function (e.g., Wechsler Similarities Subtest, Wisconsin card sorting test, category test, stroop test, neurological test batteries (e.g., the flexible battery, Halstead-Reitan neuropsychological battery, Luria-Nebraska neuropsychological battery), and psychometric exam. The evaluating can include comparing the value of the parameter from the subject to a standard (e.g., a standard described herein, e.g., a negative control), and optionally determining if a difference exists between the value of the parameter from the subject and the value from the standard. The standard can be, e.g., a value of the parameter measured in a subject with Type III Gaucher disease being treated with a different therapy for Type III Gaucher disease (e.g., a treatment described herein), or a mean value for a cohort of such subjects (e.g., after the same length of therapy), or the baseline value for the subject (or a mean baseline value for a cohort of subjects with Type III Gaucher disease) prior to commencing therapy.

The evaluating can include determining if:

the difference between one or more of the hemoglobin concentration, platelet count, liver volume, spleen volume, a neurological parameter, or a skeletal parameter (e.g., measured as bone mineral density (BMD)) and that of a standard (for the given parameter) is greater than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90%.

In some embodiments, the evaluation can be of one or more of hemoglobin concentration, platelet count and determining if antibodies (e.g., neutralizing antibodies) to a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or uplyso) are present. In some embodiments, the evaluation for one or more of these parameters is performed on a sample from the subject, e.g., a blood or serum sample. In some embodiments, the sample has been modified. For example, the values were obtained by contacting the sample with an analytical reagent and/or a substrate or cell, e.g., a substrate or cell that binds to an antibody to the glucocerebrosidase enzyme replacement therapy. In some embodiments, the values were obtained from a sample that has been

enriched for, e.g., hemoglobin, platelets and/or antibodies, e.g., a concentrated portion of a blood or serum sample. In some embodiments, the evaluation was obtained by a method described herein.

5 In another aspect, the disclosure provides a method of prescribing a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso), the method comprising:

receiving an identifier for the glucocerebrosidase enzyme replacement therapy, e.g., the chemical structure, chemical name, trade name or generic name of the glucocerebrosidase enzyme replacement therapy;

10 receiving information that the glucocerebrosidase enzyme replacement therapy has one or more of the following properties: can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase in bone mineral density), change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment;

20 selecting a subject in need of the glucocerebrosidase enzyme replacement therapy, e.g., on the basis that the subject is in need of one or more of: an increase hemoglobin concentration, an increase platelet count, a decrease liver volume, a decrease spleen volume, a decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the

likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment; and

causing the glucocerebrosidase enzyme replacement therapy to be
5 prescribed, dispensed, or administered to a subject.

In some embodiments, the subject is in need of one or more of an increase in hemoglobin concentration, an increase in platelet count and decrease in the likelihood of the production of antibodies (e.g., neutralizing antibodies) to a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or uplyso), or a change in a neurological
10 parameter (e.g., improvement in a neurological parameter). In some embodiments, a determination that a subject is in need of one or more of these changes is based upon an evaluation of one or more of these parameters performed on a sample from the subject, e.g., a blood or serum sample. In some embodiments, the sample has been modified. For example, the values were obtained by contacting the sample with an analytical reagent
15 and/or a substrate or cell, e.g., a substrate or cell that binds to an antibody to the glucocerebrosidase enzyme replacement therapy. In some embodiments, the values were obtained from a sample that has been enriched for, e.g., hemoglobin, platelets and/or antibodies, e.g., a concentrated portion of a blood sample. In some embodiments, the evaluation was obtained by a method described herein.

20 In another aspect, the disclosure provides a method of providing a recipient with information about, or with guidelines for, the use of a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or velaglucerase), the method comprising:

communicating to the recipient an identifier for the glucocerebrosidase
25 enzyme replacement therapy, e.g., the chemical structure, chemical name, trade name or generic name of the glucocerebrosidase enzyme replacement therapy;

communicating to the recipient information that the glucocerebrosidase enzyme replacement therapy has one or more of the following properties: can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen
30 volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g.,

imiglucerase or uplyso) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment;

receiving a request from the recipient to purchase the glucocerebrosidase enzyme replacement therapy; and

selling, shipping or transferring the glucocerebrosidase enzyme replacement therapy to the recipient.

In another aspect, the disclosure provides a method of providing a recipient with information about a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso), or with guidelines for, the use of a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase or velaglucerase), the method comprising:

providing an identifier for the glucocerebrosidase enzyme replacement therapy, e.g., the chemical structure, chemical name, trade name or generic name of the glucocerebrosidase enzyme replacement therapy;

providing information that the glucocerebrosidase enzyme replacement therapy has one or more of the following properties: can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase) for Type III Gaucher disease) of infusion site reaction, change in a skeletal parameter (e.g., increase bone mineral density), change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment;

memorializing, e.g., in a database, the identifier and the information; and transferring the memorialization (e.g., the memorialized identifier and information) to the recipient.

5 In another aspect, the disclosure provides a method of providing a recipient with information about a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso), or with guidelines for the use of a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso), the method comprising:

10 providing an identifier for the glucocerebrosidase enzyme replacement therapy, e.g., the chemical structure, chemical name, trade name or generic name of the glucocerebrosidase enzyme replacement therapy;

providing information that the glucocerebrosidase enzyme replacement therapy has one or more of the following properties: can increase hemoglobin
15 concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), change in a neurological parameter (e.g.,
20 improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment;

25 associating the identifier with the information, e.g., in a database or by physical association; and

transferring the associated identifier and information to the recipient.

In another aspect, the disclosure provides a database, medium, or computer
30 containing or programmed to contain:

an identifier for a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso), e.g., the chemical structure, chemical name, trade name or generic name of the glucocerebrosidase enzyme replacement therapy;

information that the glucocerebrosidase enzyme replacement therapy has one or more of the following properties: can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment; and

an associative function associating the identifier with the information, e.g., in a database or by physical association.

In another aspect, the disclosure provides a method of making a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso) available to a subject, the method comprising:

providing to the subject an identifier for the glucocerebrosidase enzyme replacement therapy, e.g., the chemical structure, chemical name, trade name or generic name of the glucocerebrosidase enzyme replacement therapy;

providing to the subject information that the glucocerebrosidase enzyme replacement therapy has one or more of the following properties: can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), change in a neurological

parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment; and

placing into commerce, a dose of the glucocerebrosidase enzyme replacement therapy which can be administered to, provided to, or purchased by the subject.

In another aspect, the disclosure provides a method of causing a subject to request a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso), the method comprising:

providing to the subject an identifier for the glucocerebrosidase enzyme replacement therapy, e.g., the chemical structure, chemical name, trade name or generic name of the glucocerebrosidase enzyme replacement therapy;

providing to the subject information that the glucocerebrosidase enzyme replacement therapy has one or more of the following can increase hemoglobin concentration, increase platelet count, decrease liver volume, decrease spleen volume, decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of infusion site reaction, change a skeletal parameter (e.g., increase bone mineral density), change in a neurological parameter (e.g., improvement in a neurological parameter), and/or decrease likelihood (e.g., relative to a standard, e.g., a standard described herein, e.g., the likelihood for a cohort of subjects receiving a different treatment (e.g., imiglucerase or uplyso) for Type III Gaucher disease) of production of antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the treatment; and

placing into commerce, a dose of the glucocerebrosidase enzyme replacement therapy which can be administered to, provided to, or purchased by the subject.

In another aspect, the disclosure features a method of selecting a payment class for a course of treatment with a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso) for a subject with Type III Gaucher disease . The method includes providing (e.g., receiving) an evaluation of whether or not the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy; and performing at least one of (1) if the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy selecting a first payment class, and (2) if the subject does not experience an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or does not produce antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy selecting a second payment class.

In some embodiments, assignment of the subject is to the first class and the assignment authorizes payment for a course of treatment (e.g., velaglucerase).

In some embodiments, assignment of the subject is to the second class and the assignment authorizes payment for a course of treatment (e.g., imiglucerase, velaglucerase or uplyso).

In some embodiments, the evaluation is whether or not a subject produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy and the evaluation was obtained by a method described herein.

In another aspect, the disclosure features a method of selecting a payment class for a course of treatment with a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso) for a subject with Type III Gaucher disease. The method includes providing (e.g., receiving) an evaluation of whether or not a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or whether or not the subject's mean platelet count increased by less than

80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months); and performing at least one of (1) if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months), selecting a first payment class, and (2) if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme

replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months), selecting a second payment class.

In some embodiments, assignment of the subject is to the first class and the assignment authorizes payment for a course of treatment (e.g., velaglucerase).

5 In some embodiments, assignment of the subject is to the second class and the assignment authorizes payment for a course of treatment (e.g., imiglucerase, velaglucerase or uplyso).

In some embodiments, the evaluation was obtained by a method described herein.

10 In one aspect, the disclosure features a method of providing information on which to make a decision about a subject with Type III Gaucher disease, or making such a decision. The method includes providing (e.g., by receiving) an evaluation of a subject, wherein the evaluation was made by a method described herein, e.g., by optionally, administering a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, 15 velaglucerase or uplyso), to the subject; providing a determination post administration of whether or not the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy, thereby providing a post administration determination; providing a comparison 20 of the post administration determination with a standard (e.g., a standard described herein), thereby, providing information on which to make a decision about a subject, or making such a decision.

In some embodiments, the method includes making the decision.

25 In some embodiments, the method also includes communicating the information to another party (e.g., by computer, compact disc, telephone, facsimile, email, or letter).

In some embodiments, the decision includes selecting a subject for payment, making or authorizing payment for a first course of action (e.g., treatment with velaglucerase) if the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., 30 IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy and a second course of action (e.g., treatment with imiglucerase, velaglucerase or

uplyso) if the subject does not experience an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or does not produce antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement.

5 In some embodiments, the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy and the course of action is authorization of a course of therapy (e.g., treatment with velaglucerase).

10 In some embodiments, the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy and the course of action is assigning the subject to a first class. In some embodiments, assignment to the first class will enable
15 payment for a treatment (e.g., velaglucerase) provided to the subject. In some embodiments, payment is by a first party to a second party. In some embodiments, the first party is other than the subject. In some embodiments, the first party is selected from a third party payer, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity. In some embodiments, the second party is selected from
20 the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is an insurance company and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some
25 embodiments, the first party is a governmental entity and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the treatment.

In some embodiments, the subject does not experience an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or does not produce antibodies (e.g.,
30 neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a

glucocerebrosidase enzyme replacement and the course of action is authorization of a course of therapy (e.g., imiglucerase, velaglucerase or uplyso).

In some embodiments, the subject does not experience an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or does not produce antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement and the course of action is assigning the subject to a second class. In some embodiments, assignment to the second class will enable payment for a treatment (e.g., imiglucerase, velaglucerase or uplyso) provided to the subject. In some embodiments, payment is by a first party to a second party. In some embodiments, the first party is other than the subject. In some embodiments, the first party is selected from a third party payer, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity. In some embodiments, the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is an insurance company and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is a governmental entity and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the treatment.

In one aspect, the disclosure features a method of providing information on which to make a decision about a subject with Type III Gaucher disease, or making such a decision. The method includes providing (e.g., by receiving) an evaluation of a subject, wherein the evaluation was made by a method described herein, e.g., by optionally, administering a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase, velaglucerase or uplyso), to the subject; providing a determination post administration of whether or not a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or whether or not the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the

glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or whether or not the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months), thereby providing a post administration determination; providing a comparison of the post administration determination with a standard (e.g., a standard described herein), thereby, providing information on which to make a decision about a subject, or making such a decision.

In some embodiments, the method includes making the decision.

In some embodiments, the method also includes communicating the information to another party (e.g., by computer, compact disc, telephone, facsimile, email, or letter).

In some embodiments, the decision includes selecting a subject for payment, making or authorizing payment for a first course of action (e.g., treatment with velaglucerase) if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) and a second course of action (e.g., treatment with imiglucerase, velaglucerase or uplyso) if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean

platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or if a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count
5 increased by 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months).

In some embodiments, a neurological parameter of the subject has changed (e.g.,
10 a neurological parameter has improved) and/or the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or a
15 neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every
20 other week for 6 months) and the course of action is authorization of a course of therapy (e.g., treatment with velaglucerase).

In some embodiments, assignment of the subject is to the first class and the assignment authorizes payment for a course of treatment (e.g., velaglucerase).

In some embodiments, assignment of the subject is to the second class and the
25 assignment authorizes payment for a course of treatment (e.g., imiglucerase, velaglucerase or uplyso).

In some embodiments, a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of
30 treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g.,

imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) and the course of action is assigning the subject to a first class. In some embodiments, assignment to the first class will enable payment for a treatment (e.g., velaglucerase) provided to the subject. In some embodiments, payment is by a first party to a second party. In some embodiments, the first party is other than the subject. In some embodiments, the first party is selected from a third party payer, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity. In some embodiments, the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is an insurance company and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is a governmental entity and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the treatment.

In some embodiments, a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or the subject's mean platelet count increased by 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or the subject's mean platelet count increased by 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g.,

imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) and the course of action is authorization of a course of therapy (e.g., imiglucerase, velaglucerase or uplyso).

In some embodiments, a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or the subject's mean platelet count increased by 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or a neurological parameter of the subject has changed (e.g., a neurological parameter has improved) and/or if the subject's mean platelet count increased by 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) and the course of action is assigning the subject to a second class. In some embodiments, assignment to the second class will enable payment for a treatment (e.g., imiglucerase, velaglucerase or uplyso) provided to the subject. In some embodiments, payment is by a first party to a second party. In some embodiments, the first party is other than the subject. In some embodiments, the first party is selected from a third party payer, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity. In some embodiments, the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is an insurance company and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is a governmental entity and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the treatment.

In another aspect, the disclosure features a method of selecting a payment class for a course of treatment with a glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase) for a subject with Type III Gaucher disease. The method includes determining that an infusion site reaction during or within 12 hours after infusion of the therapy is present in the subject or that antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the therapy are present in the subject, e.g., by a method described herein, and approving, making, authorizing, receiving, transmitting or otherwise allowing payment of a selected course of treatment, e.g., velaglucerase.

In another aspect, the disclosure features a method of selecting a payment class for a course of treatment with a glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase velaglucerase, or uplyso) for a subject with Type III Gaucher disease. The method includes determining that an infusion site reaction during or within 12 hours after infusion of the therapy is not present in the subject or that antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to the therapy are not present in the subject, e.g., by a method described herein, and approving, making, authorizing, receiving, transmitting or otherwise allowing payment of a selected course of treatment, e.g., imiglucerase, velaglucerase or uplyso.

In one aspect, the disclosure features a method of making a data record. The method includes entering the result of a method described herein into a record, e.g., a computer readable record. In some embodiments, the record is available on the world wide web. In some embodiments, the record is evaluated by a third party payer, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity, or a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment, or is otherwise relied on in a method described herein.

In another aspect, the disclosure features a data record (e.g., computer readable record), wherein the record includes results from a method described herein. In some embodiments, the record is available on the world wide web. In some embodiments, the

record is evaluated and/or transmitted to a third party payer, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity, or a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment.

5

In one aspect, the disclosure features a method of providing data. The method includes providing data described herein, e.g., generated by a method described herein, to provide a record, e.g., a record described herein, for determining if a payment will be provided. In some embodiments, the data is provided by computer, compact disc,
10 telephone, facsimile, email, or letter. In some embodiments, the data is provided by a first party to a second party. In some embodiments, the first party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the second party is a third party payer, an insurance company, employer, employer sponsored
15 health plan, HMO, or governmental entity. In some embodiments, the first party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the treatment and the second party is a governmental entity. In some embodiments, the first party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance
20 company, or an entity which sells or supplies the treatment and the second party is an insurance company.

In one aspect, the disclosure features a method of transmitting a record described herein. The method includes a first party transmitting the record to a second party, e.g.,
25 by computer, compact disc, telephone, facsimile, email, or letter. In some embodiments, the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is an insurance company or government entity and the second party is selected from the subject, a healthcare provider, a treating
30 physician, an HMO, a hospital, a governmental entity, or an entity which sells or supplies the treatment. In some embodiments, the first party is a governmental entity or insurance

company and the second party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the treatment.

5 In one method, information, e.g., about whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter, about whether or not a subject with Type III Gaucher disease experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a
10 glucocerebrosidase enzyme replacement therapy (e.g., wherein the information is obtained as described herein) is provided (e.g., communicated, e.g., electronically communicated) to a third party, e.g., a hospital, clinic, a government entity, reimbursing party or insurance company (e.g., a life insurance company). For example, choice of medical procedure, payment for a medical procedure, payment by a reimbursing party, or
15 cost for a service or insurance can be function of the information. E.g., the third party receives the information, makes a determination based at least in part on the information, and optionally communicates the information or makes a choice of procedure, payment, level of payment, coverage, etc. based on the information.

In one method, information, e.g., whether or not a subject with Type III Gaucher
20 disease experiences a change in a neurological parameter and/or whether or not the mean platelet count of a subject with Type III Gaucher disease increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether the subject's mean platelet
25 count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) (e.g., wherein the information
30 is obtained as described herein) is provided (e.g., communicated, e.g., electronically communicated) to a third party, e.g., a hospital, clinic, a government entity, reimbursing

party or insurance company (e.g., a life insurance company). For example, choice of medical procedure, payment for a medical procedure, payment by a reimbursing party, or cost for a service or insurance can be function of the information. E.g., the third party receives the information, makes a determination based at least in part on the information, and optionally communicates the information or makes a choice of procedure, payment, level of payment, coverage, etc. based on the information.

In one embodiment, a premium for insurance (e.g., life or medical) is evaluated as a function of information about whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not a subject with Type III Gaucher disease experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy. For example, premiums can be increased (e.g., by a certain percentage) if the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy.

In one embodiment, a premium for insurance (e.g., life or medical) is evaluated as a function of information about whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not the mean platelet count of a subject with Type III Gaucher disease increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) (e.g., wherein the information is obtained as described herein). For example, premiums can be increased (e.g., by a certain percentage) if the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their

baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months).

Information about whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not a subject with Type III Gaucher disease experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy can be used, e.g., in an underwriting process for life insurance. The information can be incorporated into a profile about a subject. Other information in the profile can include, for example, date of birth, gender, marital status, banking information, credit information, children, and so forth. An insurance policy can be recommended as a function of the information on whether or not a subject with Type III Gaucher disease experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy. An insurance premium or risk assessment can also be evaluated as function of whether or not a subject with Type III Gaucher disease experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy.

Information about whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for

9 months) or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) can be used, e.g., in an underwriting process for life insurance. The information can be incorporated into a profile about a subject. Other information in the profile can include, for example, date of birth, gender, marital status, banking information, credit information, children, and so forth. An insurance policy can be recommended as a function of the information on whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months). An insurance premium or risk assessment can also be evaluated as function of whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not whether or not the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months).

In one embodiment, information about whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not a subject with Type III Gaucher disease experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy is analyzed by a function that determines whether to authorize the transfer of funds to pay for a service or treatment provided to a subject (or make another decision referred to herein). For example, the results (e.g., that the subject experiences an infusion site reaction to a glucocerebrosidase enzyme replacement therapy or produces antibodies (e.g., neutralizing antibodies) (e.g., IgE, IgM, IgG and/or IgA antibodies) to a glucocerebrosidase enzyme replacement therapy) may indicate that a subject is suitable for treatment (e.g., velaglucerase), suggesting that a treatment course (e.g., with velaglucerase) is needed, thereby triggering an outcome that indicates or causes authorization to pay for a service or treatment (e.g., velaglucerase) provided to a subject. For example, an entity, e.g., a hospital, care giver, government entity, or an insurance company or other entity which pays for, or reimburses medical expenses, can use the outcome of a method described herein to determine whether a party, e.g., a party other than the subject patient, will pay for services (e.g., a particular therapy) or treatment provided to the patient. For example, a first entity, e.g., an insurance company, can use the outcome of a method described herein to determine whether to provide financial payment to, or on behalf of, a patient, e.g., whether to reimburse a third party, e.g., a vendor of goods or services, a hospital, physician, or other care-giver, for a service or treatment (e.g., velaglucerase) provided to a patient. For example, a first entity, e.g., an insurance company, can use the outcome of a method described herein to determine whether to continue, discontinue, enroll an individual in an insurance plan or program, e.g., a health insurance or life insurance plan or program.

In one embodiment, information about whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether or not the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g.,

imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether or not a subject with Type III Gaucher disease experiences a change in a neurological parameter and/or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months) is analyzed by a function that determines whether to authorize the transfer of funds to pay for a service or treatment provided to a subject (or make another decision referred to herein). For example, the results (e.g., that the subject's mean platelet count increased by less than 80%, 75%, 70%, 65%, 60%, or 55% after 9 or 12 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 9 months) or whether the subject's mean platelet count increased by less than 40%, 35%, 30%, or 25% after 6 months of treatment as compared to their baseline mean platelet count prior to initiating the glucocerebrosidase enzyme replacement therapy (e.g., imiglucerase treatment, e.g., imiglucerase at a dose of 60 U/kg, e.g., administered every other week for 6 months)) may indicate that a subject is suitable for treatment (e.g., velaglucerase), suggesting that a treatment course (e.g., with velaglucerase) is needed, thereby triggering an outcome that indicates or causes authorization to pay for a service or treatment (e.g., velaglucerase) provided to a subject. For example, an entity, e.g., a hospital, care giver, government entity, or an insurance company or other entity which pays for, or reimburses medical expenses, can use the outcome of a method described herein to determine whether a party, e.g., a party other than the subject patient, will pay for services (e.g., a particular therapy) or treatment provided to the patient. For example, a first entity, e.g., an insurance company, can use the outcome of a method described herein to determine whether to provide financial payment to, or on behalf of, a patient, e.g., whether to reimburse a third party, e.g., a vendor of goods or services, a hospital, physician, or other care-giver, for a service or treatment (e.g., velaglucerase) provided to a patient. For example, a first entity, e.g., an insurance company, can use the outcome of a method

described herein to determine whether to continue, discontinue, enroll an individual in an insurance plan or program, e.g., a health insurance or life insurance plan or program.

In one aspect, the disclosure features a method of providing data. The method includes providing data described herein, e.g., generated by a method described herein, to provide a record, e.g., a record described herein, for determining if a payment will be provided. In some embodiments, the data is provided by computer, compact disc, telephone, facsimile, email, or letter. In some embodiments, the data is provided by a first party to a second party. In some embodiments, the first party is selected from the subject, a healthcare provider, a treating physician, a health maintenance organization (HMO), a hospital, a governmental entity, or an entity which sells or supplies the drug. In some embodiments, the second party is a third party payer, an insurance company, employer, employer sponsored health plan, HMO, or governmental entity. In some embodiments, the first party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the drug and the second party is a governmental entity. In some embodiments, the first party is selected from the subject, a healthcare provider, a treating physician, an HMO, a hospital, an insurance company, or an entity which sells or supplies the drug and the second party is an insurance company.

In some aspects, the disclosure provides the use of a glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase, imiglucerase or uplyso), alone or in combination with another agent(s) described herein (e.g., isofagomine tartrate, miglustat, or Genz112638), for use in treatment.

In some aspects, the disclosure provides the use of a glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase or imiglucerase), alone or in combination with another agent(s) described herein (e.g., isofagomine tartrate, miglustat, or Genz112638), for the preparation of a medicament, e.g., for treating Type III Gaucher disease.

In another aspect, the disclosure provides a pharmaceutical composition of velaglucerase. The composition comprises: velaglucerase, a lyoprotectant (e.g., a

carbohydrate (e.g., sucrose)), a buffer salt (e.g., citrate and/or citric acid, e.g., sodium citrate and citric acid), and a stabilizing agent (e.g., polysorbate, e.g., polysorbate 20).

In some embodiments, the composition can be lyophilized. In some
embodiments, the moisture content of the lyophilized composition is 1% to 6%, e.g.,
1.3% to 6.2%. In some embodiments, the moisture content of the lyophilized
composition is 1% to 5%. In some embodiments, the moisture content of the lyophilized
composition is 3% to 5%. In some embodiments, the moisture content is greater than or
equal to 3%. In some embodiments, the moisture content is 3%.

In some embodiments, the lyophilized composition can be evaluated. For
example, the secondary structure of the lyophilized composition can be evaluated, e.g.,
by FT-IR.

In other embodiments, the composition can be a reconstituted solution. For
example, the composition is a reconstituted solution in a pharmaceutically acceptable
carrier such as Sterile Water for Injection (e.g., a 200 unit vial with 2.2 mL Sterile Water
for Injection or a 400 unit vial with 4.3 mL Sterile Water for Injection). In some
embodiments, the composition can further comprise, or consist of, sodium chloride
solution suitable for intravenous administration (e.g., 0.9% sodium chloride solution
suitable for intravenous administration).

In some embodiments, the reconstituted solution can be evaluated, e.g., for
degradation. For example, the reconstituted solution can be evaluated by SE-HPLC
and/or RP-HPLC, e.g., for the presence of degradation products.

In some embodiments, the reconstituted solution can be evaluated for oxidation.
For example, the reconstituted solution can be evaluated by peptide mapping.

In some aspects, the disclosure features an assay (e.g., method) for detecting an
anti-glucocerebrosidase antibody in a sample (e.g., a patient sample, e.g., blood or
serum). The method includes:

providing a glucocerebrosidase (e.g., velaglucerase, imiglucerase or uplyso)
immobilized on a surface (e.g., a microwell) (for example, the surface can be coated with
a coupling agent such as streptavidin and the glucocerebrosidase (e.g., velaglucerase,
imiglucerase or uplyso) can be bound to an agent (e.g., biotin) which associates with,

e.g., binds to, the coupling agent, e.g., the glucocerebrosidase (e.g., velaglucerase, imiglucerase or uplyso) is immobilized to the surface via the biotin binding to the strepavidin;

contacting the sample to the immobilized glucocerebrosidase (e.g., velaglucerase, imiglucerase or uplyso), under conditions that allow an anti-glucocerebrosidase antibody in the sample, if present, to bind to the immobilized glucocerebrosidase, thereby forming a mixture;

optionally performing a wash step to remove from the mixture any material in the sample that is not bound to the immobilized glucocerebrosidase;

adding labeled glucocerebrosidase (e.g., velaglucerase or imiglucerase), wherein the labeled glucocerebrosidase is labeled with a detectable label (e.g., ruthenium-labeled glucocerebrosidase), to the mixture under conditions that allow the labeled glucocerebrosidase to bind to the anti-glucocerebrosidase antibody (e.g., that is bound to the immobilized glucocerebrosidase), if present, (preferably, the label is not the same as the coupling agent and/or the agent that binds to the coupling agent, e.g., if biotin is used to immobilize glucocerebrosidase to the surface, the label is not biotin);

optionally performing a wash step to remove labeled glucocerebrosidase that is not bound to the anti-glucocerebrosidase antibody from the mixture; and

detecting (and optionally quantifying) the label in the mixture, e.g., wherein detection of the label indicates that an anti-glucocerebrosidase antibody is present in the sample.

In some embodiments, the detected label is quantified to a value and compared to a control, e.g., a negative control, wherein if the value of detected label is greater than the negative control, the sample contains anti-glucocerebrosidase antibody. In some embodiments, the negative control is the average negative control value (e.g., background) for a plurality of negative controls. For example, the negative control can be normal human serum (NHS), and the average negative control value can be the average for a plurality of NHS lots, or the average of the negative control values obtained from a plurality of assays. For example, the negative control value can be a value of 1, 2, 3, 4, 5 or 6 ng/ml of antibody, e.g., a value of greater than that number for a sample indicates that an anti-glucocerebrosidase antibody is present in the sample. As another

example, the negative control value can be 200, 250, 300 (e.g., 306), 350, or 400, e.g., a value of greater than that number for a sample indicates that an anti-glucocerebrosidase antibody is present in the sample.

In some embodiments, the assay is for detecting anti-velaglycerase antibodies. In
5 another embodiment, the assay is for detecting anti-imiglycerase antibodies. In some
embodiments, the assay is for detecting anti-uplyso antibodies.

In some aspects, the disclosure features an assay for detecting an anti-
glucocerebrosidase antibody (e.g., IgG anti-glucocerebrosidase antibody) in a sample
10 (e.g., a patient sample, e.g., blood or serum). The method includes:

contacting the sample to labeled glucocerebrosidase (e.g., velaglycerase or
imiglycerase), wherein the glucocerebrosidase is labeled with a detectable label (e.g., the
glucocerebrosidase is ¹²⁵I labeled), under conditions that allow an anti-glucocerebrosidase
antibody in the sample, if present, to bind to labeled glucocerebrosidase, thereby forming
15 a mixture;

applying the mixture to a resin (e.g., Protein G, Protein A, Protein A/G, or Protein
L resin) (e.g., a Protein G spin column) under conditions that allow the anti-
glucocerebrosidase antibody, if present, to bind to the resin;

optionally performing a wash step to remove labeled glucocerebrosidase that is
20 not bound to the anti-glucocerebrosidase antibody from the mixture; and

detecting (and optionally quantifying) the label in the mixture (e.g., on the resin),
e.g., wherein detection of the label indicates that anti-glucocerebrosidase antibody is
present in the sample.

In some embodiments, the assay is for detecting anti-velaglycerase antibodies. In
25 another embodiment, the assay is for detecting anti-imiglycerase antibodies. In some
embodiments, the assay is for detecting anti-uplyso antibodies.

In some embodiments, the detected label is quantified to a value and compared to
a control, e.g., a negative control, wherein if the value of detected label is greater than the
negative control, the sample contains anti-glucocerebrosidase antibody. In some
30 embodiments, the negative control is the average negative control value (e.g.,
background) for a plurality of negative controls. For example, the negative control can

be normal human serum (NHS), and the average negative control value can be the average for a plurality of NHS lots, or the average of the negative control values obtained from a plurality of assays.

5 In some aspects, the disclosure features an assay for detecting a human anti-glucocerebrosidase antibody in a sample (e.g., a patient sample, e.g., blood or serum). The method includes:

providing glucocerebrosidase (e.g., velaglycerase, imiglycerase or uplyso) immobilized on a surface (e.g., a microwell) (for example, the surface can be coated with
10 a coupling agent such as strepavidin and the glucocerebrosidase can be bound to an agent (e.g., biotin) that associates with, e.g., binds to, the coupling agent, e.g., the glucocerebrosidase is immobilized to the surface via the biotin binding to the strepavidin);

contacting the sample to the immobilized glucocerebrosidase, under conditions
15 that allow a human anti-glucocerebrosidase antibody in the sample, if present, to bind to the immobilized glucocerebrosidase, thereby forming a mixture;

optionally performing a wash step to remove from the mixture any material in the sample that is not bound to the immobilized glucocerebrosidase;

adding an antibody that binds to the human anti-glucocerebrosidase antibody to
20 the mixture, wherein the antibody that binds to the human anti-glucocerebrosidase antibody is labeled with a detectable label (e.g., ruthenium or biotin), under conditions that allow the labeled antibody that binds to the human anti-glucocerebrosidase antibody to bind to the human anti-glucocerebrosidase antibody (e.g., that is bound to the immobilized glucocerebrosidase), if present, (preferably, the label is not the same as the
25 coupling agent and/or the agent that binds to the coupling agent, e.g., if biotin is used to immobilize glucocerebrosidase to the surface, the label is not biotin);

optionally performing a wash step to remove labeled antibody that binds to the human anti-glucocerebrosidase antibody that is not bound to the human anti-glucocerebrosidase antibody from the mixture; and

detecting (and optionally quantifying) the label in the mixture, e.g., wherein detection of the label indicates that human anti-glucocerebrosidase antibody is present in the sample.

In some embodiments, the assay detects anti-velaglycerase antibodies. In some
5 embodiments, the assay detects anti-imiglycerase antibodies. In some embodiments, the assay detects anti-uplyso antibodies.

In some embodiments, the detected label is quantified to a value and compared to a control, e.g., a negative control, wherein if the value of detected label is greater than the negative control, the sample contains anti-velaglycerase antibody. In some embodiments,
10 the negative control is the average negative control value (e.g., background) for a plurality of negative controls. For example, the negative control can be normal human serum (NHS), and the average negative control value can be the average for a plurality of NHS lots, or the average of the negative control values obtained from a plurality of assays.

15 In some embodiments, the antibody that binds to the human anti-glucocerebrosidase antibody is isotype specific, wherein the isotype specific antibody that binds to the human anti-glucocerebrosidase antibody binds specifically to a human antibody of the isotype to which it is specific.

In some embodiments, the antibody that binds to the human anti-
20 glucocerebrosidase antibody is an IgA specific antibody and binds to an IgA human anti-glucocerebrosidase antibody in the sample.

In some embodiments, the antibody that binds to the human anti-
glucocerebrosidase antibody is an IgE specific antibody and binds to an IgE human anti-
glucocerebrosidase antibody in the sample.

25 In some embodiments, the antibody that binds to the human anti-glucocerebrosidase antibody is an IgM specific antibody and binds to an IgM human anti-glucocerebrosidase antibody in the sample.

In some embodiments, the antibody that binds to the human anti-
glucocerebrosidase antibody is an IgG specific antibody and binds to an IgG human anti-
30 glucocerebrosidase antibody in the sample.

In some aspects, the disclosure features a method of determining if an anti-glucocerebrosidase antibody (e.g., in a sample) neutralizes (e.g., inhibits) glucocerebrosidase (e.g., velaglucerase or imiglucerase) activity. The method includes: providing a cell (e.g., a human cell, e.g., a human fibroblast cell) that expresses human macrophage mannose receptor (MMR);

contacting the anti-glucocerebrosidase antibody to the cell, thereby forming a mixture;

contacting labeled glucocerebrosidase (e.g., velaglucerase, imiglucerase or uplyso) to the mixture, wherein the glucocerebrosidase is labeled with a detectable label (e.g., the glucocerebrosidase is labeled with a fluorescent label, e.g., a green fluorescent dye, such as Alexa FLUOR® 488 or fluorescein isothiocyanate (FITC)), under conditions that allow the labeled glucocerebrosidase to bind to the MMR in the absence of an anti-glucocerebrosidase antibody (e.g., wherein binding of glucocerebrosidase to MMR allows cellular uptake of the glucocerebrosidase);

removing unbound labeled glucocerebrosidase and labeled glucocerebrosidase bound to the cell surface (e.g., via trypsin digestion); and measuring the amount of labeled glucocerebrosidase in the cell.

In some embodiments, the levels of labeled glucocerebrosidase are compared to a control, e.g., the level of labeled glucocerebrosidase detected in the absence of the anti-glucocerebrosidase antibody under identical conditions.

In some embodiments, the cells do not express an Fc receptor (e.g., human Fc receptor).

In some embodiments, the method detects whether neutralizing anti-velaglucerase antibodies are present. In some embodiments, the method detects whether neutralizing anti-imiglucerase antibodies are present. In some embodiments, the method detects whether neutralizing anti-uplyso antibodies are present.

In some embodiments, the method detects whether an anti-imiglucerase antibody neutralizes imiglucerase activity. In some embodiments, the method detects whether an anti-velaglucerase antibody neutralizes velaglucerase activity. In some embodiments, the method detects whether an anti-uplyso antibody neutralizes uplyso activity.

In some embodiments, the method detects whether an anti-imiglucerase antibody neutralizes velaglucerase and/or uplyso activity. In some embodiments, the method detects whether an anti-velaglucerase antibody neutralizes imiglucerase and/or uplyso activity. In some embodiments, the method detects whether an anti-uplyso antibody neutralizes imiglucerase and/or velaglucerase activity.

In some aspects, the disclosure features a method of determining if an anti-velaglucerase antibody (e.g., in a sample) neutralizes (e.g., inhibits) imiglucerase activity. The method includes:

providing a cell (e.g., a human cell, e.g., a human fibroblast cell) that expresses human macrophage mannose receptor (MMR);
contacting the anti-velaglucerase antibody to the cell, thereby forming a mixture;
contacting labeled imiglucerase to the mixture, wherein the imiglucerase is labeled with a detectable label (e.g., the imiglucerase is labeled with a fluorescent label, e.g., a green fluorescent dye, such as Alexa FLUOR® 488 or fluorescein isothiocyanate (FITC)), under conditions that allow the labeled imiglucerase to bind to the MMR in the absence of an anti-velaglucerase antibody (e.g., wherein binding of imiglucerase to MMR allows cellular uptake of the imiglucerase);
removing unbound labeled imiglucerase and labeled imiglucerase bound to the cell surface (e.g., via trypsin digestion); and
measuring the amount of labeled imiglucerase in the cell.

In some embodiments, the levels of labeled imiglucerase are compared to a control, e.g., the level of labeled imiglucerase detected in the absence of the anti-velaglucerase antibody under identical conditions.

In some embodiments, the cells do not express an Fc receptor (e.g., human Fc receptor).

In some aspects, the disclosure features a method of determining if an anti-imiglucerase antibody (e.g., in a sample) neutralizes (e.g., inhibits) velaglucerase activity. The method includes:

providing a cell (e.g., a human cell, e.g., a human fibroblast cell) that expresses human macrophage mannose receptor (MMR);

contacting the anti-imiglucerase antibody to the cell, thereby forming a mixture;

contacting labeled velaglucerase to the mixture, wherein the velaglucerase is
5 labeled with a detectable label (e.g., the velaglucerase is labeled with a fluorescent label, e.g., a green fluorescent dye, such as Alexa FLUOR® 488 or fluorescein isothiocyanate (FITC)), under conditions that allow the labeled velaglucerase to bind to the MMR in the absence of an anti-imiglucerase antibody (e.g., wherein binding of velaglucerase to MMR allows cellular uptake of the velaglucerase);

10 removing unbound labeled velaglucerase and labeled velaglucerase bound to the cell surface (e.g., via trypsin digestion); and

measuring the amount of labeled velaglucerase in the cell.

In some embodiments, the levels of labeled velaglucerase are compared to a control, e.g., the level of labeled velaglucerase detected in the absence of the anti-
15 imiglucerase antibody under identical conditions.

In some embodiments, the cells do not express an Fc receptor (e.g., human Fc receptor).

In some aspects, the disclosure features a hybrid antibody, wherein the hybrid
20 antibody comprises a non-human anti-drug antibody and a human immunoglobulin (Ig).

In some embodiments, the non-human anti-drug IgG antibody is a sheep anti-drug IgG antibody.

In some embodiments, the anti-drug antibody binds to velaglucerase.

In some embodiments, the anti-drug antibody binds to imiglucerase.

25 In some embodiments, the anti-drug antibody binds to uplyso.

In some embodiments, the human Ig is IgA.

In some embodiments, the human Ig is IgE.

In some embodiments, the human Ig is IgM.

In some embodiments, the human Ig is IgG.

30 In some embodiments, the non-human anti-drug antibody is an IgG antibody.

In some embodiments, the non-human anti-drug antibody and the human Ig are conjugated together by a chemical crosslinker, e.g., a long spacer arm cross linker, e.g., succinimidyl 6-[3'-2-pyridyldithio-propionamido] hexanoate (LC-SPDP).

In some embodiments, the hybrid antibody is used as a positive control in assays that detect and/or measure levels and/or isotypes of anti-drug antibody in a sample, e.g., in a method described herein.

In some embodiments, the hybrid antibody is used as a positive control in assays for determining if an anti-glucocerebrosidase antibody (e.g., in a sample) neutralizes (e.g., inhibits) glucocerebrosidase (e.g., velaglucerase or imiglucerase) activity, e.g., in a method described herein. For example, the hybrid antibody is used as an anti-glucocerebrosidase antibody (e.g., in a sample) in the assay.

In some aspects, the disclosure features a method of measuring cellular uptake (e.g., internalization) of glucocerebrosidase (e.g., velaglucerase or imiglucerase) into a cell. The method includes:

contacting glucocerebrosidase (e.g., velaglucerase or imiglucerase) to a cell (e.g., a cell of a human leukemic monocyte lymphoma cell line (e.g., U937) or a cell of a murine macrophage cell line (e.g., J774)) to thereby form a mixture;

incubating the mixture (e.g., for 1, 2, 3, 4, 5, 6, or 7 hours or overnight), e.g., to allow cellular uptake of the glucocerebrosidase (e.g., velaglucerase or imiglucerase) into the cell; and

measuring the amount of uptake of glucocerebrosidase (e.g., velaglucerase or imiglucerase) into the cell.

In some embodiments, the amount of uptake is measured by measuring glucocerebrosidase enzymatic activity in the cell. In some embodiments, a synthetic substrate that fluoresces upon cleavage (e.g., 4-MU-glc) is used.

In some embodiments, the amount of uptake is measured by measuring intracellular glucocerebrosidase protein levels. In some embodiments, Western blot analysis is used. In some embodiments, immunohistochemistry analysis is used (e.g., immunohistochemistry on permeabilized cells).

In some embodiments, the cell is washed one or more times prior to the measuring step.

In some embodiments, the pH of the mixture is 7.5.

In some embodiments, mannose-6-phosphate (M6P) is present in the mixture.

5 In some embodiments, mannan is present in the mixture.

In some embodiments, calcium is present in the mixture.

In some embodiments, the amount of uptake is compared to a standard, e.g., the measured amount of uptake in the absence of contacting glucocerebrosidase (e.g., velaglucerase or imiglucerase) to the cell, or comparing the measured amount of uptake
10 in the presence and absence of mannose-6-phosphate, or comparing the measured amount of uptake in the presence and absence of mannan, or comparing the measured amount of uptake in the presence and absence of calcium.

In some embodiments, the glucocerebrosidase is velaglucerase.

In some embodiments, the glucocerebrosidase is imiglucerase.

15 In some embodiments, the amount of uptake of velaglucerase is compared to the amount of uptake of imiglucerase (e.g., under the same conditions).

The term “subject” refers to any mammal, including but not limited to, any animal classified as such, including humans, non human primates, primates, baboons,
20 chimpanzees, monkeys, rodents (e.g., mice, rats), rabbits, cats, dogs, horses, cows, sheep, goats, pigs, etc. The term “subject” can be used interchangeably with the term “patient.”

The term “isolated” refers to a molecule that is substantially free of its natural environment. For instance, an isolated protein is substantially free of cellular material or other proteins from the cell or tissue source from which it is derived. The term refers to
25 preparations where the isolated protein is sufficiently pure to be administered as a therapeutic composition, or at least 70% to 80% (w/w) pure, more preferably, at least 80% 90% (w/w) pure, even more preferably, 90 to 95% pure; and, most preferably, at least 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8% or 100% (w/w) pure.

As used herein, the term “about” refers to up to $\pm 10\%$ of the value qualified by
30 this term. For example, about 50 mM refers to 50 mM ± 5 mM; about 4% refers to 4% $\pm 0.4\%$.

The terms “therapeutically effective dose,” and “therapeutically effective amount,” refer to that amount of a compound that results in prevention of symptoms (e.g., prevention of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of symptoms, e.g., symptoms of Type III Gaucher disease in a subject diagnosed as having Type III Gaucher disease), delay of onset of symptoms, or amelioration of symptoms of Type III Gaucher disease. A therapeutically effective amount will, for example, be sufficient to treat, prevent, reduce the severity, delay the onset, and/or reduce the risk of occurrence of one or more symptoms of a disorder associated with Type III Gaucher disease. The effective amount can be determined by methods well known in the art and as described in subsequent sections of this description.

The terms “treatment” and “therapeutic method” refer to treatment of an existing disorder and/or prophylactic/preventative measures. Those in need of treatment may include individuals already having a particular medical disorder, as well as those at risk or having, or who may ultimately acquire the disorder. The need for treatment is assessed, for example, by the presence of one or more risk factors associated with the development of a disorder, the presence or progression of a disorder, or likely receptiveness to treatment of a subject having the disorder. Treatment may include slowing or reversing the progression of a disorder.

The term “treating” refers to administering a therapy in an amount, manner, and/or mode effective to improve or prevent a condition, symptom, or parameter associated with a disorder (e.g., a disorder described herein) or to prevent onset, progression, or exacerbation of the disorder, to either a statistically significant degree or to a degree detectable to one skilled in the art. Accordingly, treating can achieve therapeutic and/or prophylactic benefits. An effective amount, manner, or mode can vary depending on the subject and may be tailored to the subject.

“Infusion site reaction” as used herein refers to one or more symptom of hypersensitivity developed by a subject during or shortly after an infusion of a glucocerebrosidase enzyme replacement therapy (e.g., within 12 hours of infusion of a glucocerebrosidase enzyme replacement treatment to the subject). Symptoms include, for example, pruritus, burning, swelling or abscess at the site of infusion, flushing,

urticaria/angioedema, chest discomfort, tachycardia, cyanosis, respiratory symptoms and paraesthesia.

The term “combination” refers to the use of the two or more agents or therapies to treat the same patient, wherein the use or action of the agents or therapies overlap in time. The agents or therapies can be administered at the same time (e.g., as a single formulation that is administered to a patient or as two separate formulations administered concurrently) or sequentially in any order.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, controls. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 (a)-(f) depicts mean % change in hematological values, organ values, and biomarkers in phase I/II trial.

FIG. 2 depicts mean % change of key clinical parameters in phase I/II and extension trials.

FIG. 3 depicts TKT025EXT (N=10) mean hematological parameters change from baseline in TKT025.

FIG. 4 depicts TKT025EXT (N=10) mean normalized organ volumes change from baseline in TKT025.

FIG. 5 depicts TKT025 and TKT025EXT temporal change in mean Z-scores of lumbar spine from baseline.

FIG. 6 depicts TKT025 and TKT025EXT temporal change in mean Z-score of femoral neck from baseline.

FIG. 7 depicts TKT032 (N=25) mean hemoglobin concentration change from baseline.

FIG. 8 depicts TKT032 (N=25) mean platelet count change from baseline.

FIG. 9 depicts TKT032 (N=25) mean normalized spleen volume change from baseline.

FIG. 10 depicts TKT032 (N=25) mean normalized liver volume change from baseline.

FIG. 11 depicts HGT-GCB-039 (N=34) mean hemoglobin concentration change from baseline.

FIG. 12 depicts HGT-GCB-039 (N=34) mean platelet count change from baseline.

FIG. 13 depicts HGT-GCB-039 (N=34) mean change from baseline of platelet count in patients without spleen.

FIG. 14 depicts HGT-GCB-039 (N=34) mean normalized liver volume change from baseline.

FIG. 15 depicts TKT034 mean change from baseline in hemoglobin concentration.

FIG. 16 depicts TKT034 mean n percent change from baseline in platelet count.

FIG. 17 depicts TKT034 mean percent change from baseline in normalized liver volume.

FIG. 18 depicts TKT034 mean percent change from baseline in normalized spleen volume.

FIG. 19 depicts TKT034 mean percent change from baseline in plasma chitotriosidase.

FIG. 20 depicts TKT034 mean percent change from baseline in plasma CCL18.

FIG. 21 depicts an immunogenicity evaluation of patients in velaglucerase alfa clinical studies.

FIG. 22 depicts an anti-drug antibody screening by electro-chemiluminescence (ECL) immunoassay.

FIG. 23 depicts a screening assay dose response curve.

FIG. 24 depicts an IgG ADA confirmatory assay.

FIG. 25 depicts an RIP assay dose response curve.

FIG. 26 depicts IgA, IgM and IgE ADA confirmatory assays.

DETAILED DESCRIPTION

The disclosure is based, in part, on the discovery that velaglucerase elicits less of an immune response (e.g., less production of antibody, e.g., less production of neutralizing antibody) than imiglucerase upon administration to a subject (e.g., a subject with Gaucher disease). It was discovered that velaglucerase elicits less infusion site reaction upon administration to a subject (e.g., a subject with Gaucher disease) than imiglucerase and velaglucerase can result in an increase in platelet count when administered to a subject (e.g., a subject having Gaucher disease) than imiglucerase. The invention relates, *inter alia*, to compositions and methods for selecting a treatment for a subject with Gaucher disease, selecting subjects for treatment with velaglucerase (e.g., alone or in combination with another therapy), and methods for reducing injection site reaction in subjects undergoing treatment for Gaucher disease.

Velaglucerase

Velaglucerase is human β -glucocerebrosidase produced by gene-activation in a human cell line. Gene activation refers to targeted recombination with a promoter that activates the endogenous β -glucocerebrosidase gene in the selected human cell line. Velaglucerase is secreted as a monomeric glycoprotein of approximately 63 kDa and is composed of 497 amino acids with a sequence identical to the natural human protein. The amino acid sequence of velaglucerase is described in Zimran et al. (2007) *Blood Cells Mol Dis*, 39: 115-118.

Glycosylation of velaglucerase alfa is altered by using kifunensine, a mannosidase I inhibitor, during cell culture, which results in the secretion of a protein containing primarily high-mannose type glycans having 6-9 mannose units per glycan. A summary of the glycan structure of velaglucerase is provided below.

Glycosylation Site	Predominant Glycan	Other Glycans
Asn19	<u>High-mannose</u> (Man) ₉ (GlcNAc) ₂	<u>High-mannose</u> (Man) ₆₋₈ (GlcNAc) ₂ <u>Phosphorylated high-mannose</u> (Phos) ₁ (Man) _{8,9} (GlcNAc) ₂ <u>GlcNAc capped phosphate</u> (Phos) ₁ (Man) _{8,9} (GlcNAc) ₃ <u>Hybrid</u> (Hex) ₂ (Man) ₃ (GlcNAc) ₃ (Fuc) ₁
Asn59	<u>High-mannose</u> (Man) ₉ (GlcNAc) ₂	<u>High-mannose</u> (Man) ₅₋₈ (GlcNAc) ₂ <u>Phosphorylated high-mannose</u> (Phos) ₁ (Man) _{7,9} (GlcNAc) ₂ <u>GlcNAc capped phosphate</u> (Phos) ₁ (Man) _{8,9} (GlcNAc) ₃ <u>Hybrid</u> (NeuAc) ₁ (Gal) ₁ (Man) ₅ (GlcNAc) ₃ (Fuc) ₁ <u>Complex</u> (NeuAc) ₀₋₂ (Gal) ₂ (Man) ₃ (GlcNAc) ₄ (Fuc) ₁ (Gal) ₃ (Man) ₃ (GlcNAc) ₅ (Fuc) ₁
Asn146	<u>High-mannose</u> (Man) ₉ (GlcNAc) ₂	<u>High-mannose</u> (Man) ₆₋₈ (GlcNAc) ₂ <u>Phosphorylated high-mannose</u> (Phos) ₁ (Man) _{7,9} (GlcNAc) ₂ <u>GlcNAc capped phosphate</u> (Phos) ₁ (Man) ₉ (GlcNAc) ₃ <u>Hybrid</u> (NeuAc) ₁ (Gal) ₁ (Man) ₅ (GlcNAc) ₃ (Fuc) ₁
Asn270	<u>High-mannose</u> (Man) ₉ (GlcNAc) ₂	<u>High-mannose</u> (Man) ₆₋₈ (GlcNAc) ₂ <u>Phosphorylated high-mannose</u> (Phos) ₁ (Man) _{6,9} (GlcNAc) ₂ <u>GlcNAc capped phosphate</u> (Phos) ₁ (Man) ₉ (GlcNAc) ₃ <u>Hybrid</u> (Gal) ₁ Man ₇ GlcNAc ₃ Fuc ₁ (NeuAc) ₁ (Gal) ₁ (Man) ₅ (GlcNAc) ₃ (Fuc) ₁ <u>Complex</u> (NeuAc) ₂ (Gal) ₂ (Man) ₃ (GlcNAc) ₄ (Fuc) ₁
Asn462	Not Detected	Not Detected

Velaglucerase has three non-contiguous domains, with the catalytic site located in domain III (residues 76-381 and 416-430), a (β/α)₈ (TIM) barrel.

Velaglucerase (VPRIVTM) is commercially available from Shire Human Genetics Therapies, Inc. Methods of making velaglucerase are described, for example, in U.S. Patent No.: 7,138,262.

Pharmaceutical Form. Velaglucerase (also referred to herein as velaglucerase alfa) is a sterile, white to off-white, preservative-free lyophilized powder for solution in single-use vials for intravenous (IV) infusion after reconstitution with Sterile Water for Injection.

Qualitative and Quantitative Composition. Upon reconstitution with Sterile Water for Injection, each vial contains approximately 2.5 mg/mL (40 U/mL) of velaglucerase alfa, 50 mg/mL sucrose, 12.9 mg/mL sodium citrate dihydrate, 1.3 mg/mL citric acid monohydrate and 0.11 mg/mL polysorbate 20. Each vial contains an extractable volume of 2.0 mL for the 200 U vial and 4.0 mL for the 400 U vial. Velaglucerase is supplied in a 200 U/vial (5 mg) or 400 U/vial (10mg) of velaglucerase alfa, one unit (U) of enzyme activity being defined as the quantity of enzyme required to convert one micromole of p-nitrophenyl β -D-glucopyranoside to p-nitrophenol per minute at 37°C.

Container and Contents. Velaglucerase is a sterile, lyophilized powder for solution supplied in either a 5 mL (200 U/vial presentation) or 20 mL (400 U/vial presentation) type I glass vial. Each vial contains either 200 U (5 mg) or 400 U (10 mg) of velaglucerase alfa. The vials are closed with a butyl rubber stopper with a fluoro-resin coating and are sealed with an aluminum overseal with a flip-off plastic cap.

Instructions for Use. Velaglucerase is a lyophilized powder for solution intended for intravenous infusion. Vials are single-use vials. Velaglucerase is not infused with other products in the same infusion. The total volume of infusion is delivered over a period of 60 minutes. Velaglucerase should be handled as follows:

1. Determine the number of vials to be reconstituted based on the individual patient's weight and the prescribed dose.

2. Remove the required number of vials from the refrigerator. Reconstitute 200 unit vials with 2.2 mL of Sterile Water for Injection and 400 unit vials with 4.3 mL Sterile Water for Injection. Do not shake.

3. Prior to dilution, visually inspect the solution in the vials. Do not use if the solution is discolored or if particulate matter is present.

4. Withdraw the calculated volume of drug from the appropriate number of vials.

5. Dilute the total volume required in 100mL of 0.9% sodium chloride solution suitable for IV administration, rock gently, but do not shake.

Dose. Velaglucerase is administered at doses between (and including) 2.5 U/kg and 60 U/kg of subject body weight, e.g., 15 U/kg to 60 U/kg (e.g., 15 U/kg, 30 U/kg, 45 U/kg, or 60 U/kg). Velaglucerase can be administered at a rate of 2 U/kg/minute, 1.5 U/kg/minute, 1 U/kg/minute or 0.5 U/kg/minute. The dose of velaglucerase is administered to the subject every other week.

Velaglucerase can be administered at a dose of 15 to 60 U/kg (e.g. 30 U/kg to 60 U/kg, e.g., 15 U/kg, 30 U/kg, 45U/kg, or 60 U/kg), at a dose equal to or below 22.5 U/kg, at a dose between 22.5 and 37.5 U/kg, at a dose between 37.5 and 52.5 U/kg, or at a dose equal to or above 52.5 U/kg. In some embodiments, velaglucerase can be administered at a dose of 2.5 U/kg to 60 U/kg. In some embodiments, the velaglucerase can be administered every other week by intravenous infusion. In other embodiments, the velaglucerase can be administered every week by intravenous infusion. In some embodiments, the velaglucerase can be administered three times a week by intravenous infusion, e.g., at a dose of 2.5 U/kg.

In some embodiments, the infusion of the dose (e.g., a dose described herein occurs over less than 2 hours, e.g., less than 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50 minutes or 45 minutes.

Recombinantly-produced human glucocerebrosidase. Other forms of recombinantly-produced human glucocerebrosidase that can be used in the compositions, assays and methods described herein. For example, imiglucerase (Cerazyme®) is recombinately produced in Chinese Hamster Ovary (CHO) cells and is commercially available. In addition, uplyso, a recombinant glucocerebrosidase (prGCD) expressed in

plant cells, can be used. Plant recombinant glucocerebrosidase can be obtained by methods described, e.g., in U.S. Publication Nos: US 20090208477 and US 20080038232 and PCT Publication Nos.: WO 2004/096978 and WO 2008/132743.

5 Maintenance Dose

Upon improvement of a subject's condition, a maintenance dose of a treatment may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. Subjects may, however, require intermittent
10 treatment on a long-term basis upon any recurrence of disease symptoms.

Subjects treated with a dose of 60 U/kg of velaglucerase every other week, and after about 15-18 months of total treatment with velaglucerase, upon showing improvements in 2 of 4 recommended therapeutic criteria (hemoglobin concentration, platelet counts, liver volume, spleen volume), were switched to a reduced dose of 30U/kg every other week. In spite of this
15 dose reduction, these subjects showed clinically meaningful changes in the 4 parameters (hemoglobin concentration, platelet counts, spleen volume, and liver volume) and Biomarkers (Chitotriosidase and CCL18) over the course of 48 months.

Qualitative comparison between velaglucerase and imiglucerase indicates potential additional benefit for Gaucher subjects in that, in spite of a dose reduction to almost half of that
20 of imiglucerase, patients continued to improve clinically in the 4 parameters demonstrating a marked increase in hemoglobin concentration, continued increase in platelet count after 36 months, and a rate of decline in liver/spleen volume.

Alternative Therapy

25 The administration of velaglucerase (with or without the additional agent) can be used as an alternative treatment, e.g., for subjects who were previously treated with another therapy (i.e., a therapy other than velaglucerase, e.g., imiglucerase, alglucerase, uplyso, isofagomine tartrate, miglustat, or Genz112638). For example, a subject who is undergoing treatment for Gaucher disease with another therapy can be transferred to
30 treatment with velaglucerase, e.g., if the subject is experiencing a side effect or adverse effect from the other therapy. For example, a subject who is undergoing treatment for

Gaucher disease with imiglucerase can be transferred to treatment with velaglucerase, e.g., velaglucerase can be administered at the same dose and with the same frequency at which the imiglucerase was administered. For example, the subject may have experienced an infusion site reaction upon or after administration of imiglucerase and/or developed anti-imiglucerase antibodies (e.g., neutralizing antibodies to imiglucerase).

Combination Therapy

A subject who has Gaucher disease can be administered a therapy that includes velaglucerase in an amount and for a time to provide an overall therapeutic effect. The velaglucerase can be administered alone or in combination with an additional agent(s). In the case of a combination therapy, the amounts and times of administration can be those that provide, e.g., a synergistic therapeutic effect, or an additive therapeutic effect.

In some embodiments, velaglucerase can be used in combination with another therapy for Gaucher disease, e.g., a therapy other than enzyme replacement therapy, e.g., isofagomine tartrate, miglustat, or Genz112638.

Isofagomine tartrate. Isofagomine tartrate (AT-2101, HGT-34100, PLICERA®) ((3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)piperidine L-(+)-tartrate; CAS No. 957230-65-8) selectively binds to and stabilizes glucocerebrosidase and facilitates proper trafficking of the enzyme to the lysosomes, the compartments in the cell where it is needed to break down glucocerebroside. See also U.S. Pat. No. 7,501,439.

Miglustat. Miglustat (ZAVESCA®) ((2*R*,3*R*,4*R*,5*S*)-1-butyl-2-(hydroxymethyl)piperidine-3,4,5-triol; CAS No. 72599-27-0) is an N-alkylated imino sugar, a synthetic analogue of D-glucose and a white to off-white crystalline solid that has a bitter taste. Miglustat exhibits a large volume of distribution and has the capacity to access deep organs such as the brain, bone and lung.

Miglustat inhibits glucosylceramide synthase an essential enzyme for the synthesis of most glycosphingolipids. Miglustat is a glucosylceramide synthase inhibitor. It works by blocking an enzyme that reduces the formation of certain chemicals in the body (glucosylceramide-based glycosphingolipids). Miglustat is used to treat adults with mild to moderate type 1 Gaucher disease.

Genz112638. Genz112638 is glucosylceramide analog given orally, and is designed to partially inhibit glucosylceramide synthase, which results in reduced production of glucosylceramide.

In some embodiments, when velaglucerase is administered in combination with an additional agent, the combination can result in a lower dose of the additional agent or velaglucerase being needed, such that side effects are reduced. The combination may result in enhanced delivery and efficacy of one or both agents.

The agents or therapies can be administered at the same time (e.g., as a single formulation that is administered to a patient or as two separate formulations administered concurrently) or sequentially in any order. Sequential administrations are administrations that are given at different times. The time between administration of the one agent and another agent can be minutes, hours, days, or weeks. The use of velaglucerase can also be used to reduce the dosage of another therapy, e.g., to reduce the side-effects associated with another agent that is being administered, e.g., to reduce the side-effects of a therapy other than enzyme replacement therapy. Accordingly, a combination can include administering a second agent at a dosage at least 10, 20, 30, or 50% lower than would be used in the absence of velaglucerase.

A combination therapy can include administering an agent that reduces the side effects of other therapies. For example, a corticosteroid can be administered to a subject prior to administration of the treatment for Gaucher disease to decrease infusion site reaction. As another example, iron supplement therapy can be given during the course of velaglucerase therapy.

Gaucher disease

Gaucher disease is the most common of the lysosomal storage diseases. It is caused by a hereditary deficiency of the enzyme glucocerebrosidase (also known as acid β -glucosidase). The enzyme acts on a fatty substance glucocerebroside (also known as glucosylceramide). When the enzyme is defective, the substance accumulates, particularly in cells of the mononuclear cell lineage. Fatty material can collect in the spleen, liver, kidneys, lungs, brain and bone marrow. Symptoms may include enlarged spleen and liver, liver malfunction, skeletal disorders and bone lesions that may be

painful, severe neurologic complications, swelling of lymph nodes and (occasionally) adjacent joints, distended abdomen, a brownish tint to the skin, anemia, low blood platelets and yellow fatty deposits on the white of the eye (sclera). Persons affected most seriously may also be more susceptible to infection. The disease is caused by a recessive gene on chromosome 1 and affects both males and females.

Gaucher disease has three common clinical subtypes:

Type I (or non-neuropathic type) is the most common form of the disease, occurring in approximately 1 in 50,000 live births. It occurs most often among persons of Ashkenazi Jewish heritage. Symptoms may begin early in life or in adulthood and include enlarged liver and grossly enlarged spleen (together hepatosplenomegaly); the spleen can rupture and cause additional complications. Skeletal weakness and bone disease may be extensive. Spleen enlargement and bone marrow replacement cause anemia, thrombocytopenia and leucopenia. The brain is not affected, but there may be lung and, rarely, kidney impairment. Patients in this group usually bruise easily (due to low levels of platelets) and experience fatigue due to low numbers of red blood cells. Depending on disease onset and severity, type 1 patients may live well into adulthood. Many patients have a mild form of the disease or may not show any symptoms. In some embodiments, the methods and compositions described herein are used to treat type I Gaucher disease.

Type II (or acute infantile neuropathic Gaucher disease) typically begins within 6 months of birth and has an incidence rate of approximately 1 in 100,000 live births. Symptoms include an enlarged liver and spleen, extensive and progressive brain damage, eye movement disorders, spasticity, seizures, limb rigidity, and a poor ability to suck and swallow. Affected children usually die by age 2.

Type III (the chronic neuropathic form) can begin at any time in childhood or even in adulthood, and occurs in approximately 1 in 100,000 live births. It is characterized by slowly progressive but milder neurologic symptoms compared to the acute or type 2 version. Major symptoms include an enlarged spleen and/or liver, seizures, poor coordination, skeletal irregularities, eye movement disorders, blood disorders including anemia and respiratory problems. Patients often live into their early teen years and adulthood.

Bone Mineral Density

As used herein bone density (or bone mineral density) refers to the amount of matter per square centimeter of bones. Bone density can be used in clinical medicine as an indirect indicator of osteoporosis and/or fracture risk. BMD can be measured by a number of procedures, e.g., dual energy X-ray absorptiometry (DXA or DEXA), quantitative computed tomography (QCT), qualitative ultrasound (QUS), single photon absorptiometry (SPA), dual photon absorptiometry (DPA), digital X-ray radiogrammetry (DXR), and single energy X-ray absorptiometry (SEXA). Measurements can be made, e.g., over the lumbar spine, the upper part of the hip, or the forearm.

Average bone mineral density can be defined as BMC / W [g/cm^2], wherein BMC = bone mineral content = g/cm , and W = width at the scanned line

Densitometry results can be reported in, e.g., measured density in $g\ cm^{-3}$, z-score, and t-score. Negative scores indicate lower bone density, and positive scores indicate higher.

Z-score refers to the number of standard deviations above or below the mean for the patient's age, sex and ethnicity.

T-score refers to the number of standard deviations above or below the mean for a healthy 30 year old adult of the same sex and ethnicity as the patient. The criteria of the World Health Organization are:

- Normal is a T-score of -1.0 or higher.
- Osteopenia is defined as less than -1.0 and greater than -2.5.
- Osteoporosis is defined as -2.5 or lower, meaning a bone density that is two and a half standard deviations below the mean of a thirty year old woman.

Administration

The glucocerebrosidase enzyme replacement therapy described herein can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or subcutaneously. Preferably the glucocerebrosidase enzyme replacement therapy is administered intravenously, with a dosage ranging from about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or 80 U/kg, administered every

other week, or according to the requirements of the particular compound. The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Typically, the glucocerebrosidase enzyme replacement therapy can be administered as a continuous infusion, e.g., a continuous infusion over 60 minutes, 90 minutes, 120 minutes, or 150 minutes. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from 5% to 95% active compound (w/w). Alternatively, such preparations contain from 20% to 80% active compound.

Lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular subject will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the subject's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

Upon improvement of a subject's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. Subjects may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

A compound, composition or combination of this invention may be administered as a home therapy (e.g., in the subject's home, workplace, or other non-clinical (e.g., non-hospital) setting). It can be administered (e.g., via infusion) by a health care professional (e.g., nurse or physician's assistant). For example, if the subject has not experienced an adverse event (AE) (e.g., a drug-related serious AE or an infusion-related AE, e.g., an event described herein), e.g., after one, two, or three administrations (e.g., via infusion) of the compound, composition or combination, the subject is eligible to receive home therapy for subsequent administrations.

Pharmaceutical Compositions

A glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase) can be incorporated into a pharmaceutical composition for administration to a subject. Such compositions typically include the glucocerebrosidase enzyme replacement therapy (e.g.,
5 velaglucerase) and a pharmaceutically acceptable carrier.

As used herein, the language “pharmaceutically acceptable carrier or adjuvant” includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the
10 compositions. The term “pharmaceutically acceptable carrier or adjuvant” refers to a carrier or adjuvant that may be administered to a subject, together with glucocerebrosidase, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the glucocerebrosidase.

A pharmaceutical composition is formulated to be compatible with its intended
15 route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal and subcutaneous. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols,
20 glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium
25 hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

For intravenous administration, suitable carriers include Sterile Water for Injection, physiological saline, bacteriostatic water, CREMOPHOR EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must
30 be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the

contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars such as sucrose, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

The pharmaceutical composition can include, for example, sterile water for injection, sucrose, sodium citrate, citric acid and polysorbate.

Sterile injectable solutions can be prepared by incorporating the glucocerebrosidase in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a 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 freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Kit

A glucocerebrosidase enzyme replacement therapy (e.g., velaglucerase) can be provided in a kit. The kit includes (a) the glucocerebrosidase enzyme replacement
5 therapy, e.g., a composition that includes the glucocerebrosidase enzyme replacement therapy, and (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of the glucocerebrosidase enzyme replacement therapy for the methods described herein. For example, the informational material describes methods for
10 administering the glucocerebrosidase enzyme replacement therapy to treat Gaucher disease.

In one embodiment, the informational material can include instructions to administer the glucocerebrosidase enzyme replacement therapy in a suitable manner, e.g., in a suitable dose, dosage form, or mode of administration (e.g., a dose, dosage form, or
15 mode of administration described herein). In another embodiment, the informational material can include instructions for identifying a suitable subject, e.g., a human. The informational material of the kits is not limited in its form. In many cases, the informational material, e.g., instructions, is provided in printed matter, e.g., a printed text, drawing, and/or photograph, e.g., a label or printed sheet. However, the informational
20 material can also be provided in other formats, such as Braille, computer readable material, video recording, or audio recording. In another embodiment, the informational material of the kit is a link or contact information, e.g., a physical address, email address, hyperlink, website, or telephone number, where a user of the kit can obtain substantive information about the modulator and/or its use in the methods described herein. Of
25 course, the informational material can also be provided in any combination of formats.

In addition to the glucocerebrosidase enzyme replacement therapy, the composition of the kit can include other ingredients, such as a solvent or buffer, a stabilizer or a preservative, and/or a second agent for treating Gaucher disease. Alternatively, the other ingredients can be included in the kit, but in different
30 compositions or containers than the glucocerebrosidase enzyme replacement therapy. In such embodiments, the kit can include instructions for admixing the glucocerebrosidase

enzyme replacement therapy and the other ingredients (e.g., reconstituting a lyophilized therapy and/or diluting the reconstituted therapy prior to administration), or for using the glucocerebrosidase enzyme replacement therapy together with the other ingredients.

The glucocerebrosidase enzyme replacement therapy can be provided in any form, e.g., liquid, dried or lyophilized form. It is preferred that the glucocerebrosidase enzyme replacement therapy be substantially pure and/or sterile. When the glucocerebrosidase enzyme replacement therapy is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When the glucocerebrosidase enzyme replacement therapy is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing the glucocerebrosidase enzyme replacement therapy. In some embodiments, the kit contains separate containers, dividers or compartments for the glucocerebrosidase enzyme replacement therapy (e.g., in a composition) and informational material. For example, the glucocerebrosidase enzyme replacement therapy (e.g., in a composition) can be contained in a bottle, vial, or syringe, and the informational material can be contained in a plastic sleeve or packet. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the glucocerebrosidase enzyme replacement therapy (e.g., in a composition) is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of the glucocerebrosidase enzyme replacement therapy (e.g., in a composition). For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of the glucocerebrosidase enzyme replacement therapy. The containers of the kits can be air tight and/or waterproof.

The glucocerebrosidase enzyme replacement therapy (e.g., in a composition) can be administered to a subject with Gaucher disease. The method can include evaluating a subject, e.g., as described herein and thereby identifying a subject being in need of treatment with the glucocerebrosidase enzyme replacement therapy.

The following examples provide illustrative embodiments of the invention. One of ordinary skill in the art will recognize the numerous modifications and variations that may be performed without altering the spirit or scope of the present invention. Such modifications and variations are encompassed within the scope of the invention. The Examples do not in any way limit the invention.

EXAMPLES

The introduction of enzyme therapy has significantly impacted the natural history of type 1 Gaucher disease. Unfortunately, the existence of a single therapeutic option represents an inherent vulnerability in the treatment of patients with type 1 Gaucher disease. Approximately 15% of imiglucerase treated patients have been reported to develop IgG antibodies and approximately half of these patients reported symptoms of hypersensitivity (Starzyk K *et al.*, *Molec Genet Metab.* 2007; 90:157-163). Globally, the dependence upon a single product in the treatment of Gaucher disease has been underscored by the recent shortage of imiglucerase (Steinbrook R *et al.*, *N Engl J Med.* 2009; 361:1525-1527). Among those receiving imiglucerase infusions, an unknown number of patients require pre-medication to mitigate a potential immune-mediated response. In some instances patients require hydrocortisone, which itself is associated with medical risks including AVN. Furthermore, recently published data suggest that 59% of GD1 patients treated with imiglucerase fail to achieve at least one therapeutic goal following a minimum of 4 years of treatment regardless of dose and duration of treatment (Weinreb N *et al.*, *Am J Hematol.* 2008; 83: 890-895).

Velaglucerase alfa is a novel enzyme replacement therapy (ERT) with unique characteristics (wild type amino acid sequence and high α -mannosyl content) that distinguishes it from imiglucerase. Some of the examples provided below describe clinical trials and extension studies to evaluate the safety and efficacy of velaglucerase alfa. These are the first clinical trial involving an ERT to implement dose reduction and home therapy.

Example 1: TKT025 and TKT025EXT StudiesSummary

This example describes a nine-month Phase I/II open-label, single center trial of velaglucerase alfa (TKT025) and ongoing extension (TKT025EXT) study conducted to
5 evaluate safety and efficacy of velaglucerase alfa.

The primary objective of the Phase I/II trial was to assess the safety of velaglucerase alfa administered intravenously at a dose of 60U/kg every-other-week for nine months in adult patients with symptomatic type 1 (non-neuronopathic) Gaucher disease (GD1). The secondary objective of this trial was to assess the clinical activity of
10 velaglucerase alfa on key disease features (Barton NW *et al. N Engl J Med.* 1991; 324:1464-1470). The extension study was similarly designed to evaluate the long-term safety and assess the effects of velaglucerase alfa on four disease measures, hemoglobin concentration, platelet count, liver volume, and spleen volume (Barton NW *et al. N Engl J Med.* 1991; 324:1464-1470).

Twelve symptomatic adult patients with type 1 Gaucher disease and intact spleens received velaglucerase alfa (60U/kg/infusion) during the Phase I/II study. Originally thirteen patients were screened to participate in the study, but one was excluded because of the presence of anti-imiglucerase antibody. An extension study was offered to patients who completed the trial and a step-wise dose reduction (to 30U/kg/infusion) was
20 instituted. Eleven patients completed Phase I/II; ten entered the extension study; nine patients reached 39 months in the extension. No drug-related serious adverse events or withdrawals, and no antibodies were observed. Home therapy was successfully implemented during the extension. Statistically significant improvements ($p < 0.004$) were noted in mean percent change from baseline to nine months and baseline to 48 months for
25 hemoglobin (+19.2%, +21.7% respectively), platelet counts (+67.6%, +157.8% respectively), normalized liver volume (-18.2%, -42.8% respectively), and normalized spleen volume (-49.5%, -79.3% respectively). These significant clinical changes and safety profile led to Phase III trials and highlight the potential of velaglucerase alfa as alternative therapy for type 1 Gaucher disease.

Methods

The Phase I/II and extension study was conducted in a single center (Gaucher Clinic, Shaare Zedek Medical Center; Jerusalem, Israel).

Patients: Adult, symptomatic, enzymatically confirmed patients with GD1 were screened. Eligibility criteria included age ≥ 18 years-old, an intact spleen, disease-related anemia (hemoglobin values at least 1g/dL below lower limit of normal (LLN) for gender), thrombocytopenia (platelet counts below the LLN), and a negative result for hepatitis B and C antigen and human immunodeficiency virus. Patients were eligible if they were naïve to ERT or had not received imiglucerase within the 12 months prior to enrollment and were imiglucerase antibody-negative. Patients were excluded if they had received an investigational therapy for any other indication ≤ 30 days prior to enrollment or if they could not comply with the protocol for either medical or non-medical reasons.

Preparation and Dosing: Velaglucerase alfa was supplied by Shire HGT as a lyophilized product and shipped at 2°-8°C. The product was reconstituted with preservative-free, sterile water for injection. The appropriate amount of velaglucerase alfa (based on body weight) was slowly mixed with normal saline to a final volume of 100mL. The diluted velaglucerase alfa was administered intravenously across a 0.2 μ m filter for a period of 60 minutes (maximum rate of 1.5mg/kg/hour; 1U/kg/min).

During the Phase I/II study, the first three patients received velaglucerase alfa on an every-other-week schedule at the trial site. Dose escalation was undertaken for the first three patients whereby dosing doubled from an initial dose of 15U/kg until a final dose of 60U/kg was achieved. The second and third patients received their initial 15U/kg infusion only after a 7-day observation period was completed for the first and second patients respectively. Once the third patient received a single dose of 60U/kg and was observed for a period of 7 days, nine additional patients were enrolled and received infusions of 60U/kg every-other-week for a total of 20 doses. The patients who had undergone dose escalation were continued on an every-other-week schedule for 17 further infusions at 60U/kg for a total of 20 infusions.

During the extension study, all patients were continued at 60U/kg/infusion every-other-week. After approximately 6-9 months of the extension study, patients who achieved at least 2 of the 4 therapeutic goals for improvement in anemia,

thrombocytopenia, hepatomegaly, and/or splenomegaly (Pastores GM *et al.*, *Semin Hematol.* 2004; 41:4-14) were transitioned to 45U/kg/infusion every-other-week for three months and then transitioned to 30U/kg/infusion every-other-week. This convention of dose reduction based on achievement of therapeutic goals is in accordance with recommendations for individualization of ERT in patients on imiglucerase (Andersson HC *et al.*, *Genet Med.* 2005; 7:105-110).

In addition, the seven patients residing in Israel were transitioned to home therapy during the extension phase.

Safety Assessments: Safety was evaluated throughout the study by every-other-week assessments of adverse events (including infusion-related reactions), concomitant medications, and vital signs performed before, during, and after infusions. Additional safety assessments were conducted approximately every 12 weeks and included physical examinations, clinical laboratory tests (hematology, serum chemistry, urinalysis, and pregnancy test), 12-lead electrocardiograms and echocardiograms at the trial site. Determination of the presence of anti-velaglucerase alfa antibodies were conducted at 3-month intervals at Shire HGT.

Antibody Assays: All participants were screened for circulating anti-velaglucerase alfa antibodies using a validated indirect ELISA. Microwell plates were coated with velaglucerase alfa, washed, and blocked with bovine serum albumin to limit non-specific antibody binding. They were incubated with patient serum samples diluted 100-fold in phosphate buffered saline containing 0.05% Tween 20 for 60 minutes at 37°C. The microwells were washed and then incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody. They were separately probed with the following HRP-antibody, isotype-specific conjugates: 1) goat anti-human IgG Fc, 2) goat anti-human IgA α -chain, 3) goat anti-human IgM μ -chain, or 4) goat anti-human IgE ϵ -chain secondary antibodies. The microwells were washed one final time and incubated with the HRP chromogenic substrate 3, 3', 5, 5' tetramethyl benzidine. The reaction was stopped by the addition of 2N sulfuric acid, and the absorbance of each well was quantified at 450nm (A_{450}) using a Molecular Devices SPECTRAmax Plus384 plate reader and

SOFTMax PRO software. Antibody-positive serum samples were obtained from patients receiving imiglucerase. These patient antibodies cross-reacted with velaglucerase alfa and were used as human positive controls in the screening assay for anti-velaglucerase antibodies. These sera were therefore both anti-imiglucerase and anti-velaglucerase alfa positive. Negative, as well as positive, human serum controls were included within every assay plate.

A robust ELISA antibody positive cutpoint absorbance for anti-velaglucerase alfa antibodies was established from the mean absorbance of ERT-naïve Gaucher patient serum samples (N=108). Parametric analysis of ELISA absorbance data was used to calculate the antibody-positive lower limit (mean plus 1.645 x standard deviations; where 1.645 is the 95th percentile of the normal distribution, thus potentially accepting a 5% false-positive rate) for each antibody isotype (Mire-Sluis AR *et al.*, *J Immunol Methods*. 2004; 289:1-16). The ELISA A₄₅₀ background for ERT-naïve Gaucher serum samples was calculated to be 0.040 for all anti-velaglucerase alfa antibody isotype assays. The ELISA antibody positive cutoff was established as a ratio ≥ 2.0 and an A₄₅₀ ≥ 0.040 , where ratio is the A₄₅₀ of a patient sample taken at any time point, divided by the A₄₅₀ of the patient sample taken at baseline prior to the first ERT treatment. Any sample exceeding the ELISA positive cutoff would have been confirmed by a quantitative radioimmunoprecipitation assay and tested for neutralizing antibodies; however no sample achieved the established cutoff criteria (Mire-Sluis AR *et al.*, *J Immunol Methods*. 2004; 289:1-16).

Clinical Activity: The main efficacy assessments of hemoglobin concentration and platelet counts were evaluated at pre-determined tri-monthly intervals. Liver and spleen volumes were measured using quantitative abdominal MRI (on the same model apparatus) performed at baseline, six- and nine-months (at the Hadassah-Hebrew University Medical Center), and at 24, 33, and 45 months (at the MOR-MAR Imaging unit) during the extension study. Liver and spleen volumes were assessed at the end of the trial by a radiologist blinded to the patient's identity and the sequence by which the quantitative abdominal MRIs were performed. Chitotriosidase and CCL18 were measured at the Academic Medical Center; Amsterdam, the Netherlands.

Statistical Analysis: The safety population, which was also the intent-to-treat population, was defined as all enrolled patients receiving at least one infusion (partial or full) of velaglucerase alfa, and was used for all clinical activities. No imputation was utilized.

For the primary clinical activity parameters of hemoglobin concentration, platelet count, and liver and spleen volumes, the null hypothesis is that there is no difference between the baseline value and end-of-the study value (nine months), and baseline value and end-of-48 month value. (i.e., the difference between the members of each pair of observation has median value zero.) Comparisons were performed using two-tailed hypothesis testing at the 5% level of significance. Differences between baseline and end of period values were analyzed using the Wilcoxon signed-rank test. Changes from baseline were calculated and the percentage changes from baseline are summarized using descriptive statistics.

For secondary clinical activity parameters, observed data, change from baseline, and percent change from baseline were summarized by visit including mean, standard error (SE) and median. No formal statistical testing was applied to these data sets.

Results from TKT025 study (9M, 60 U/kg velaglucerase alfa)

Velaglucerase alfa was well tolerated and no patient developed antibodies. Clinically and statistically significant improvements in hematological parameters and organ volumes were observed as early as 3 to 6 months (**Table 1**).

Table 1 TKT025 Clinical Results (N=12)

Clinical Activity Parameter	Median Baseline Value	Mean Change from Baseline $\pm$ standard deviation		
		Month 3	Month 6	Month 9
Hemoglobin concentration (g/dL)	10.95	1.24 $\pm$ 0.90	1.92 $\pm$ 0.82	2.24 $\pm$ 0.89
Platelet count ($\times 10^9$ /L)	57.5	8.9 $\pm$ 11.20	23.4 $\pm$ 24.63	40.6 $\pm$ 30.68
Liver (% of body weight)	4.43	NA	-0.67 $\pm$ 0.64	-0.79 $\pm$ 0.48

Spleen (% of body weight)	3.63	NA	-1.62 ± 0.67	-1.89 ± 0.75

Demographics and disposition: A total of thirteen patients were screened and all consented to participate in this study; one patient (0004) was excluded because of imiglucerase antibodies. All the patients were treatment-naïve at advent according to the protocol by virtue of not having been exposed to any Gaucher-specific therapy in the 12 months prior to enrolment although in the more distant past, 2 patients (0008 and 0009) had each received 3 infusions of imiglucerase, one patient (0003) had been exposed to miglustat, and two patients (0005 and 0007) had been exposed to both miglustat and imiglucerase.

The intent-to-treat population (**Table 2**) included twelve patients who received at least one dose of velaglucerase alfa; of these, 11 patients (92%) completed the Phase I/II study (one patient, 0006, withdrew after a sudden death in the family withdrew consent after receiving three infusions).

At enrollment of the Phase I/II trial, seven patients (58%) were female; mean age was 41.7 (SD±17.3; range 19-70) years; mean weight was 59.6 (SD±9.1 range 50-73) kg and mean height was 169 (SD±8.0; range 160-184) cm. Two patients (16.7%) had avascular necrosis (AVN) of the hip joint at enrollment and another had a destructive lesion in each ankle. **Table 2** provides the demographic, genotypic, and clinical characteristics at baseline, as well as the clinical findings of each intent-to-treat patient at key data collection points within the Phase I/II studies.

Table 2 Patient Demographics and Characteristics

				Hemoglobin g/dL						Platelets, 10/L						Normalized spleen volume, multiple of normal						Normalized liver volume, multiple of normal					
Patient	Age	Sex	Genotype	BL	6 Mo	9 Mo	24 Mo	48 Mo	BL	6 Mo	9 Mo	24 Mo	48 Mo	BL	6 Mo	9 Mo	24 Mo	45 Mo	BL	6 Mo	9 Mo	24 Mo	45 Mo				
1	24	F	N370S/N370S	10.8	12.2	12.5	12.4	12.7	80	123	149	155	130	17.5	11.0	10.0	8.0	6.5	2.3	1.8	1.9	1.5	1.2				
2	62	M	N370S/L444P	12.7	14.1	14.8	14.3	14.2	69	74	90	127	142	11.0	7.5	6.0	4.0	3.0	1.0	1.0	0.9	1.0	0.8				
3	35	M	N370S/Other	13.5	15.7	16.5	15.7	16.0	48	95	128	140	151	17.5	8.0	6.5	*	*	2.2	1.6	1.7	1.3	1.2				
5	35	F	N370S/N370S	10.6	11.7	12.0			52	68	72			32.5	23.0	24.5			1.7	1.8	1.6						
6	44	F	N370S/IVS2+1	10.9					56					16.0					2.1								
7	42	F	N370S/N370S	10.5	12.5	12.5	13.8	13.0	37	32	39	42	95	21.0	13.5	13.0	7.0	4.0	1.8	1.7	1.6	1.4	1.0				
8	23	F	N370S/RecNc1	10.0	12.1	11.8	12.7	14.2	65	137	150	154	203	19.0	9.0	7.0	3.5	3.5	2.2	1.6	1.6	1.2	1.2				
9	25	M	N370S/RecNc1	12.6	15.8	15.9	15.8	15.1	69	97	120	178	183	28.0	12.5	10.0	4.0	2.5	2.0	1.4	1.4	1.1	0.9				
10	60	F	N370S/N370S	10.1	11.1	12.5	12.4	12.4	48	60	86	121	139	19.0	11.0	8.5	3.5	3.0	1.4	1.2	1.0	0.8	0.8				
11	18	F	N370S/Other	11.0	13.8	13.6	13.6	12.6	59	98	110	122	130	15.0	9.0	8.0	5.0	3.5	1.4	1.1	1.0	1.1	0.8				
12	69	M	N370S/N370S	13.5	14.4	14.1			59	65	99			13.0	9.0	7.5			1.0	1.0	1.0						
13	56	M	N370S/V394L	12.9	15.9	16.6	15.4	16.0	46	40	36	111	135	19.5	10.0	8.0	4.5	2.5	1.0	0.8	0.8	0.8	0.7				

Patient demographics and characteristics at baseline and at select points of evaluation during the Phase I/II and

- 5 extension trials including the last values collected for each clinical parameter for the cohort. Normalized liver and spleen volumes are defined as 2.5% and 0.2% respectively of total body weight in kilograms (Pastores GM *et al.*, *Semin Hematol.* 2004; 41:4-14). Multiples of normal is the observed organ volume divided by the normalized organ volume. By 24 months, all patients demonstrated normalization of hemoglobin concentrations and all but one patient demonstrated platelet counts greater than $100 \times 10^9 /L$. BL, baseline; Mo, month; M, male; F, Female; *Spleen volume not interpretable due to technical artifact.

10

Results of liver and spleen magnetic resonance imaging (MRI) scans were blinded and interpreted by a single reviewer to minimize bias. Collection of baseline data from other clinical parameters including PFTs (pulmonary function tests), MRI of the femora and lumbar spine, and bone densitometry were also performed at various time points throughout the study.

The study was comprised of 2 phases, a dose escalation phase and a continuous dose phase. During the dose escalation phase, the first patient was enrolled and administered Intravenous (IV) velaglucerase alfa 15 U/kg, then was followed for 7 days for assessment of safety. After safety was confirmed for the first patient, 2 additional patients were enrolled and administered IV velaglucerase alfa 15 U/kg and followed for 7 days for assessment of safety. After safety was confirmed for all 3 patients who received the 15 U/kg dose, the next two higher doses (30 and 60 U/kg) were administered in the same fashion. Once safety was confirmed for all 3 patients, an additional 9 patients were enrolled and administered the 60 U/kg dose. All patients then received the 60 U/kg dose every other week for a total of 20 doses of velaglucerase alfa.

Pharmacokinetics (PK) in humans were evaluated at Weeks 1, 3, 5, and 37/39 in TKT025. Additional PK studies were conducted at Week 65 of Study TKT025EXT. Velaglucerase alfa was rapidly cleared from blood with first-order elimination kinetics at each evaluation. Elimination half-life values for patients following initial exposure to velaglucerase alfa and following repeat administration of velaglucerase alfa were similar. In contrast, clearance and apparent volume of distribution values were reduced following repeat administration of velaglucerase alfa.

Statistically significant mean increases from baseline in hemoglobin concentration and platelet count were observed 3 months after initiation of biweekly dosing of 60U/kg velaglucerase alfa in Study TKT025. By Week 25 (Month 6) in TKT025, mean hemoglobin concentrations were 13.57 g/dL (within normal range) and remained within normal ranges for the rest of the study. At the end of the study, 10 of 11 patients experienced normalization of hemoglobin concentration. Statistically significant increases from baseline ($57.3 \times 10^3/\text{mm}^3$) in mean platelet count were observed by Week 13 (Month 3) and continued throughout the study. At Week 37, mean platelet counts had increased to $98.1 \times 10^3/\text{mm}^3$.

Additionally, statistically significant reductions from baseline in mean and normalized (corrected by percentage of body weight) liver and spleen volumes were observed 6 (Week 25) and 9 months (Week 37) after initiating velaglucerase alfa treatment. Marked reductions in biomarker values (serum chitotriosidase and Chemokine (C-C motif) ligand 18 [CCL18]) were observed by Month 3. Overall, mean improvement in all of these parameters was continual during the course of velaglucerase alfa administration. Moreover, each patient experienced improvement in at least 2 of the 4 therapeutic parameters specific for Gaucher disease (e.g., hemoglobin concentrations, platelet counts, and spleen and liver volumes).

In summary, **FIGS. 1A-1F** presents the mean percent change in hematological parameters, organ volumes, and biomarkers for the Phase I/II study. A marked increase in (a) hemoglobin concentration and (b) platelet count is observed during weeks 25 and 37 along with a marked reduction in (c) liver and (d) spleen volumes. Although biomarker sampling for (e) chitotriosidase and (f) CCL18 was incomplete, a general decrease in both biomarkers relative to baseline is observed per patient over time. Statistically significant improvements ($p < 0.003$) were noted in the mean percent change from baseline to nine months for hemoglobin concentration (+21.7%), platelet counts (+67.6%), normalized liver volume (-18.2%), and normalized spleen volume (-49.5%), with statistically significant improvements from baseline in both hemoglobin concentration and platelet counts achieved within the first 3 months (**FIG. 2**). In **FIG. 2**, the mean percent change in hemoglobin concentration, platelet counts, liver volume and spleen volume is plotted across time and demarcated for both the Phase I/II and extension studies. A statistically significant change from baseline is observed from baseline to 9 months ($p < 0.003$) for each parameter. The most marked changes were observed for platelet count and spleen volume.

In TKT025, all 12 patients experienced 1 or more AEs (Adverse events); in total, 103 AEs were reported. Two AEs (dizziness and hyperhidrosis) were reported by 1 patient during the 15 U/kg dose; the remaining 101 AEs were reported by patients receiving the 60 U/kg dose. The most frequently reported AEs were dizziness, bone pain, and headache (5 patients each; 41.7%). Arthralgia, back pain, pain in the extremities, influenza, upper abdominal pain, and asthenia each occurred in 3 patients (25%). All

AEs were mild (Grade 1) except 1 event of bone pain, which was of moderate (Grade 2) severity and unrelated to study drug. None of the 103 AEs was considered severe or life threatening (Grade 3 or 4). No patient was withdrawn from the study due to an adverse event.

The majority of AEs observed during TKT025 were determined by the investigator as not related to administration of study drug (**Table 3A**). Ten (10) patients experienced 22 AEs that were considered possibly or probably related to study drug; most commonly dizziness (3 patients); back pain, bone pain, headache, increased body temperature, and nausea (each 2 patients). Nine (9) patients experienced a total of 17 infusion-related adverse events, which were defined in the protocol as an AE that occurred on the day of the infusion, began either during or after the infusion, and was judged as possibly or probably related to study drug. These AEs included dizziness, headache, back pain, bone pain, body temperature increased, each occurring in 2 patients. All infusion-related adverse events were reported for patients receiving the 60 U/kg dose. One patient was dose-increased to 60 U/kg/infusion every-other-week 24 months following dose reduction because of worsening bone pain but experienced no relief following dose increase. Therefore, there was no drug-related serious adverse events, regardless of infusion setting. No pre-medications were administered and no patient withdrew due to an adverse event. No patient developed antibodies to velaglucerase alfa.

Table 3A Treatment emergent adverse events determined to be possibly or probably related to velaglucerase alfa (GA-GCB) administration observed during the phase I/II Trial (TKT025)

System Organ Class Preferred Term	velaglucerase alfa 60 U/kg EOW n = 12 patients	
	Patients, n (%)	Events, n (%)
Any Adverse Event	10 (83.3)	22 (21.8)
Nervous System Disorders		
Dizziness	3 (25.0)	4 (4.0)
Headache	2 (16.7)	2 (2.0)
Burning sensation	1 (8.3)	1 (1.0)

Migraine	1 (8.3)	3 (3.0)
Tremor	1 (8.3)	1 (1.0)
Gastrointestinal Disorders		
Nausea	2 (16.7)	2 (2.0)
Upper abdominal pain	1 (8.3)	1 (1.0)
Musculoskeletal and Connective Tissue Disorders		
Back pain	2 (16.7)	2 (2.0)
Bone pain	2 (16.7)	2 (2.0)
Pain in extremity	1 (8.3)	1 (1.0)
General Disorders and Administration Site Conditions		
Asthenia	1 (8.3)	1 (1.0)
Investigations		
Body temperature increased	2 (16.7)	2 (2.0)

Percentages of patients are based on the total number of patients in the treatment group. Adverse events are coded using MedDRA Version 7.0 dictionary. EOW: every other week.

5 Results from TKT025EXT (Long-Term, 30-60 U/Kg Velaglucerase Alfa)

Summary: TKT025EXT is an open-label extension study of velaglucerase alfa therapy in patients with type 1 Gaucher disease who completed study TKT025. The primary objective of TKT025EXT is to evaluate the long-term safety of velaglucerase alfa when administered IV at a dose of 30 or 60 U/kg every other week for a total of 4 years. The secondary objective is to continue to assess the effects of velaglucerase alfa on clinical activity in these patients as measured in hematological parameters and reductions in liver and spleen volumes. Plasma chitotriosidase and CCL18 as well as PFTs, MRI of the femoral neck and lumbar spine, skeletal survey, and bone densitometry are also being evaluated.

15 Ten patients were elected to participate in this clinical study. After 60 months of cumulative treatment (TKT025 + TKT025EXT) velaglucerase alfa was well tolerated and no patient developed antibodies. Continued and sustained clinically and statistically

significant improvements in hematological parameters and organ volumes continue to be seen (Table 4). FIG. 3 shows the increase of mean hemoglobin concentration and platelet count from baseline in the patients treated with velaglucerase alfa for 60 months. FIG. 4 shows the decrease of mean liver and spleen volumes from baseline in patients treated with velaglucerase alfa for 60 months.

Table 4 TKT025EXT Clinical Results (N=10)

Clinical Activity Parameter	Median Baseline Value	Mean Change from Baseline [95% Confidence Interval]			
		Month 24	Month 36 ^a	Month 48 ^a	Month 60 ^a
Hemoglobin concentration (g/dL)	10.90	2.30 [1.78, 2.82]	2.13 [1.59, 2.67]	2.31 [1.66, 2.96]	2.38 [1.60, 3.16]
Platelet count (x 10 ⁹ /L)	55.5	66.5 [45.3, 87.7]	71.1 [53.6, 88.6]	82.3 [60.1, 104.5]	85.1 [59.8, 110.4]
Liver (% of body weight)	4.40	-1.29 [-1.91, -0.67]	-1.37 [-1.90, -0.84]	-1.74 [-2.40, -1.08]	-1.70 [-2.33, -1.07]
Spleen (% of body weight)	3.80	-2.63 [-3.44, -1.83]	-2.69 [-3.51, -1.87]	-2.88 [-3.72, -2.03]	-2.89 [-3.72, -2.06]

a - Organ volumes were assessed at Months 33, 45 and 57 from the start of

TKT025

Demographics and disposition: Upon completion of Week 41 evaluations in TKT025, patients were eligible to participate in TKT025EXT. Of the 11 patients who completed Study TKT025, 10 patients entered Study TKT025EXT. One patient (0012) did not consent to enter the extension study because of the inconvenience of every-other-week hospital attendance, and one patient (0005) withdrew from the extension study because of pregnancy. Table 2 provides the demographic, genotypic, and clinical characteristics at baseline, as well as the clinical findings of each intent-to-treat patient at key data collection points within the extension studies.

All patients enrolled initially received velaglucerase alfa 60 U/kg every other week. Doses were titrated down to 30 U/kg provided patients had improvements in

hematological parameters (hemoglobin concentration or platelet levels) and/or improvements in organ volumes (liver or spleen) after 1 year of cumulative treatment. All 10 patients initially enrolled in TKT025EXT met the required dose reduction criteria and were transitioned to the 30 U/kg dose.

5 All 9 patients in TKT025EXT had hemoglobin concentration and platelet count data available up to Month 42, while 3 patients had data available up to Month 45. The mean (standard error [SE]) changes in hemoglobin from pretreatment baseline at 42 months were 2.18 (0.25) g/dL ($p = 0.004$), corresponding to a mean percent changes from pretreatment baseline of 19.0%. With respect to platelet count, the mean (SE) change
10 from pretreatment baseline was $82.1 (8.4) \times 10^3/\text{mm}^3$ ($p = 0.004$) at Month 42, corresponding to a mean percent change from pretreatment baseline of 149.8%.

Liver and spleen volumes normalized for percent of body weight were analyzed through Month 33; all 9 patients had liver data available and 8 patients had spleen data available for this assessment. The mean (SE) change from pretreatment baseline in
15 normalized liver and spleen volumes at Month 33 was -1.5 (0.22) and -2.8 (0.37), respectively. The results corresponded to -34.0% and -73.5% mean percent changes from pretreatment baseline for normalized liver and spleen volumes, ($p = 0.004$ and $p = 0.008$, respectively). Further improvements were also observed for both plasma chitotriosidase and CCL18 parameters.

20 In summary, **FIG. 2** presents the mean percent change in hemoglobin concentration, platelet counts, liver volume and spleen volume that is plotted across time and demarcated for the extension studies. A statistically significant change is observed from baseline to 48 months ($p < 0.004$) for each parameter. The most marked changes were observed for platelet count and spleen volume. Hemoglobin values normalized for
25 all patients by 24 months. Liver volumes approached normal. Continuous improvement in these clinical parameters was noted throughout the extension study (**FIG. 2**) and normalization of hemoglobin was observed in all patients by 24 months (**Table 2**). The mean percent change from baseline to 48 months was statistically significant ($p < 0.004$) for hemoglobin concentration (+21.7%), platelet counts (+157.8%), liver volume (-
30 42.8%), and spleen volume (-79.3%).

Safety and clinical activity data for Study TKT025EXT available up to a certain date were analyzed. Patients enrolled in this study had a cumulative mean duration of exposure of 43.2 months (range: 13.2-45.0 months). A total of 248 adverse events were reported among all 10 patients enrolled. The majority of AEs were mild to moderate in severity. Three severe (Grade 3) adverse events, considered unrelated to study drug by the Investigator, were reported for 2 patients (arthralgia and aseptic necrosis bone in 1 patient, and headache in 1 patient). Three (3) SAEs have been reported (2 events of aseptic bone necrosis in 1 patient, and 1 event of scar in 1 patient). Each of these SAEs was considered unrelated to study drug by the Investigator, and each resolved without sequelae. The most frequently reported treatment-emergent adverse events were influenza (8 patients, 24 events); arthralgia (8 patients, 21 events); headache (6 patients, 13 events); back pain (6 patients, 10 events); pharyngolaryngeal pain (5 patients, 7 events); abdominal pain upper (5 patients, 7 events); and gingival bleeding, pyrexia and fatigue (each 4 patients, 4 events).

The majority of adverse events reported in TKT025EXT were considered by the Investigator to be unrelated to study drug (**Table 3B**). A total of 7 treatment-related adverse events were reported in 4 patients, including epistaxis (2 patients, 1 event each) abdominal pain (1 patient, 1 event), abdominal pain upper (1 patient, 1 event), fatigue (1 patient, 1 event), and pain exacerbated (1 patient, 1 event). Two patients experienced 1 infusion-related AE each: moderate (Grade 2) pain in extremity in 1 patient, and mild (Grade 1) tremor in 1 patient. Therefore, there was no drug-related serious adverse events regardless of infusion setting. In addition, no patient had an infusion-related adverse event requiring interruption of administration of velaglucerase alfa.

No patient developed antibodies to velaglucerase alfa. During the first year of the extension study all seven residents of Israel were successfully transitioned to home therapy.

Table 3B Treatment emergent adverse events determined to be possibly or probably related to velaglucerase alfa (GA-GCB) administration observed during the extension study (TKT025 EXT)

System organ class preferred term	velaglucerase alfa 30 U/kg – 60 U/kg EOW	
	Patients, n (%) N=10	Events, n (%) N=311
Any Related Adverse Event	4 (40.0)	7 (2.8)
Nervous System Disorders		
Tremors	1 (10.0)	1 (0.32)
Respiratory, Thoracic or Mediastinal disorders		
Epistaxis	2 (20.0)	2 (0.6)
Gastrointestinal Disorders		
Abdominal pain	2 (20.0)	2 (0.6)
Musculoskeletal and Connective Tissue Disorders		
Pain in extremity	1 (10.0)	1 (0.3)
General Disorders and Administration Site Conditions		
Fatigue	1 (10.0)	1 (0.3)

Percentages of patients are based on the total number of patients in the safety population. Percentages of events are based on the total number of events experienced by patients in the safety population. Adverse events are coded using MedDRA Version 9.0 dictionary. EOW: every other week.

5

Results of bone mineral density in Type 1 Gaucher disease patients in TKT025 and TKT025EXT

Long term bone mineral density (BMD) changes were evaluated in Type 1 Gaucher disease patients treated with velaglucerase alfa. Patients demonstrated significant and continuous improvement in BMD.

10

Assessments: During TKT025 enrollment, skeletal surveys and dual energy X-ray absorptiometry (DEXA) were used to evaluate skeletal pathology. Z-scores of the lumbar spine and femoral neck were analyzed at predefined times throughout the study. For

some patients, DEXA scans were performed, but Z-scores could not be assessed from the scans. Missing Z-scores included: one patient – femoral neck at baseline and Month 9; one patient – lumbar spine at baseline; and one patient – femoral neck at Month 9. Lumbar spine and femoral neck Z-scores were also not evaluated for two patients after they withdrew from the study: one patient before Month 24 and one patient before Month 57.

T-scores were used to compare bone density to a “young, normal” healthy 30-year-old adult with peak bone density. Clinical bone status at baseline and 69 months was characterized according to the WHO criteria for T-scores: ≥ -1 is normal; > -2.5 and < -1 is osteopenia; ≤ -2.5 is osteoporosis.

Methods:

Analysis population: The primary analysis was conducted in the intent-to-treat (ITT) population (N=10), defined as all patients who signed the informed consent to participate in the long-term extension study and received ≥ 1 full or partial dose of velaglugerace alfa. The effect of velaglugerace alfa on BMD was also assessed in subgroups who did or did not receive bisphosphonates concomitant with ERT.

Statistical methodology: Missing baseline Z-scores were replaced with the next value. Last observation carried forward (LOCF) analysis was used for subsequent missing Z-scores. Linear mixed models were used as a repeated measures analysis to analyze Z- scores over time. The y-intercept and the slope estimate, showing the annual increase in Z-score units, are displayed. A shift from osteoporosis to osteopenia or normal, and from osteopenia to normal was recorded and reported.

Results:

Baseline characteristics: All patients enrolled in TKT025EXT had GD1-related bone pathology. Clinical status of lumbar spine (LS) bone pathology was: 1 patient (10%) was in the normal range; 8 patients (80%) had osteopenia; and 1 patient (10%) had osteoporosis. Clinical status of femoral neck (FN) bone pathology was: 1 patient (10%) was in the normal range; 5 patients (50%) had osteopenia; and 4 patients (40%) patients

had osteoporosis. DXA Z-scores were (median [range]): LS, -1.8 [-2.9 to -0.4], FN -1.5 [-2.9 to -0.2]. Through 69 months the average velaglucerase alfa dose was 40 U/kg. Four of ten patients were also treated with bisphosphonates.

5 **Z-scores:** Z-scores at baseline and various timepoints for ITT population (n=10), patients who received concomitant bisphosphonates, and patients who did not received bisphosphonates are shown in **Tables 5-7**, respectively. Temporal changes in mean Z-scores of lumbar spine and femoral neck are also shown in **FIGs. 5 and 6**, respectively.

10 **Table 5** Z-scores; N=10, ITT

Time	Z-Scores	
	Lumbar Spine Mean [95% CI]	Femur Mean [95% CI]
Baseline	-1.59 [-2.17, -1.01]	-1.46 [-2.11, -0.81]
Month 9	-1.50 [-2.05, -0.95]	-1.39 [-2.06, -0.72]
Month 24	-1.20 [-1.80, -0.60]	-1.23 [-2.04, -0.42]
Month 33	-1.16 [-1.80, -0.52]	-1.07 [-1.82, -0.32]
Month 45	-1.12 [-1.72, -0.52]	-1.14 [-1.86, -0.42]
Month 57	-1.07 [-1.68, -0.46]	-1.10 [-1.89, -0.31]
Month 69	-0.91 [-1.55, -0.27]	-1.06 [-1.83, -0.29]

Table 6 Z-scores; n=4 [with bisphosphonates]

Time	Z-Scores	
	Lumbar Spine Mean [95% CI]	Femur Mean [95% CI]
Baseline	-1.88 [-3.25, -0.50]	-1.70 [-3.13, -0.27]
Month 9	-1.90 [-3.14, -0.66]	-1.68 [-3.15, -0.20]
Month 24	-1.78 [-2.89, -0.66]	-1.75 [-3.52, 0.02]
Month 33	-1.68 [-3.14, -0.21]	-1.45 [-3.19, 0.29]
Month 45	-1.60 [-2.74, -0.46]	-1.48 [-3.23, 0.28]
Month 57	-1.50 [-2.96, -0.04]	-1.40 [-3.25, 0.45]
Month 69	-1.23 [-2.78, 0.33]	-1.48 [-3.22, 0.27]

Table 7 Z-scores; n=6 [no bisphosphonates]

Time	Z-Scores	
	Lumbar Spine Mean [95% CI]	Femur Mean [95% CI]
Baseline	-1.40 [-2.22, -0.58]	-1.30 [-2.30, -0.30]
Month 9	-1.23 [-1.97, -0.50]	-1.20 [-2.22, -0.18]
Month 24	-0.82 [-1.57, -0.06]	-0.88 [-2.03, 0.26]
Month 33	-0.82 [-1.62, -0.02]	-0.82 [-1.91, 0.28]
Month 45	-0.80 [-1.64, 0.04]	-0.92 [-1.95, 0.11]
Month 57	-0.78 [-1.55, -0.01]	-0.90 [-2.07, 0.27]
Month 69	-0.70 [-1.61, 0.21]	-0.78 [-1.91, 0.34]

Changes in Z-scores of lumbar spine (LS) and femoral neck (FN) from baseline Z-scores during a course of 69 months are shown in **Table 8**.

5

Table 8

Scheduled Visit	No bisphosphonates n=6	Bisphosphonates* n=4	ITT Population N=10
Lumbar Spine Change From Baseline Z-scores (95% CI)			
Month 9	0.17 (-0.03, 0.36)	-0.03 (-0.18, 0.13)	0.09 (-0.04, 0.22)
Month 24	0.58 (0.08, 1.09)	0.10 (-0.27, 0.47)	0.39 (0.06, 0.72)
Month 33	0.58 (-0.06, 1.22)	0.20 (-0.14, 0.54)	0.43 (0.06, 0.80)
Month 45	0.60 (0.03, 1.17)	0.28 (-0.12, 0.67)	0.47 (0.14, 0.80)
Month 57	0.62 (0.05, 1.18)	0.38 (-0.04, 0.79)	0.52 (0.20, 0.84)
Month 69	0.70 (0.16, 1.24)	0.65 (-0.04, 1.34)	0.68 (0.35, 1.01)
Femoral Neck Change From Baseline Z-scores (95% CI)			
Month 9	0.10 (-0.03, 0.23)	0.03 (-0.18, 0.23)	0.07 (-0.02, 0.16)
Month 24	0.42 (-0.04, 0.87)	-0.05 (-0.47, 0.37)	0.23 (-0.08, 0.54)
Month 33	0.48 (0.10, 0.87)	0.25 (-0.13, 0.63)	0.39 (0.16, 0.62)
Month 45	0.38 (0.18, 0.59)	0.23 (-0.15, 0.60)	0.32 (0.17, 0.47)
Month 57	0.40 (0.06, 0.74)	0.30 (-0.20, 0.80)	0.36 (0.14, 0.58)
Month 69	0.52 (0.22, 0.81)	0.23 (-0.25, 0.70)	0.40 (0.18, 0.62)

P≤0.05 vs baseline value

As shown in **Table 8**, BMD for the intent-to-treat population improved significantly by Months 24 (LS: 0.39 (0.06, 0.72)) and Months 33 (FN: 0.39 (0.16, 0.62)). BMD for the patients who did not receive concomitant bisphosphonates improved significant by Months 24 (LS: 0.58 (0.08, 1.09)) and Months 33 (FN: 0.48 (0.10, 0.87)).

5

Linear mixed model estimated Z-scores: Z-scores (ITT population, n=10, 95% CI) were significantly lower than the reference population (LS y-intercept = -1.56 [-2.09, -1.03]; $P<0.0001$) and FN (y-intercept = -1.42 [-2.06, -0.79]; $P=0.0007$). Both parameters improved significantly over time (LS slope per month = +0.011 [0.005, 0.017], $P=0.0021$ (slope +0.011 Z-score units/month corresponds to +0.132/year); FN slope per month = +0.007 [0.004, 0.009], $P=0.0005$ (slope +0.007 Z-score units/month corresponds to +0.084/year)).

10

Among patients who only received velaglucerase alfa (n=6, 95% CI), the Z-score LS y-intercept was -1.29 [-1.97, -0.62] ($P=0.0045$), FN y-intercept was -1.24 [-2.21, -0.27] ($P=0.0216$) and significant improvement was seen over time [LS slope per month = +0.013 [0.002, 0.024] ($P=0.028$) (corresponds to +0.158 per year), FN slope per month = +0.009 [0.004, 0.013] ($P=0.0055$) (corresponds to +0.103 per year)].

15

Among patients who received bisphosphonates concomitantly (n=4, 95% CI), the Z-score LS y-intercept was -1.97 [-3.06, -0.88] ($P=0.0104$), FN y-intercept was -1.71 [-3.02, -0.40] ($P=0.0252$) and improvement was seen over time [LS slope per month = +0.009 [0.001, 0.017] ($P=0.0351$) (corresponds to +0.111 per year), FN slope per month = +0.004 [-0.001, 0.010] ($P=0.0867$) (corresponds to +0.048 per year)].

20

In a linear mixed model based upon an analysis of an observational database (International Collaborative Gaucher Group Gaucher Registry), GD1 patients treated with the ERT imiglucerase had significantly lower Z-scores than the reference population at the start of follow-up (n=340, Y-intercept -1.17, $P<0.001$) (Wenstrup *et al. J Bone Min Res.* 2007; 22: 119-26). However, a dose-response improvement was observed in Z-score slopes per year (15 U/kg [n=113]: +0.064; 30 U/kg [n=116]: +0.086; 60 U/kg [n=111]: +0.132; all $P<0.001$) (Wenstrup *et al. J Bone Min Res.* 2007; 22: 119-26).

25

30

BMD status at baseline and 69 months: Clinical bone status at baseline and 69 months was characterized using WHO criteria (normal = T-scores: ≥ -1 ; osteopenia = T-score > -2.5 and < -1 ; osteoporosis = T-score ≤ -2.5). Status change was observed in patients who received velaglucerase alfa without concomitant bisphosphonates (n=6). By 69 months, two LS and one FN osteopenic patients normalized and one FN osteoporotic patient became osteopenic. All four patients on bisphosphonates had no change in WHO category.

Repeated measures analysis (longitudinal) slope estimates: In the repeated measures analysis, Z-score estimates [95% CI] for ITT population (N=10) were: LS intercept = -1.56 [$-2.09, -1.03$], $P < 0.0001$; LS slope (per month) = 0.011 [$0.005, 0.017$], $P = 0.0021$; FN intercept = -1.42 [$-2.06, -0.79$], $P = 0.0007$; LS slope (per month) = 0.007 [$0.004, 0.009$], $P = 0.0005$. A lumbar spine slope of 0.011 increases per month corresponds to a 0.132 increase per year, and a femoral neck slope of 0.007 increase per month corresponds to a 0.084 increase per year.

Z-score estimates [95% CI] for patients who did not receive bisphosphonates (N=6) were: LS intercept = -1.29 [$-1.97, -0.62$], $P = 0.0045$; LS slope (per month) = 0.013 [$0.002, 0.024$], $P = 0.0280$; FN intercept = -1.24 [$-2.21, -0.27$], $P = 0.0216$; LS slope (per month) = 0.009 [$0.004, 0.013$], $P = 0.0055$. A lumbar spine slope of 0.013 increase per month corresponds to a 0.158 increase per year, and a femoral neck slope of 0.009 increase per month corresponds to a 0.103 increase per year.

Z-score estimates [95% CI] for patients who received bisphosphonates (N=4) were: LS intercept = -1.97 [$-3.06, -0.88$], $P = 0.0104$; LS slope (per month) = 0.009 [$0.001, 0.017$], $P = 0.0351$; FN intercept = -1.71 [$-3.02, -0.40$], $P = 0.0252$; LS slope (per month) = 0.004 [$-0.001, 0.010$], $P = 0.0867$. A lumbar spine slope of 0.009 increase per month corresponds to a 0.111 increase per year, and a femoral neck slope of 0.004 increase per month corresponds to a 0.048 increase per year.

The statistical model used all available data and no data imputation was used.

Conclusions: In patients with Gaucher disease and baseline osteopenia/osteoporosis who were treated with velaglucerase alfa, BMD improved in

both lumbar spine (Month 24) and femoral neck (Month 36). Since the velaglucerase alfa dose was reduced from 60 to 30 unit/kg/infusion during Year 2, the improvement in bone pathology was not dependent upon continuous high-dose therapy.

5 Achievement of long-term therapeutic goals

Therapeutic goals have been described to monitor achievement, maintenance and continuity of therapeutic response in patients with type 1 Gaucher disease receiving ERT (Pastores G *et al.*, (2004) *Seminars in Hematology*, 41 (suppl 5): 4–14)

10 To benchmark the impact of velaglucerase alfa treatment against therapeutic goals for 5 key clinical parameters of type 1 Gaucher disease (anemia, thrombocytopenia, hepatomegaly, splenomegaly and skeletal pathology), the proportion of patients at goal for anemia, thrombocytopenia, hepatomegaly and splenomegaly at baseline was compared with the proportion achieving each of these goals at 4 years. Complete data for anemia, thrombocytopenia, hepatomegaly, splenomegaly and skeletal pathology at
15 baseline and 4 years are available for 8 patients (3 male, 5 female). The proportion achieving the skeletal pathology goal at 4 years was determined on the basis of Z-score improvement from baseline to 4 years. In addition, the proportion of patients who achieved all 5 goals at 4 years was compared with the proportion at goal for all 5 parameters at baseline.

20 At baseline, no patient was at goal for all clinical parameters (**Table 9**). After 1 year of treatment, all patients maintained goals present at baseline, and all achieved ≥ 2 goals. All 8 patients began step-wise dose reduction to velaglucerase alfa 30 U/kg EOW between 15 and 18 months. By year 4 of treatment, all patients met goal for all 5 clinical parameters; therefore, 100% achievement was seen for each of the 5 long-term,
25 therapeutic goals (**Table 9**).

Table 9

	Baseline	Year 4
Anemia	4/8 (50%)	8/8 (100%)
Thrombocytopenia	0/8 (0%)	8/8 (100%)
Hepatomegaly	4/8 (50%)	8/8 (100%)

Splenomegaly	0/8 (0%)	8/8 (100%)
Skeletal pathology	-----	8/8 (100%)
All 5 goals	0/8 (0%)	8/8 (100%)

Skeletal pathology was measured as improvement in bone mineral density (BMD) at year 4 relative to baseline.

In this velaglucerase alfa Phase I/II and extension study, clinically meaningful achievement of each long-term, therapeutic goal was observed for each patient, despite dose reduction after 1 year. This is the first report of a cohort where all patients receiving ERT for type 1 Gaucher disease achieved all 5 of these long-term, therapeutic goals within 4 years of starting treatment.

Summary of Results from TKT025 and TKT025EXT

The findings reported herein demonstrate that adverse events associated with velaglucerase alfa were generally mild in severity and were mostly unrelated to therapy. Treatment-emergent adverse events were mild to moderate and were mostly not drug-related. No patient enrolled in these studies developed antibodies, no drug related serious adverse events were observed regardless of infusion setting or duration of exposure, and no patient withdrew from the study because of adverse events. Following an initial period of observation at the study site, eligible patients were successfully transitioned to home-based, nurse-administered velaglucerase alfa.

Velaglucerase alfa demonstrated efficacy in the four disease parameters studied with statistically significant and clinically meaningful improvements from baseline observed within the first six months of treatment and throughout the course of the trial and extension study. Within 24 months of initiation of therapy, all patients achieved normalization of hemoglobin level, all but one patient achieved platelet counts of greater than 100,000/mm³, all patients achieved near normalization in liver volumes, and all patients but one exhibited a reduction of more than 50% in spleen volume. Moreover, these improvements were observed throughout the duration of the studies, including the dose-reduction phase. The only patient who was returned to the original dose of 60U/kg/every-other-week did so at 39 months secondary to bone pain following an initial

dose reduction at 15 months. This patient had boney destructive lesions in both her ankles at enrollment (imaging pathology could not rule out AVN) and had a prior history of osteomyelitis. The principle investigator (AZ) attributed the worsening pain to the preexisting destructive lesion and prior pathology, and likely not related to the dose reduction or treatment failure.

The observed safety profile including the transition to home treatment and the significant changes observed in the clinical parameters despite dose reduction, have led to further three subsequent Phase III trials for velaglucerase alfa (which allowed children), as well as a global early access program, and an FDA-accepted treatment protocol.

Example 2: TKT032 Study (12M, 45 or 60 U/kg velaglucerase alfa)

Example 2.1: Summary

This example describes a global, multicenter trial to evaluate the efficacy and safety of velaglucerase alfa in type 1 GD. Twenty-five treatment-naïve, anemic, type 1 GD patients (age 4–62 years) were randomized to intravenous velaglucerase alfa 60 U/kg (n=12) or 45 U/kg (n=13) every other week for 12 months.

Patients were randomized in 1:1 ratio to receive 45 U/kg (N=13) or 60 U/kg (N=12) velaglucerase alfa. Stratification factors included age (2-17; ≥ 18) and gender (male; female). The baseline characteristics of the patients are listed in **Table 10**.

Clinically and statistically significant improvements in hematological parameters and spleen volumes were observed at 12 months (**Table 11**). **FIGS. 7** and **8** show the increase of mean hemoglobin concentration and platelet count, respectively, from baseline in the patients treated with 45 U/kg or 60 U/kg velaglucerase alfa for 12 months. **FIGS. 9** and **10** show the decrease of mean normalized spleen volume and liver volume, respectively, from baseline in patients treated with 45 U/kg or 60 U/kg velaglucerase alfa for 12 months.

Table 10 TKT032 Baseline Characteristics

	Velaglucerase alfa	Velaglucerase alfa	
	45 U/kg	60 U/kg	Total
Baseline Factor	N = 13	N = 12	N = 25

2 to 17 years	n (%)	3 (23.1)	4 (33.3)	7 (28.0)
2 to 4 years	n (%)	0	1 (8.3)	1 (4.0)
5 to 17 years	n (%)	3 (23.1)	3 (25.0)	6 (24.0)
≥ 18 years	n (%)	10 (76.9)	8 (66.7)	18 (72.0)
Male	n (%)	8 (61.5)	7 (58.3)	15 (60.0)
Female	n (%)	5 (38.5)	5 (41.7)	10 (40.0)
Hemoglobin concentration (g/dL)		10.90 [8.45, 12.85]	10.83 [7.05, 12.25]	10.85 [7.05, 12.85]
Median [Min, Max]				
Platelet count (x 10⁹/L)		58.00 [13.0, 264.0]	66.75 [47.0, 438.0]	65.50 [13.0, 438.0]
Median [Min, Max]				

Table 11 TKT032 Clinical Results (N=25)

	n	Baseline Median	Mean Change from Baseline to Month 12	95% CI	P-value
Primary Endpoint					
Hemoglobin 60 U/kg	12	10.83	2.43	[1.72, 3.14]	< 0.0001
Secondary Endpoints					
Hemoglobin 45 U/kg	13	10.90	2.44	[1.49, 3.39]	0.0001
Platelets 60 U/kg	12	66.75	50.88	[23.97, 77.78]	0.0016
Platelets 45 U/kg	13	58.00	40.92	[11.20, 70.64]	0.0111
Spleen 60 U/kg	12	2.80	-1.92	[-3.04, -0.79]	0.0032
Spleen 45 U/kg	13	2.90	-1.87	[-3.17, -0.57]	0.0085
Liver 60 U/kg	12	3.65	-0.84	[-1.58, -0.11]	0.0282a
Liver 45 U/kg	13	3.50	-0.30	[-0.92, 0.32]	0.3149

a - Not statistically significant after adjusting for multiple testing.

Velaglucerase alfa was well tolerated. One patient developed antibodies at the
5 end of the study (12 months). **Table 12** summarizes the results of TKT032 safety test.

Table 12 TKT032 Safety

	<u>Patients n(%)</u>		
DESCRIPTION	velaglucerase alfa 45 U/kg N = 13	velaglucerase alfa 60 U/kg N = 12	Total N = 25
Experienced No Adverse Events	2 (15.4)	0	2 (8.0)
Experienced At Least 1 Adverse Event	11 (84.6)	12 (100.0)	23 (92.0)
Experienced At Least 1 Drug-Related Adverse Event	9 (69.2)	6 (50.0)	15 (60.0)
Experienced At Least 1 Infusion-Related Adverse Event	8 (61.5)	6 (50.0)	14 (56.0)
Experienced At Least 1 Severe Or Life-Threatening Adverse Event	2 (15.4)	0	2 (8.0)
Experienced At Least 1 Serious Adverse Event	0	1 (8.3)	1 (4.0)
Discontinued Due To An Adverse Event	0	0	0
Deaths	0	0	0
Developed anti-velaglucerase alfa antibodies	1 (7.7)	0	1 (4.0)

At 12 months, mean hemoglobin concentration increased (60 U/kg: +23%; +2.4g/dL [95% confidence interval (CI): 1.5, 3.4; P<0.001]; 45 U/kg: +24%; +2.4g/dL [95% CI: 1.5, 3.4; P<0.001]), as did mean platelet count (60 U/kg: +65.9%; +51×10⁹/L [95% CI: 24, 78; P=0.002]; 45 U/kg: +66%; +41×10⁹/L [95% CI: 11, 71; P=0.01]). Mean spleen volume decreased (60 U/kg: -50% [95% CI: -62, -39%] from 14.0 to 5.8 multiples of normal [MN] [P=0.003]; 45 U/kg: -40% [95% CI: -52, -28%], from 14.5 to 9.5 MN [P=0.009]); as did mean liver volume (60 U/kg: -17% [95% CI: -27, -7%], from 1.5 to 1.2 MN [P=0.03]; 45 U/kg: -6% [95% CI: -18, 6%], from 1.4 to 1.2 MN [P=0.32]. No drug-related serious adverse events or withdrawals were observed. One patient developed antibodies.

Study Objectives

The primary objective of this study is to determine the efficacy of every other week dosing of velaglucerase alfa at a dose of 60 U/kg in patients with type 1 Gaucher disease as measured by increases in hemoglobin concentration.

5 The secondary objectives of this study are to evaluate the safety of every other week dosing of velaglucerase alfa at doses of 60 and 45 U/kg; to evaluate the efficacy of every other week dosing of velaglucerase alfa at a dose of 45 U/kg as measured by increases in hemoglobin concentration; to evaluate the efficacy of every other week dosing of velaglucerase alfa at doses of 60 and 45 U/kg by assessing increases in platelet
10 counts, decreases in spleen and liver volumes, and decreases in levels of plasma chitotriosidase and Chemokine (C-C motif) ligand 18 (CCL18); to evaluate the effect of every other week dosing of velaglucerase alfa at doses of 60 and 45 U/kg on overall quality of life (QoL); and to evaluate the single- and repeat-dose pharmacokinetics of every other week dosing of velaglucerase alfa when administered at doses of 60 and 45
15 U/kg.

 The tertiary objectives of this study are to determine the time from Baseline to achieve a hemoglobin response, defined as an increase in hemoglobin concentration of $\geq 1\text{g/dL}$, after every other week dosing with velaglucerase alfa at doses of 60 or 45 U/kg; to evaluate the effect of every other week dosing of velaglucerase alfa at doses of 60 and 45
20 U/kg on pulmonary function tests (PFTs) in patients ≥ 18 years-old; to evaluate growth velocity and Tanner staging in patients between 2 and 17 years-old; to evaluate changes in skeletal age in patients between 2 and 17 years-old by radiography of the left hand and wrist; to establish a Baseline from which to evaluate bone disease in patients between 2 and 17 years-old by magnetic resonance imaging (MRI) of the lumbar spine and femoral
25 neck; and to establish a Baseline from which to evaluate the long-term effect of velaglucerase alfa therapy on Gaucher-related local and systemic bone disease in patients ≥ 18 years-old by: DXA of the lumbar spine and femoral neck (including coronal imaging); and serum alkaline phosphatase, N-Telopeptide cross-links (NTx), and C-Telopeptide cross-links (CTx).

30

Overall Study Design

This is a multicenter, Phase III randomized, double-blind, parallel group, 2-dose study designed to evaluate the efficacy and safety of velaglucerase alfa therapy for patients with type I Gaucher disease.

5 This study was comprised of 5 phases as follows: (1) Screening: Day –21 through Day –4; Baseline: Day –3 through Day 0 (prior to first dose); Treatment Phase: Week 1 (Day 1; first dose) through Week 51 (a total of 26 infusions were administered per patient); End of Study Visit: Week 53; Follow-up: 30 days after the final infusion (for
10 complete this study but do not elect to enroll in the subsequent long-term clinical study).

 At Screening, patients who provided written informed consent to participate in this study were reviewed against the study entrance criteria to determine study eligibility and underwent Screening evaluations. In particular, patients provided a blood sample to measure hemoglobin concentration. Only those patients who had a hemoglobin
15 concentration that was at least 1 g/dL below the lower limit of normal for age and gender were eligible to continue into the baseline phase. For statistical analysis purposes, an additional blood sample was collected at screening for evaluation of hemoglobin concentration.

 Patients who were eligible for study participation after completing the Screening
20 evaluations underwent Baseline procedures and evaluations (i.e., Days –3 to 0) prior to the first dose. To confirm that their hemoglobin concentration was at least 1 g/dL below the lower limit of normal for age and gender, patients provided a blood sample at Baseline. Hemoglobin concentration were analyzed and reported. Only those patients
25 who had a hemoglobin concentration that was at least 1 g/dL below the lower limit of normal for age and gender at both Screening and Baseline were eligible for randomization. For statistical analysis purposes, an additional blood sample was collected at Baseline for evaluation of hemoglobin concentrations. Additional Baseline procedures and evaluations were conducted prior to administration of the first dose.

 Following completion of Baseline evaluations and confirmation of eligibility,
30 patients were randomized in a 1:1 ratio to receive either velaglucerase alfa 60 U/kg or velaglucerase alfa 45 U/kg via a computer generated randomization schedule.

Patients received a total of 26 IV infusions of double-blind study medications at the clinical site once every other week for a total of 51 weeks. Safety and efficacy assessments were made at regular intervals during the treatment period. The final assessments of safety and efficacy were made at the Week 51 and Week 53 visits.

5 Safety was assessed throughout the study by assessments of adverse events (including infusion-related adverse events), concomitant medications, and vital signs. Additional safety assessments, including, 12-lead electrocardiograms, physical examinations, clinical laboratory tests (hematology, serum chemistry, and urinalysis), were made at Weeks 13, 25, 37, and 53. Determination of the presence of anti-velaglucerase alfa
10 antibodies and enzyme neutralizing antibodies were conducted approximately every 6 weeks until Week 53.

Efficacy was assessed via hemoglobin concentration and platelet count, liver and spleen volume, and plasma chitotriosidase and CCL18 level. Additional efficacy assessments included growth velocity and Tanner staging, QoL indicators, skeletal
15 growth and pulmonary function testing.

Single-dose and repeat-dose velaglucerase alfa pharmacokinetic profiles were also evaluated during the study. Blood samples were collected at Week 1 (Day 1) and Week 37, respectively, for these analyses.

Patients who completed this study were provided the opportunity to enroll in a
20 subsequent long-term clinical study. For patients who elected to enroll in this long-term study, certain assessments from the Week 51 and the Week 53 visits in TKT032 were used as the Baseline assessments; patients received their first velaglucerase alfa infusion for the long-term clinical study at the Week 53 visit, after the Week 53 procedures scheduled for TKT032 were completed. Therefore, it was intended that patients would
25 receive continuous velaglucerase alfa treatment across the 2 studies. Patients who completed this study and did not elect to enroll in the subsequent long-term clinical study would have a safety evaluation by site visit or telephone 30 days after their last infusion of velaglucerase alfa.

Selection of Study Population

Of 39 patients assessed, 14 were not eligible for randomization (12 did not meet inclusion criteria; 2 did not meet exclusion criteria). Twenty five participants were randomized to velaglucerase alfa at a dose of 45 U/kg (n=13) or 60 U/kg (n=12). All randomized patients who received at least 1 infusion (or partial infusion) were included in the intent-to-treat (ITT) patient population.

Eligible participants were males or females age ≥ 2 years with diagnosed type 1 Gaucher disease (deficient glucocerebrosidase activity in leukocytes, or by genotype analysis), and disease-related anemia (hemoglobin levels ≥ 1 g/dL below the local laboratory's lower limit of normal for age and gender). Participants also had 1 or more of the following: at least moderate splenomegaly (2 to 3 cm below the left costal margin) by palpation; disease-related thrombocytopenia (platelet count $< 90 \times 10^3$ platelets/mm³); or readily palpable enlarged liver. Participants could not have received treatment for Gaucher disease within 30 months prior to study entry.

Participants were excluded if they had a splenectomy; had (or were suspected of having) type 2 or 3 Gaucher disease; were antibody-positive or had experienced an anaphylactic shock to imiglucerase. Other exclusion criteria included treatment with any non-Gaucher disease-related investigational drug or device within 30 days prior to study entry; positive test for HIV, or hepatitis B or C; exacerbated anemia (vitamin B12, folic acid, or iron deficiency-related), or any significant co-morbidity that could affect study data. Pregnant or lactating women were excluded and women of child-bearing potential were required to use a medically acceptable method of contraception at all times.

Study Treatments

Treatment Assignment: Patients were randomized in a 1:1 ratio to receive: velaglucerase alfa 60 U/kg every other week for 51 weeks (12 patients, 26 infusions); or velaglucerase alfa 45 U/kg every other week for 51 weeks (12 patients, 26 infusions).

Randomization: Following the completion of Baseline evaluations and confirmation of eligibility, patients were randomized in a ratio of 1:1 via a computer

generated randomization schedule to receive either velaglucerase alfa 45 U/kg or 60 U/kg every other week infusions for 51 weeks.

The pursuit of balance on prognostic factors was important in small trials on the grounds of statistical efficiency for the primary analyses. To achieve this balance across a number of prognostic factors (e.g., age and gender) dynamic allocation techniques were used. For the dynamic randomization procedure, the allocation of patients was influenced by the current balance on the stratifying factors for the patient in question. This technique used the approach as suggested by Pocock and Simon and was very consistent with the guidance provided in ICH-9 (Pocock *et al. Biometrics*. 1975; 31:105-115).

Treatment Schedule: Patients received their first infusion at Week 1. All patients were treated every other week for 12 months (51 weeks); therefore, a total of 26 infusions of velaglucerase alfa were administered.

Dose Calculation: The actual dose of study drug was calculated based on the patient's weight at Baseline. A change in weight of $\geq 5\%$ noted at Weeks 13, 25, or 37 from the prior assessment would have required recalculation of the dose of study medication.

Velaglucerase alfa Administration: Velaglucerase alfa was administered as a continuous IV infusion at both the 60 and 45 U/kg dose levels. All infusions were administered over a 1-hour duration. All infusions were reconstituted in 4.3 mL of preservative-free, Sterile Water for Injection, and then diluted in normal saline (0.9% sodium chloride) to yield a 100 mL total volume. Study drug infusions occurred on approximately the same day of the week but might occur every 14 days (± 3 days) of the scheduled day in order to facilitate patient scheduling.

Study Procedures and Data Collection Methods

Genotyping: All patients provided a blood sample at Screening for Gaucher disease genotyping and plasma chitotriosidase genotyping.

Medical History: At Screening, the patient's complete medical history was recorded. This included a review of body systems, documentation of current and prior medical procedures, and documentation of current and prior concomitant medication usage, and documentation that the patient had not been treated for Gaucher disease within the 30 months prior to study entry.

Vital Signs: Vital signs parameters that were recorded included pulse, blood pressure, respiration rate, and temperature. The following schedule was followed for recording vital signs at infusion visits: start of infusion (within 10 minutes prior to starting the infusion), during infusion (30 minutes (± 5 minutes)), after infusion (within 5 minutes, 30 minutes (± 5 minutes), and 60 minutes (± 5 minutes) after completing the infusion). At Screening, Baseline, and Week 53, vital signs were collected at one time point only.

Physical Examinations: Physical examinations were performed at Screening, Baseline and at Study Weeks 13, 25, 37, and 53. Physical examinations included the following: general appearance, endocrine, head and neck, cardiovascular, eyes, abdomen, ears, genitourinary, nose, skin, throat, musculoskeletal, chest and lungs, and neurological. In addition, liver and spleen palpations were performed during Screening to confirm that the patient had moderate splenomegaly (2 to 3 cm below the left costal margin) and a Gaucher disease-related enlarged liver.

Height and Weight: Height and weight were recorded at Baseline and at Study Weeks 13, 25, 37, and 53.

12-Lead Electrocardiograms: A 12-Lead ECG was performed at Baseline and Study Weeks 13, 25, 37, and 53. Each 12-lead ECG included assessment of PR, QRS, QT, and QTc intervals, and heart rate.

Clinical Laboratory Testing: Blood and urine samples were collected as described below for clinical laboratory testing. All blood samples were collected via venipuncture.

5 **Hematology:** Blood samples were collected at Screening, Baseline, and Weeks 13, 25, 37, and 53 for complete hematology testing. The following hematology parameters were evaluated: complete blood count (CBC) with differential, activated partial thromboplastin time (aPPT), reticulocyte count (analyzed and reported by the clinical site's local laboratory), platelet count, and prothrombin time (PT). At Screening,
10 Baseline, and at every study visit (except at the Week 1 visit), blood samples were collected to measure hemoglobin concentration and platelet count.

15 **Serum Chemistry:** Blood samples were collected for serum chemistry testing at Screening, Baseline, and at Study Weeks 13, 25, 37, and 53. The following serum chemistry parameters were evaluated: sodium, alanine aminotransferase, potassium, aspartate aminotransferase, glucose, lactate dehydrogenase, total calcium, gammaglutamyltransferase, total protein, creatinine phosphokinase, albumin, NTx*, creatinine, CTx*, urea nitrogen, folic acid (screening only), total bilirubin, vitamin B₁₂ (screening only), alkaline phosphatase* (* results were used for assessments of bone
20 biomarkers).

25 **Urinalysis:** Urine samples were collected for urinalysis at Screening, Baseline, and at Study Weeks 13, 25, 37, and 53. The following urinalysis parameters were evaluated: pH, macroscopic evaluation, microscopic evaluation.

30 **Serum Anti-imiglucerase Antibody Determination:** All patients had a blood sample collected during Screening only for determination of serum anti-imiglucerase antibodies. Patients with a positive result were excluded from the study.

30 **Serum Anti-velaglucerase alfa Antibody Determination:** Patients provided blood samples to measure anti-velaglucerase alfa antibodies in serum at Baseline and

approximately every 6 weeks during the treatment phase (Weeks 7, 13, 19, 25, 31, 37, 43, and 49), and at Week 53. During the treatment phase, these blood samples were collected prior to the infusion.

5 ***Adverse Events:*** Adverse events were monitored throughout the study from informed consent/assent through 30 days after the last infusion for patients who completed the study and did not elect to enroll in the long-term clinical study from the study prior to the Week 53 visit. For patients who completed this study and elected to enroll in the long-term clinical study, adverse events were monitored from informed
10 consent/assent through the Week 53 visit.

Prior and Concomitant Illnesses: Additional illnesses present at Baseline were regarded as concomitant illnesses and were documented on the appropriate pages of the medical history CRF. Illnesses first occurring or detected during the study, or worsening
15 of a concomitant illness during the study, were regarded as AEs and documented as such in the CRF.

Liver and Spleen MRI: Patients had MRI of the liver and spleen at Baseline, Week 25 and Week 51. Liver and spleen size were measured using quantitative
20 abdominal MRI.

Plasma Chitotriosidase Levels: Blood samples (approximately 2.5 mL) were collected for the evaluation of plasma chitotriosidase levels at Baseline, Weeks 13, 25, 37, and at Week 53.
25

Plasma CCL18 Levels: Blood samples (approximately 2.5 mL) were collected for the evaluation of plasma CCL18 levels at Baseline, Week 13, 25, 37 and at Week 53.

Quality of Life Testing: At Baseline and at Week 53, patients' quality of life was
30 evaluated using validated questionnaires, including the Short Form 36 (SF-36), version 2

(for patients ≥ 18 years-old) and the Childhood Health Questionnaire (CHQ), PF50 (for patients 5 to 17 years-old).

Growth Velocity and Tanner Staging: For patients 2 to 17 years-old, growth was assessed at Baseline and Weeks 13, 25, 37, and 53. Growth velocity was calculated using height and weight measurements, recorded at regular time points during this study. Growth rates of patients in this study were measured against growth rates for normal individuals of comparable ages obtained from the Centers for Disease Control and Prevention height and weight data. Tanner stage was recorded at Baseline and Weeks 13, 25, 37, and 53.

Skeletal Growth: Patients between 2 and 17 years-old underwent radiography of the left hand and wrist at Baseline and Week 51 for evaluation of skeletal age.

Pulmonary Function Testing: At Baseline and Week 53, patients ≥ 18 years-old who were enrolled at study sites with the capability to perform spirometry had PFTs.

Spirometry was performed according to the guidelines published by the American Thoracic Society or European Respiratory Society for standardization of spirometry (American Thoracic Society. Standardization of Spirometry. *Am J Respir Crit Care Med.* 1995; 152:1107-1136; Quanjer *et al.* *Eur Respir J.* 1993; 16(Suppl):5-40) Both Forced Vital Capacity (FVC) and Forced Expiratory Volume/second (FEV₁) were expressed as absolute values and % predicted of normal, which were calculated based on published reference values for adults (Hankinson *et al.* *Am J Respir. Crit Care Med.* 1999; 159:179-187). Current standing height was used for the calculations.

Lung volume and Diffusion Capacity (DL_{CO}) determinations were performed at the same time as spirometric testing. Lung volume measurements were include Total Lung Capacity (TLC), and Residual Volume (RV), which were recorded as absolute values and % predicted of normal based on published reference values. DL_{CO} was also expressed as absolute values and % predicted of normal based on published reference values.

Pharmacokinetic Evaluations: For patients ≥ 18 years-old, blood samples were collected at the following times at Week 1 (Day 1) and Week 37: immediately before first dose, during infusion (sample collected at 5, 10, 15, 20, 40 and 60 (end of infusion) minutes), and after infusion (sample collected at 65, 70, 80, 90, 105 and 120 minutes).

5 For patients 2 to 17 years-old, blood samples were collected at the following times at Week 1 (Day 1) and Week 37: immediately before first dose, during infusion (sample collected at 10, 20, 40 and 60 (end of infusion) minutes), and after infusion (sample collected at 70, 80 and 90 minutes).

Serum samples were evaluated for the presence of administered velaglucerase alfa using a glucocerebrosidase antigen immunoassay. The following PK parameters were evaluated: AUC (Area under the curve), $C_{\max}$ (Maximum serum concentration), $T_{\max}$ (Time to maximum serum concentration), CL (mL/min/kg) (Serum clearance, normalized for body weight), V_{ss} (mL) (Apparent volume of distribution at steady-state), V_{ss} (%BW) (V_{ss} normalized for body weight), MRT (Mean residence time), and $T_{1/2}$ (Elimination half-life (analyzed with appropriate PK models)

10
15

Bone Biomarkers: At Baseline only, patients ≥ 18 years-old underwent DXA of the lumbar spine and femoral neck, including coronal imaging, to determine Gaucher-related local and systemic bone disease. Bone loss and demineralization were also evaluated by measuring serum alkaline phosphatase, NTx, and CTx. Results for these parameters were obtained from blood samples collected for clinical laboratory testing at Baseline only.

20

For patients 2 to 17 years-old, MRI of the femoral neck and lumbar spine was obtained at Baseline (i.e., at the same time patients underwent MRI of the liver and spleen). It was not expected that any treatment effect would be apparent for these parameters during this study; therefore, measurements were collected at Baseline only to establish a reference point from which to monitor these biomarkers during the long-term clinical study.

25

Adverse Events

Adverse Event Definition: An adverse event (AE) is any noxious, pathologic, or unintended change in anatomical, physiologic, or metabolic function as indicated by physical signs, symptoms, and/or laboratory changes occurring in any phase of a clinical trial, and whether or not considered study drug-related. This includes an exacerbation of a pre-existing condition. Adverse events were collected from informed consent/assent until 30 days after the last dose of study medication and/or until the event had been resolved/stabilized or an outcome was reached, whichever came first. For patients who completed this study and elected to enroll in the subsequent long-term clinical study, adverse events were monitored from the time the patient provided informed consent through the Week 53 visit of TKT032.

AEs include: worsening (change in nature, severity, or frequency) of conditions present at the onset of the study; intercurrent illnesses; drug interactions; events related to or possibly related to concomitant medications; abnormal laboratory values (this includes significant shifts from Baseline within the range of normal that the Investigator considers to be clinically important); clinically significant abnormalities in physical examination, vital signs, weight, and ECG.

In addition, AEs might also include unexpected laboratory values that became significantly out of range and determined to be clinically significant by the Investigator.

Infusion-Related Adverse Event Definition: An infusion-related adverse event is defined as an adverse event that 1) begins either during or within 12 hours after the start of the infusion, and 2) is judged as possibly or probably related to study medication. Other AEs which occurred prior to the infusion, along with AEs associated with protocol-defined testing and assessments (e.g., laboratory testing, ECGs, and physical examinations) which were performed prior to the infusion, are not defined as infusion-related adverse events.

Serious Adverse Event Definition: A serious AE (SAE) is any AE occurring at any dose that results in any of the following outcomes: death, is life-threatening, requires

inpatient hospitalization, requires prolongation of existing hospitalization, a persistent or significant disability/incapacity, and a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

A life-threatening AE is defined as an AE that placed the patient, in the view of the initial reporter, at immediate risk of death from the AE as it occurred (i.e., it does not include an AE that, had it occurred in a more severe form, might have caused death).

Classification of Adverse Events and Serious Adverse Events: The National Cancer Institute Common Toxicity Criteria (NCI CTC) Version 3.0 grading scale was referenced when assessing the severity of an AE. If an AE was not described in the NCI CTC, the severity was recorded based on the scale below. The severity of all AEs/SAEs were recorded on the appropriate CRF page as Grade 1, 2, 3 or 4 corresponding, respectively, to a severity of mild, moderate, severe, or life-threatening. Grade 1 (mild) is defined as no limitation of usual activities. Grade 2 (moderate) is defined as some limitation of usual activities; Grade 3 (severe) is defined as inability to carry out usual activities; and Grade 4 (life-threatening) is defined as immediate risk of death.

Relationship of an adverse event or serious adverse event to blinded study medication was determined by the Investigator based on the following definitions. “Not related” is defined as unrelated to study drug. “Possibly related” is defined as a clinical event/laboratory abnormality with a reasonable time sequence to administration of study drug, but which could also be explained by concurrent disease or other drugs/chemicals. “Probably related” is defined as a clinical event/laboratory abnormality with a reasonable time sequence to administration of study drug, unlikely to be attributable to concurrent disease or other drugs and chemicals and which follows a clinically reasonable response on dechallenge. The association of the clinical event/laboratory abnormality must also have some biologic plausibility, at least on theoretical grounds.

Clarification between Serious and Severe: The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious,” which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) and causality serve as a guide for defining regulatory reporting obligations.

Adverse Event Monitoring and Period of Observation: For the purposes of this study, the period of observation extended from informed consent/assent until the patient’s final evaluation of the study. For safety purposes, the final evaluation was defined as the post-study safety evaluation performed approximately 30 days after the last infusion for patients who completed the study and did not elect to enroll in the long-term study. For patients who completed this study and elected to enroll in the long-term clinical study, adverse events were monitored from the time the patient provides informed consent through the Week 53 visit of TKT032.

Statistical Methods

General Statistical Methodology: Statistical analyses were based on the ITT principle for all efficacy variables. The ITT analysis was based on all randomized patients who received at least one infusion (full or partial infusion). Summary statistics were provided for the changes and percent changes from Baseline for each parameter by treatment group. Two-sided 95% confidence intervals in the mean changes and mean percent changes from Baseline were presented by treatment group for each endpoint.

Continuous data collected at Baseline and subsequent study visits were summarized, and the mean, standard deviation, minimum, maximum, and median values for each variable were tabulated to facilitate the search for trends over time which might be attributable to study drug. Categorical variables were presented in terms of frequencies and percent. Within group changes were examined using paired t-tests. Statistical significance was defined at the 0.05 level.

Demographic and Baseline characteristics were summarized as frequencies and percentages, and data were presented using descriptive statistics. Additional analyses were conducted specifically for patients between 2 and 17 years old.

In general, descriptive statistics and graphs were used for presentation of study results, including, if relevant, graphs showing the development over time for patients individually and for each treatment group.

Safety was evaluated on the basis of AEs reported, clinical laboratory data, ECG recordings, medical histories, vital signs, and physical examinations. In addition, blood samples were analyzed for determination of the presence of anti-velaglucerase alfa antibodies.

Hypothesis Testing: All hypothesis testing was 2-sided and was performed at the 0.05 level of significance. Each variable was quantified as a mean change from Baseline or mean percent change from Baseline. The null hypothesis for each variable being tested is that, at Week 51 or Week 53, there is no change from Baseline. The alternative hypothesis is that there is a change in either direction from Baseline.

Screen Failures: The disposition of all patients screened for entry into the study was tabulated along with reasons for screen failure. The disposition of all randomized patients was tabulated by treatment and visit, and reasons for discontinuation were tabulated by treatment.

Sample Size Justification: The sample size for this study was chosen to have a high power to detect a clinically significant difference in mean hemoglobin concentrations from Baseline to 12 months. A total of 12 patients per treatment arm were required for the primary analysis. This number was based on results from the Phase I/II Study TKT025, examining the within patient change from Baseline results. It was observed that at Week 25, the average hemoglobin increase from Baseline was 1.92 g/dL with a standard deviation of 0.824. The assumption was that the standard deviation of the mean change was approximately the same. Using a two-sided alpha level of 0.05 and assuming a 1-unit change in hemoglobin is considered clinically significant, the standard

deviation of the change from Baseline is 0.824, then 10 patients would be needed for the trial to have a power of 90%. Assuming a 20% drop out rate, then 12 patients per treatment group would be needed. To gather additional safety data and to protect against possible patient dropout(s), and to achieve the target patient population, up to 30 patients could be enrolled in this study

Analysis Populations: The primary population for analyses of efficacy data was the ITT patient population, defined as all enrolled and treated patients who receive at least one velaglucerase alfa infusion (or partial infusion). It was anticipated that attrition from the original random sample due to lack of post-Baseline data would be sufficiently small (5% or less) so as to minimize concerns regarding bias due to the exclusion of such patients.

The safety population consisted of all randomized patients who received at least one study infusion (or partial infusion). Any patient in the safety population who did not receive the study infusion to which he or she was randomized was analyzed according to the infusion they predominantly received rather than the randomized treatment. Such patients were excluded from the Per-Protocol (PP) patient population. The PP patient population is defined as all randomized patients who have received $\geq 80\%$ of the scheduled infusions, and who have valid Baseline and Week 51 and/or Week 53 evaluations.

Efficacy Analyses: The disposition of all patients Screened for entry into the study was tabulated, along with reason(s) for Screening failure. The disposition of all randomized patients was tabulated by treatment group and visit, and reason(s) for discontinuation(s) was tabulated.

The primary clinical activity variable is hemoglobin concentrations in the patients randomized to 60 U/kg of velaglucerase alfa. The primary objective is to demonstrate efficacy by showing a mean change in hemoglobin from Baseline to 12 months in patients randomized to 60 U/kg of velaglucerase alfa. For analysis purposes, hemoglobin values collected at Screening and Baseline were averaged to establish the Baseline used to calculate change. The null hypothesis is that there will be no change from Baseline in

hemoglobin concentrations to 12 months. The mean difference from Baseline to 12 months was tested using a paired t-test or Wilcoxon signed rank test. A 95% confidence interval for the mean difference was also presented.

Secondary and tertiary clinical activity variables are: hemoglobin concentrations (change from Baseline to 12 months (Week 53) was assessed for the 45 U/kg group); platelet counts (change from Baseline to 12 months (Week 53) was assessed for both treatment groups); spleen volume (percent change from Baseline to 12 months (Week 51) was assessed for both treatment groups; in addition to observed values, spleen volumes were normalized by body weight and also presented by multiples of normal); liver volume (percent change from Baseline to 12 months (Week 51) was assessed for both treatment groups; in addition to observed values, liver volumes were normalized by body weight and also presented by multiples of normal); plasma chitotriosidase (change from Baseline to 12 months (Week 53) was assessed for both treatment groups; a statistically significant decrease expected after 12 months of treatment); plasma CCL18 (change from Baseline to 12 months (Week 53) was assessed for both treatment groups; a statistically significant decrease expected after 12 months of treatment); quality of life (SF-36 and CHQ) (change from Baseline to 12 months (Week 53) was assessed for both treatment groups); hemoglobin response (time to achieve a hemoglobin response, defined as an increase in hemoglobin concentration of ≥ 1 g/dL was assessed for both treatment groups); growth velocity and Tanner staging (change from Baseline to 12 months (Week 53) was evaluated for both treatment groups in patients between 2 and 17 years-old); skeletal age (change from Baseline to 12 months (Week 51) was evaluated for both treatment groups, as measured by radiography of the left hand and wrist, in patients 2 to 17 years-old); and PFTs (change from Baseline to 12 months (Week 53) was assessed for both treatment groups in patients ≥ 18 years-old).

For the secondary objective used to demonstrate a mean change in hemoglobin from Baseline to 12 months (Week 53) in patients randomized to 45 U/kg of velaglucerase alfa, the null hypothesis is that there will be no change from Baseline to 12 months (Week 51 or Week 53). For analysis purposes, hemoglobin values collected at Screening and Baseline were averaged to establish the Baseline used to calculate change. The mean difference from Baseline to 12 months (Week 51 or Week 53) was tested using

a paired t-test or Wilcoxon signed rank test. A 95% confidence interval for the mean difference was also presented.

For the remaining secondary parameters, the null hypothesis is that there will be no change from Baseline to 12 months (Week 51 or Week 53) for each treatment group. For analysis purposes, platelet values collected at Screening and Baseline were averaged to establish the Baseline used to calculate change for both the treatment groups. The mean difference from Baseline to 12 months (Week 51 or Week 53) was tested using a paired t-test or Wilcoxon signed rank test. A 95% confidence interval for the mean difference was also presented.

For time to hemoglobin response, Kaplan-Meier (product limit) survival curves were presented for each treatment group. The median time and 95% confidence interval were obtained. Patients who did not experience the event at the end of the study (i.e., at Week 53) were censored at Week 53. In addition, the proportion of patients who achieved a hemoglobin level within the normal range during this study was presented.

For the remaining tertiary parameters that examine change from Baseline, the null hypothesis is that there will be no change from Baseline to 12 months (Week 51 or Week 53) for each treatment group. The mean difference between Baseline to 12 months (Week 51 or Week 53) was tested using a paired t-test or Wilcoxon signed rank test. A 95% confidence interval for the mean difference was also presented.

Safety Analyses: All patients who received at least one dose of study drug (or partial dose) were assessed for clinical safety and tolerability. No formal statistical tests were performed on the safety parameters. Vital signs, 12-lead ECG, clinical chemistry, hematology, and urinalysis safety monitoring were listed for each patient and abnormal values were flagged. For categorical variables, such as AEs, the number and percentage of patients experiencing each AE were tabulated. AEs were summarized by severity of event. The number and percentage of patients experiencing drug related AEs as well as AEs that were not considered related to study drug were also displayed.

Clinical laboratory evaluations (hematology, serum chemistry, urinalysis, and determination of anti-velaglycerase alfa antibodies) were used to assess the safety of velaglycerase alfa.

Analysis of Subgroups: Additional analyses were conducted specifically for patients between 2 to 17 years old. Also, consideration was given in the analysis to disease severity with regard to hemoglobin Baseline values.

Pharmacokinetic Analyses: The single- and repeat-dose pharmacokinetic profiles for velaglucerase alfa were established by analyzing standard PK parameters at Week 1 and Week 37, respectively.

Results

At 12 months, mean hemoglobin concentration increased in both groups (60 U/kg: 23.3% increase, $+2.4 \pm 0.3$ g/dL, $P=0.0001$; 45 U/kg: 23.8% increase, $+2.4 \pm 0.5$ g/dL, $P=0.0001$), as did mean platelet count (60 U/kg: 66% increase, $+51 \pm 12 \times 10^9$ /L, $P=0.0016$; 45 U/kg: 66% increase, $+41 \pm 12 \times 10^9$ /L; $P=0.0111$). Mean spleen volume decreased in both groups (60 U/kg: 50% decrease, $-1.9 \pm 0.5\%$ body weight, $P=0.0032$, from 14.0 multiples of normal [MN] at baseline to 5.6 MN; 45 U/kg: 40% decrease, $-1.9 \pm 0.6\%$ body weight, $P=0.0085$; from 14.5 to 9.5 MN) as did liver volume (60 U/kg: 17% decrease, $0.8 \pm 3.0\%$ body weight, $P=0.0282$, from 1.5 to 1.2 MN; 45 U/kg: 6% decrease, $-0.3 \pm 0.3\%$ body weight, $P=0.3149$, from 1.4 to 1.2 MN).

In both groups, three-quarters of patients achieved ≥ 1 g/dL increase in hemoglobin concentration by Week 15; in the 60U/kg group, all patients achieved ≥ 1 g/dL increase by Week 27 vs Week 37 for the 45U/kg group.

Patients were excluded from the analysis of chitotriosidase if they had 2 copies of the chitotriosidase mutation (patient 6, 45 U/kg) or if they had baseline chitotriosidase activity less than 5700 (patient 4, 60 U/kg; patient 15, 45 U/kg). Following 12 months of treatment, mean plasma chitotriosidase activity decreased from baseline for both treatment groups: by 83% (95% CI: -91.15, -74.08%; $N=11$; $p<0.001$) in the 60 U/kg group and by 60% (95% CI: -73.26, -46.63%; $N=11$; $p<0.001$) in the 45 U/kg group. Mean CCL18 levels also decreased over 1 year of treatment with velaglucerase alfa 60 U/kg and 45 U/kg by 66% (95% CI: -77.81, -54.22; $p<0.001$) and 47% (95% CI: -63.37, -30.15%; $p<0.001$), respectively.

Velaglucerase alfa was generally well tolerated with no drug-related serious AEs, and no patient withdrew due to an AE. The most common AEs were headache, nasopharyngitis, injury, arthralgia, cough, and pyrexia. A single patient developed antibodies.

5 In conclusion, in this global, multicenter study, velaglucerase alfa 60 U/kg and 45 U/kg was generally well tolerated and effective as a first-line treatment for adults and children with type 1 Gaucher disease. Both doses were associated with rapid improvement in hemoglobin values, with the majority of patients responding as early as 15 weeks. All clinical parameters measured demonstrated clinically meaningful
10 improvements after 12 months, with a greater response seen with velaglucerase alfa 60 U/kg.

Example 3: HGT-GCB-039 (9M, 60 U/kg velaglucerase alfa or imiglucerase)

Summary

15 This example describes a multicenter, Phase III, randomized, double-blind, parallel-group study designed to compare the safety and efficacy of the enzyme replacement therapy velaglucerase alfa with imiglucerase in the treatment of patients with type 1 Gaucher disease.

The primary objective of this example is to demonstrate that velaglucerase alfa is
20 not inferior to imiglucerase as measured by a change from baseline in hemoglobin concentration to Week 41 (9M). The key secondary objective is to demonstrate that there are no differences in increases in platelet counts or reductions in liver/spleen volumes to Week 41 between the two groups.

Patients were randomized in 1:1 ratio to receive 60 U/kg of velaglucerase alfa
25 (N=17) or imiglucerase (N=17). The baseline characteristics of the patients are listed in **Tables 13** and **14**. Stratification factors included age (2-17; ≥ 18) and splenectomy status (Y; N). Both primary and secondary objectives were met. The results for the primary and secondary efficiency assessments are shown in **Tables 15** and **16**, respectively.

FIGS. 11 and **12** show the comparable increase of mean hemoglobin concentration and
30 platelet count, respectively, from baseline in the patients treated with 60 U/kg velaglucerase alfa or imiglucerase for 41 weeks. **FIG. 13** shows the comparable increase

of mean platelet count from baseline in the patients without spleen treated with 60 U/kg velaglucerase alfa or imiglucerase for 41 weeks. **FIG. 14** shows the comparable decrease of mean normalized liver volume from baseline in the patients treated with 60 U/kg velaglucerase alfa or imiglucerase for 41 weeks. There were no significant differences in safety between velaglucerase alfa and imiglucerase. Treatment emergent adverse events are summarized in **Table 17**. No patient receiving velaglucerase alfa developed antibodies (**Table 18**). Four patients receiving imiglucerase developed anti-imiglucerase antibodies (**Table 18**).

Table 13 HGT-GCB-039 Baseline Characteristics of ITT

Baseline Factor	velaglucerase alfa 60 U/kg N = 17	imiglucerase 60 U/kg N = 17
2 to 17 years n (%)	4 (23.5)	5 (29.4)
2 to 4 years n (%)	0	4 (23.5)
5 to 17 years n (%)	4 (23.5)	1 (5.9)
≥ 18 years n (%)	13 (76.5)	12 (70.6)
Male n (%)	8 (47.1)	8 (47.1)
Female n (%)	9 (52.9)	9 (52.9)
Splenectomized	10 (58.8)	10 (58.8)
With intact spleen	7 (41.2)	7 (41.2)
BL Hemoglobin concentration (g/dL) Median [Min, Max]	11.40 [9.65, 14.35]	10.60 [8.10, 13.05]
BL Platelet count (x 10 ⁹ /L) Median [Min, Max]	172.00 [44.0, 310.5]	188.00 [63.0, 430.5]

Table 14 HGT-GCB-039 Baseline Characteristics by Age Group

Baseline Factor	2-4 Years Old N = 4	>5 Years Old N = 30
Male n (%)	4 (100.0)	12 (40.0)
Female n (%)	0	18 (60.0)
Splenectomized	1 (25.0)	19 (63.3)

With intact spleen	3 (75.0)	11 (36.7)
BL Hemoglobin concentration (g/dL) Median [Min, Max]	9.275 [8.10, 9.70]	11.300 [8.95, 14.35]
BL Platelet count ($\times 10^9/L$) Median [Min, Max]	70.75 [63.0, 188.0]	176.25 [44.0, 430.5]
BL Spleen Volume (% body Weight) Median [Min, Max]	8.90 [7.3, 8.9]	1.70 [0.6, 6.3]
BL Liver Volume (% body Weight) Median [Min, Max]	5.8 [5.4, 7.0]	3.85 [1.7, 12.2]

Table 15 HGT-GCB-039 Primary Efficacy Assessments – Mean Change at Week 41 from Baseline in Hgb One-sided CI for Non-inferiority (velaglucerase alfa - imiglucerase)

Treatment Difference in the Change from Baseline to Week 41						
ITT Population				Per Protocol Population		
Parameter/ Endpoint	n	Mean Treatment Difference	Lower Bound of a 97.5% One-sided CI	n	Mean Treatment Difference	Lower Bound of a 97.5% One-sided CI
Hemoglobin Concentration (g/dL)						
	34	0.135	-0.596	30	0.157	-0.599

5

Table 16 HGT-GCB-039 Secondary Efficacy Assessments – Difference in Mean Change at Week 41 from Baseline (velaglucerase alfa - imiglucerase)

Change from Baseline to Week 41			
Parameter	n	Mean Treatment Difference	95% CI
Platelets^a ($\times 10^9/L$)	34	-38.71	(-88.42, 10.99)

Normalized Liver Volume ^a (% of Body Weight)	34	-0.07	(-0.43, 0.29)
Normalized Spleen ^b Volume ^c (% of Body Weight)	14	0.08	(-0.52, 0.68)
Chitotriosidase ^d (nmol/mL/h)	21	-703.6	(-11762.3, 10355.1)
Chemokine (C-C motif) Ligand 18 (ng/mL)	34	145.7	(-188.6, 480.0)

a - Based on a mixed model adjusting for age at informed consent, splenectomy status and baseline values.

b - There are 20 splenectomized patient(s) excluded (10 velaglucerase alfa 60 U/kg; 10 imiglucerase 60 U/kg).

5 c - Based on a mixed model adjusting for age at informed consent and baseline values.

d - There are 13 patient(s) deficient in chitotriosidase activity excluded (7 velaglucerase alfa 60 U/kg; 6 imiglucerase 60 U/kg).

10 **Table 17** HGT-GCB-039 Overall Summary of Treatment Emergent Adverse Events

DESCRIPTION	<u>Patients n(%)</u>	
	velaglucerase alfa 60 U/kg N = 17	imiglucerase 60 U/kg N = 17
Experienced No Adverse Events	1 (5.9)	1 (5.9)
Experienced At Least 1 Adverse Event	16 (94.1)	16 (94.1)
Experienced At Least 1 Drug-Related Adverse Event	8 (47.1)	6 (35.3)
Experienced At Least 1 Infusion-Related Adverse Event	5 (29.4)	4 (23.5)
Experienced At Least 1 Severe Or Life-Threatening Adverse Event	3 (17.6)	2 (11.8)
Experienced At Least 1 Serious Adverse Event	3 (17.6)	0
Experienced at least 1 drug-related SAE	1 (5.9)	0

Table 18 HGT-GCB-039 Antibodies

Anti-imiglucerase Antibody Result	velaglucerase alfa 60 U/kg N = 17 n (%)	imiglucerase 60 U/kg N = 17 n (%)
Negative	17 (100.0)	13 (76.5)
Positive ^a	0	4 (23.5)
IgG	0	4 (23.5)
IgA	0	0
IgM	0	0
IgE	0	0
Neutralizing antibodies	0	1 (5.9)
Anti-velaglucerase alfa Antibody Result		
Negative	17 (100.0)	16 (94.1)
Positive ^a	0	1 (5.9)
IgG	0	1 (5.9)
IgA	0	0
IgM	0	0
IgE	0	0
Neutralizing antibodies	0	1 (5.9)

Study Objectives

The primary objective of this study was to compare the effects of velaglucerase alfa and imiglucerase on hemoglobin concentration in patients with type 1 Gaucher disease.

The secondary objectives of this study were: to compare the effects of velaglucerase alfa and imiglucerase on platelet count; to compare the effects of velaglucerase alfa and imiglucerase on liver and spleen volumes (by MRI); to compare the effects of velaglucerase alfa and imiglucerase on Gaucher disease-specific

biomarkers (plasma chitotriosidase and CCL18 levels); to evaluate the safety of velaglucerase alfa and imiglucerase in patients with type 1 Gaucher disease, as measured by standard clinical laboratory assessments (including rates of antibody formation and enzyme neutralizing antibody activity) and safety evaluations (including rates of infusion-related adverse events and the proportion of patients requiring premedication use to manage infusion-related adverse events) for each treatment group; and to compare the effects of velaglucerase alfa and imiglucerase on the earliest time to response for hemoglobin (defined as a ≥ 1 g/dL improvement in hemoglobin levels relative to Baseline).

The tertiary objectives of this study are: to evaluate the effects of velaglucerase alfa and imiglucerase on growth velocity and Tanner staging in patients between 2 and 17 years-old; to evaluate the effects of velaglucerase alfa and imiglucerase on changes in skeletal age in patients between 2 and 17 years-old by radiography of the left hand and wrist; to evaluate the effects of velaglucerase alfa and imiglucerase on changes in overall QoL, as measured by the SF-36 for patients ≥ 18 years of age and the CHQ PF-50 for patients 5 to 17 years-old; to evaluate the effects of velaglucerase alfa and imiglucerase on immune and inflammatory responses in patients ≥ 18 years of age as measured by selected cytokine assessments (TNF- α , IL6, IL1b, IL8, IL13, CD14, and GM-CSF); to establish a baseline from which to evaluate bone disease in patients between 2 and 17 years-old by MRI of the lumbar spine and femoral neck; and to establish a baseline from which to evaluate the long-term effect of velaglucerase alfa therapy on Gaucher-related local and systemic bone disease in patients ≥ 18 years-old by: dual energy X-ray absorptiometry (DXA) of the lumbar spine and femoral neck, including coronal imaging ; and serum alkaline phosphatase, N-telopeptide cross-links (NTx), and C-telopeptide cross-links (CTx).

Study Endpoints

The primary endpoint of this study is to measure the mean change from Baseline to week 41/End of Study (EOS) in hemoglobin concentration between the two treatment groups.

The secondary endpoints of this study are: to evaluate the safety of velaglucerase alfa and imiglucerase, as assessed by adverse events and infusion-related adverse events (and the proportion of patients requiring premedication use to manage infusion-related adverse events), clinical laboratory values, vital signs, 12-lead electrocardiograms (ECG), antibody formation and enzyme neutralizing antibody activity; to compare the mean and percent changes from Baseline in platelet count between treatment groups; to compare the mean and percent changes from Baseline in liver and spleen volumes by MRI between treatment groups; to compare the mean and percent changes from Baseline in plasma chitotriosidase and plasma CCL18 levels between treatment groups; and to compare time to response for hemoglobin concentration (defined as a ≥ 1 g/dL improvement in hemoglobin levels relative to Baseline) between treatment groups.

The tertiary endpoints of this study are: to evaluate change from Baseline in growth velocity and Tanner staging for patients between 2 and 17 years-old within each treatment group; to evaluate change from Baseline in the SF-36 parameters for patients ≥ 18 years-old within each treatment group; to evaluate the effects of velaglucerase alfa and imiglucerase on immune and inflammatory responses in patients ≥ 18 years-old as measured by selected cytokine assessments (TNF- α , IL6, IL1b, IL8, IL13, CD14, and GM-CSF); to evaluate change from Baseline in the CHQ (PF-50) parameters for patients 5 to 17 years-old within each treatment group; and to evaluate change from Baseline in skeletal age as measured by radiography of the left hand and wrist for patients between 2 and 17 years old within each treatment group.

Overall Study Design

This study was comprised of 5 phases as follows: Screening: Day -21 through Day -4; Baseline: Day -3 through Day 0 (through patient randomization); Treatment: Week 1 (Day 1, i.e., day of first dose) through Week 39; End of Study Visit: Week 41; Follow-up Contact: 30 days after the final infusion (for patients who discontinue/withdraw prior to the Week 41 evaluation, or for patients who do not elect to enroll in the long-term clinical study).

At Screening, patients who provided written informed consent to participate in this study were reviewed against the study entrance criteria to determine eligibility.

Patients provided blood samples to measure hemoglobin concentration. Only those patients who had a hemoglobin concentration that was below the lower limit of normal for age and gender were eligible for enrollment. For statistical analysis purposes, an additional blood sample was collected at screening for evaluation of hemoglobin concentration.

Patients who were eligible for study participation after completing the Screening evaluations underwent Baseline procedures and evaluations (i.e., Days -3 to 0). To confirm that their hemoglobin concentration was below the lower limit of normal for age and gender, patients provided a blood sample at Baseline. Hemoglobin concentration was analyzed and reported. Only those patients who had a hemoglobin concentration that was below the lower limit of normal for age and gender at both Screening and Baseline were eligible for enrollment. For statistical analysis purposes, an additional blood sample was collected at Baseline for evaluation of hemoglobin concentrations. Additional Baseline procedures and evaluations were conducted prior to administration of the first dose of blinded study medication.

Upon completion of Screening and Baseline procedures and confirmation of patient eligibility, patients were randomized in a 1:1 ratio to receive double-blind study medication (either velaglucerase alfa 60 U/kg or imiglucerase 60 U/kg). Randomization was accomplished via a centralized procedure. A computer generated randomization schedule was utilized to allocate patients to treatment groups. An attempt was made to obtain treatment groups that were comparable in certain prognostic variables, such as age, hemoglobin concentration, and whether the patient had undergone splenectomy. The randomization schedule was prepared prior to the study.

Patients received a total of 20 IV infusions of double-blind study medications at the clinical site once every other week for a total of 39 Weeks. Safety and efficacy assessments were made at regular intervals during the treatment phase. The final assessments of safety and efficacy were made at the Week 41 visit (2 weeks after the last infusion).

Safety was assessed throughout the study by assessments of adverse events (including infusion-related adverse events), concomitant medications, and vital signs. Additional safety assessments, including, 12-lead electrocardiograms, physical

examinations, clinical laboratory tests (hematology, serum chemistry, and urinalysis), were made at Weeks 13, 25, and 41. Determination of the presence of anti-velaglycerase alfa or anti-imiglycerase antibodies and enzyme neutralizing antibodies was conducted approximately every 6 weeks until Week 41.

5 Efficacy was assessed via hemoglobin concentration and platelet count, liver and spleen volume, and plasma chitotriosidase and CCL18 level. Additional efficacy assessments included growth velocity and Tanner staging, QoL indicators, skeletal growth. Immune and inflammatory response (as measured by selected cytokine parameters) was measured in patients who are ≥ 18 years of age at study entry.

10 The duration of treatment in this study was 39 weeks and the duration of patient participation in this study was up to 11 months (from Screening through follow-up). Patients who completed this study were provided the opportunity to enroll in a subsequent open-label long-term clinical study, in which all patients would receive velaglycerase alfa. For patients who elected to enroll in the subsequent open-label long-
15 term clinical study, certain assessments from the Week 41 visit were used as the baseline assessments for that clinical study; patients would receive their first velaglycerase alfa infusion for the long-term clinical study following completion of the Week 41 procedures and evaluations scheduled for this study. Therefore, it was intended that patients would receive continuous treatment across the 2 studies. Patients who completed this study and
20 did not elect to enroll in the long-term clinical study would have a safety evaluation by site visit or telephone 30 days after their last infusion in this study.

Selection of Study Population

34 patients were enrolled (17 patients assigned to each treatment group).

25 Eligible participants were males or females age ≥ 2 years with diagnosed type 1 Gaucher disease (deficient glucocerebrosidase activity in leukocytes, or by genotype analysis), and disease-related anemia (hemoglobin levels below the local laboratory's lower limit of normal for age and gender). Participants also had 1 or more of the following: at least moderate splenomegaly (2 to 3 cm below the left costal margin) by
30 palpation; disease-related thrombocytopenia (platelet count $< 120 \times 10^3$ platelets/mm³); or

readily palpable enlarged liver. Participants could not have received treatment for Gaucher disease within 12 months prior to study entry.

Participants were excluded if they had a splenectomy; had (or were suspected of having) type 2 or 3 Gaucher disease; were antibody-positive or had experienced an anaphylactic shock to imiglucerase. Other exclusion criteria included treatment with any non-Gaucher disease-related investigational drug or device within 30 days prior to study entry; positive test for HIV, or hepatitis B or C; exacerbated anemia (vitamin B12, folic acid, or iron deficiency-related), or any significant co-morbidity that could affect study data. Pregnant or lactating women were excluded and women of child-bearing potential were required to use a medically acceptable method of contraception at all times.

Study Treatments

Treatment Assignment: Patients were randomized in a 1:1 ratio prior to administration of the first dose of: velaglucerase alfa 60 U/kg every other week for 39 Weeks (up to 16 patients, 20 infusions), or imiglucerase 60 U/kg every other week for 39 Weeks (up to 16 patients, 20 infusions). All study medication was administered by IV infusion over 1 hour to maintain the treatment blind.

Treatment Administration

Study Medication Infusions: Double-blind study medication infusions were administered at the clinical site as a continuous 1-hour IV infusion to maintain the treatment blind. Study medication infusions occurred on approximately the same day of the week but occurred every 14 days (± 3 days) in order to facilitate patient scheduling.

Dose Calculation: The first dose of double-blind study medication was based on the patient's weight at Baseline. A change in weight of $\geq 5\%$ from Baseline or from the most recent recorded measurement (Week 13 or Week 25) would require recalculation of the dose of study medication.

Description of Study Medications

VELAGLUCERASE ALFA: Velaglucerase alfa is a lyophilized product that was supplied and shipped to the clinical study site to be stored at 2 to 8°C.

5 **Imiglucerase (Cerezyme®):** Imiglucerase (Cerezyme) was supplied as a sterile, non-pyrogenic, white to off-white lyophilized product.

Study Procedures and Data Collection Methods

10 ***Study Entrance Criteria:*** At Screening patients were reviewed for eligibility against the study entrance criteria. Patients who did not meet the study entrance criteria were considered Screen failures.

15 ***Confirmation of Eligibility:*** At Screening, patients provided blood samples to measure hemoglobin concentration to determine study eligibility. Only those patients who had a hemoglobin concentration that was below the local laboratory's lower limit of normal for age and gender were eligible for enrollment.

20 At Baseline, patients provided a blood sample to confirm that their hemoglobin concentration was below the local laboratory's lower limit of normal for age and gender. Only those patients who had a hemoglobin concentration that was below the lower limit of normal for age and gender at both Screening and Baseline were confirmed eligible for this study.

25 ***Genotyping:*** All patients provided a blood sample at Screening for Gaucher disease genotyping and plasma chitotriosidase genotyping.

30 ***Medical History:*** At Screening, the patient's complete medical history was recorded. This included a review of body systems, documentation of current and prior medical procedures, and documentation of current and prior concomitant medication usage, and documentation that the patient had not been treated for Gaucher disease within the 12 months prior to study entry.

Vital Signs: Vital signs parameters that were recorded included pulse, blood pressure, respiration rate, and temperature. The following schedule was followed for recording vital signs at infusion visits: start of infusion (within 10 minutes prior to starting the infusion), during infusion (30 minutes (± 5 minutes)), after infusion (within 5 minutes, 30 minutes (± 5 minutes), and 60 minutes (± 5 minutes) after completing the infusion). At Screening, Baseline, and Week 41, vital signs were collected at one time point only.

Physical Examinations: Physical examinations were performed at Screening, Baseline and at Study Weeks 13, 25, and 41. Physical examinations included the following: general appearance, endocrine, head and neck, cardiovascular, eyes, abdomen, ears, genitourinary, nose, skin, throat, musculoskeletal, chest and lungs, and neurological. Any abnormal change in physical findings was recorded as an adverse event on the appropriate CRF page(s).

Height and Weight: Height and weight were recorded at Baseline and at Study Weeks 13, 25, and Week 41. For pediatric patients (i.e., 2 to 17 years-old), height and weight assessments were used to determine growth velocity.

12-Lead Electrocardiograms: A 12-lead ECG was performed at Baseline and at Study Weeks 13, 25, and 41, and included assessment of PR, QRS, QT, and QTc intervals, and heart rate.

Clinical Laboratory Testing: Blood and urine samples were collected as described below for clinical laboratory testing.

Hematology: Blood samples were collected during Screening and at Baseline to measure hemoglobin levels for statistical analysis. Blood samples were also collected at Screening, Baseline, and Weeks 13, 25, and 41 for complete hematology testing. The following hematology parameters were evaluated: complete blood count (CBC) with differential, platelet count, activated partial thromboplastin time (aPPT), reticulocyte count (analyzed and reported by the clinical site's local laboratory), and prothrombin

time (PT). Blood samples were collected at Screening, Baseline, and at every study visit (except at the Week 1 visit) to measure hemoglobin concentration and platelet count.

Serum Chemistry: Blood samples were collected for serum chemistry testing at Screening, Baseline, and at Study Weeks 13, 25, and 41. The following serum chemistry parameters were evaluated: sodium, alanine aminotransferase, potassium, aspartate aminotransferase, glucose, lactate dehydrogenase, total calcium, gammaglutamyltransferase, total protein, creatinine phosphokinase, albumin, NTx*, creatinine, CTx*, urea nitrogen, folic acid (to determine study eligibility), total bilirubin, vitamin B₁₂ (screening only), alkaline phosphatase* (* results were used for assessments of bone biomarkers). Patients who at Screening had folic acid and/or vitamin B₁₂ deficiency-related anemia, and so did not meet study entry criteria were considered a screen failure.

Urinalysis: Urine samples were collected for urinalysis at Screening, Baseline, and at Study Weeks 13, 25, and 41. The following urinalysis parameters were evaluated: pH, microscopic evaluation, and macroscopic evaluation.

Serum Anti-velaglucerase alfa Antibodies: Patients provided blood samples to measure anti-velaglucerase alfa antibodies in serum at Screening and approximately every 6 weeks during the treatment phase (Weeks 7, 13, 19, 25, 31, and 37), and at Week 41. During the treatment phase, these blood samples were collected prior to the infusion of double-blind study medication.

Blood samples collected for anti-velaglucerase alfa antibody determination were evaluated. These samples were screened using an enzyme-linked immunosorbence assay (ELISA), and all positive samples were confirmed positive using a radioimmunoassay (RIA). Positive samples were isozymed (IgG, IgA, IgM, or IgE). In addition, positive samples were tested for enzyme neutralizing activity using an *in vitro* assay.

Serum Anti-imiglucerase Antibody Determination: Patients provided blood samples at Screening to measure anti-imiglucerase antibodies. The anti-imiglucerase antibody analyses were performed using the same samples obtained for anti-velaglucerase alfa antibody analyses.

5 These blood samples were evaluated to determine the presence of anti-imiglucerase antibodies. These samples were screened using an enzyme-linked immunosorbance assay (ELISA), and all positive samples were confirmed positive using a radioimmunoassay (RIA). Positive samples were isotyped (IgG, IgA, IgM, or IgE). In addition, positive samples were tested for enzyme neutralizing activity using
10 an *in vitro* assay.

Patients who test positive for anti-imiglucerase antibodies at Screening were not eligible for this study.

Antibody cross-reactivity testing (to velaglucerase alfa) was conducted for patients who develop anti-imiglucerase antibodies during this study.

15 Immune and Inflammatory Response Testing: Patients who were ≥ 18 years of age provided blood samples for immune and inflammatory response testing at Baseline and at Weeks 13, 25, and 41. One sample was obtained at Baseline. At Weeks 13, 25, and 41, samples were obtained before, immediately following, and 1 hour after each infusion
20 with study drug.

Adverse Events: Adverse events were monitored throughout the study from the time the patient provided informed consent through 30 days after the last infusion for patients who completed the study and did not elect to enroll in the subsequent open-label
25 long-term clinical study, or for patients who discontinued or withdraw from the study prior to the Week 41 visit. For patients who completed this study and elected to enroll in the subsequent open-label long-term clinical study, adverse events were monitored from informed consent through completion of the Week 41 visit.

30 Management of Infusion-Related Adverse Events: Infusions of proteins can be associated with reactions to the infusion. An infusion-related adverse event is defined as

an adverse event that 1) begins either during or within 12 hours after the start of the infusion, and 2) is judged as possibly or probably related to blinded study medications.

Liver and Spleen MRI: Patients underwent MRI of the liver and spleen at Baseline and at Weeks 25 and 41/EOS. Liver and spleen size were measured using quantitative abdominal MRI.

Plasma Chitotriosidase Levels: Blood samples were collected for the evaluation of plasma chitotriosidase levels at Baseline and at Weeks 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, and Week 41. Chitotriosidase was analyzed using an enzyme activity assay.

Plasma CCL18 Levels: Blood samples were collected for the evaluation of plasma CCL18 levels at Baseline and at Weeks 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, and Week 41. CCL18 levels were measured by an enzyme-linked immunosorbent assay (ELISA) in a commercially available kit.

Quality of Life Testing: At Baseline and Week 41, patients' quality of life was evaluated using validated questionnaires, including the Short Form 36 (SF-36), version 2, for patients ≥ 18 years-old and the Childhood Health Questionnaire (CHQ), PF50 for patients 5 to 17 years-old (Ware *Arch Phys Med Rehabil* Vol 84, Suppl 2, April 2003:43-51; SF-36v2™ Health Survey© 1996, 2000 by QualityMetric Incorporated and Medical Outcomes Trust. All Rights Reserved; Landgraf *et al.* Child Health Questionnaire: A User's Manual. 2nd printing, Health Act, Inc., Boston MA, 1999; Landgraf *et al.* *Quality of Life Research*. 1998; 7(5):433-445).

Growth Velocity and Tanner Staging: For patients 2 to 17 years-old, growth was assessed at Baseline and Weeks 13, 25, and 41. Growth velocity was calculated using height and weight measurements that were recorded at regular time points during this study, and correlated with Tanner staging. Tanner stage was recorded at Baseline and Weeks 13, 25, and 41. The change from Baseline for each patient between 2 and 17-years old in each treatment group was evaluated as a tertiary efficacy parameter.

Skeletal Growth: Patients between 2 and 17 years-old underwent radiography of the left hand and wrist at Baseline and Week 41 for evaluation of skeletal age.

5 Bone Biomarkers: At Baseline and Week 41, patients who were ≥ 18 years-old underwent DXA of the lumbar spine and femoral neck, including coronal imaging, to determine Gaucher-related local and systemic bone disease. Bone loss and demineralization were evaluated for these patients by measuring serum alkaline phosphatase, NTx, and CTx at Baseline and Week 41.

10 For patients 2 to 17 years-old, MRI of the femoral neck and lumbar spine was obtained at Baseline and Week 41, at the same time these patients underwent MRI of the liver and spleen.

15 It was not expected that any treatment effect would be apparent for these parameters during this study, however, the measurements collected at Baseline and Week 41 would be utilized to establish a reference point from which to monitor these biomarkers during the subsequent open-label long-term clinical study.

20 Prior and Concomitant Illnesses: Additional illnesses present at Baseline were regarded as concomitant illnesses and were documented on the appropriate pages of the medical history CRF. Illnesses first occurring or detected during the study, or worsening of a concomitant illness during the study, were regarded as AEs and were documented as such in the CRF.

25 Patients did not receive treatment with red blood cell growth factor or investigational drug(s) or device(s) at any point during this study or within 30 days after the last infusion.

During the treatment phase of this study, patients might receive corticosteroids as premedications to mitigate potential infusion-related adverse events.

30 Adverse Events

Adverse Event Definition: An adverse event (AE) is any noxious, pathologic, or unintended change in anatomical, physiologic, or metabolic function as indicated by

physical signs, symptoms, and/or laboratory changes occurring in any phase of a clinical trial, and whether or not considered study drug-related. This includes an exacerbation of a pre-existing condition. Adverse events were collected from the time the patient provides signed informed consent until 30 days after the last dose of blinded study medication and/or until the event had been resolved/stabilized or an outcome was reached, whichever comes first. For patients who discontinued or were withdrawn prior to the Week 41 visit, AEs were followed up to 30 days after their last infusion. For patients who completed this study and elected to enroll in the long-term clinical study, adverse events were monitored from the time the patient provides informed consent through the Week 41 visit.

AEs include: worsening (change in nature, severity, or frequency) of conditions present at the onset of the study; intercurrent illnesses; drug interactions; events related to or possibly related to concomitant medications; abnormal laboratory values (this includes significant shifts from Baseline within the range of normal that the Investigator considers to be clinically important); clinically significant abnormalities in physical examination, vital signs, weight, and ECG.

In addition, AEs might also include unexpected laboratory values that became significantly out of range and determined to be clinically significant by the Investigator. In the event of an unexpected out-of-range value, the laboratory test was repeated until it returned to normal or could be explained and the patient's safety was not at risk.

Infusion-Related Adverse Event Definition: An infusion-related adverse event was defined as an adverse event that 1) begins either during or within 12 hours after the start of the infusion, and 2) is judged as possibly or probably related to blinded study medication. Other AEs which occurred prior to the infusion, along with AEs associated with protocol-defined testing and assessments (e.g., laboratory testing, ECGs, and physical examinations) which were performed prior to the infusion, were not defined as infusion-related adverse events.

Serious Adverse Event Definition: A serious AE (SAE) is any AE occurring at any dose that results in any of the following outcomes: death, is life-threatening, requires

inpatient hospitalization, requires prolongation of existing hospitalization, a persistent or significant disability/incapacity, and a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

A life-threatening AE is defined as an AE that placed the patient, in the view of the initial reporter, at immediate risk of death from the AE as it occurred (i.e., it does not include an AE that, had it occurred in a more severe form, might have caused death).

Classification of Adverse Events and Serious Adverse Events: The National Cancer Institute Common Toxicity Criteria (NCI CTC) Version 3.0 grading scale was referenced when assessing the severity of an AE. If an AE was not described in the NCI CTC, the severity was recorded based on the scale below. The severity of all AEs/SAEs were recorded on the appropriate CRF page as Grade 1, 2, 3 or 4 corresponding, respectively, to a severity of mild, moderate, severe, or life-threatening. Grade 1 (mild) is defined as no limitation of usual activities. Grade 2 (moderate) is defined as some limitation of usual activities; Grade 3 (severe) is defined as inability to carry out usual activities; and Grade 4 (life-threatening) is defined as immediate risk of death.

Relationship of an adverse event or serious adverse event to blinded study medication was determined by the Investigator based on the following definitions. “Not related” is defined as unrelated to study drug. “Possibly related” is defined as a clinical event/laboratory abnormality with a reasonable time sequence to administration of study drug, but which could also be explained by concurrent disease or other drugs/chemicals. “Probably related” is defined as a clinical event/laboratory abnormality with a reasonable time sequence to administration of study drug, unlikely to be attributable to concurrent disease or other drugs and chemicals and which follows a clinically reasonable response on dechallenge. The association of the clinical event/laboratory abnormality must also have some biologic plausibility, at least on theoretical grounds.

Clarification between Serious and Severe: The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious,” which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) and causality serve as a guide for defining regulatory reporting obligations.

Adverse Event Monitoring and Period of Observation: For the purposes of this study, the period of observation extended from the time the patient provided informed consent until the patient’s final evaluation of the study. For safety purposes, the final evaluation was defined as the post-study safety evaluation performed approximately 30 days after the last infusion for patients who completed the study and did not elect to enroll in the long-term study, or for patients who discontinued or withdrew from the study prior to the Week 41 visit. For patients who completed this study and elected to enroll in the long-term clinical study, adverse events were monitored from the time the patient provides informed consent through the Week 41 visit. If the Investigator considered it necessary to report an AE in a study patient after the end of the observation period, he or she would contact the Sponsor to determine how the AE should be documented and reported.

Statistical Method

General Statistical Methodology: Two data sets were considered for the statistical analyses of efficacy: 1) the intention-to-treat (ITT) data set and 2) the per-protocol (PP) data set. The ITT data set is comprised of all randomized patients who received at least one full or partial dose of study drug. The PP data set is a subset of the ITT data set, which includes patients who completed 41 weeks of the study, had both the Baseline and the Week 41 measurements of the primary efficacy variable collected, and received at least 80% of their scheduled dose of infusion.

For variables following a continuous distribution, tabular summaries consisted of n, mean, standard deviation, minimum, maximum, and median. Graphs of the key

efficacy variables were presented by treatment groups. For categorical variables, tabular summaries consisted of presenting the frequency and the percentage in each category by treatment group. The primary efficacy variable was presented by treatment group, and included: raw values: the untransformed value of the variable in the originally reported scale; the absolute change in the value from Baseline, i.e., $X - B$ (where B is the Baseline value and X is a post-Baseline value); and the percent change in the value from Baseline, i.e., $100 * (X - B) / B$ (where B is the Baseline value and X is a post-Baseline value).

Hypothesis Testing: This study compared the effect of velaglucerase alfa with imiglucerase. The intent was to show that velaglucerase alfa was clinically at least as good as imiglucerase at the 0.025 level of significance.

The null hypothesis for the primary efficacy endpoint is that the mean change in hemoglobin concentration from Baseline to Week 41 for velaglucerase alfa is at least 1 g/dL inferior to the mean change in hemoglobin concentration from Baseline to Week 41 for imiglucerase. The hypothesis to be tested can be stated as:

$$H_0: \mu_{Vela} - \mu_{IMIG} \leq -1 \quad \text{vs.} \quad H_A: \mu_{Vela} - \mu_{IMIG} > -1$$

Or

H_0 : velaglucerase alfa is inferior with respect to the mean hemoglobin response

H_A : velaglucerase alfa is non-inferior with respect to the mean hemoglobin response

Screen Failures and Patient Disposition: The disposition of all patients screened for entry into the study was tabulated along with reasons for screen failure. The disposition of all randomized patients was tabulated by treatment arm and visit, and reasons for discontinuation were tabulated by treatment arm.

Sample Size Justification: When the sample size in each treatment group is 14, a two-group 0.025 one-sided t-test will have an 80% power to reject the null hypothesis that the difference in means for hemoglobin is ≤ -1 g/dL in favor of the alternative hypothesis that the difference in means is greater than -1, assuming that the expected difference in means is 0, and the common standard deviation is 0.90.

Assuming a 15% dropout, a total of 32 patients (16 patients per treatment arm) were enrolled into the study.

Efficacy Analysis

5 Analysis Populations: Two data sets were considered for the statistical analyses of efficacy (the intention-to-treat (ITT) data set and the per-protocol (PP) data set).

10 Primary Efficacy Analyses: The primary efficacy endpoint is the mean change from Baseline to Week 41 in hemoglobin concentration between the two treatment groups. The primary analysis was carried out using the ITT population. This is a non-inferiority randomized controlled trial designed to demonstrate that velaglucerase alfa is non-inferior to imiglucerase in terms of efficacy in treating patients with type 1 Gaucher disease.

15 A one-sided 97.5% confidence interval was used. Non-inferiority was demonstrated by either a one-sided confidence interval or a hypothesis test for testing the null hypothesis that the treatment difference is less than or equal to the lower equivalence margin in hemoglobin (-1 g/dL) versus the alternative that imiglucerase treatment difference is greater than the lower equivalence margin. In other words, focusing on just one end of the confidence interval and ignoring the other results in a one-sided 97.5% confidence interval $[(a, \infty)]$, where a is the lower bound of the 1-sided confidence interval, an efficacy conclusion could be drawn.

25 Secondary Efficacy Analyses: For the secondary efficacy parameters (platelet counts, liver, and spleen volumes, chitotriosidase, and CCL18) that compare changes from Baseline between treatment groups, statistical tests evaluated if the mean changes from Baseline to Week 41 between the two treatment groups is statistically significant (statistically significant will be defined as a p-value less than 0.05). A 95% confidence interval was presented for the difference in mean changes from Baseline between the two treatment arms using an analysis of covariance (ANCOVA) model, which included, e.g., Baseline age as a covariate.

For time to event outcome measures (i.e., time to first hemoglobin response ≥ 1 g/dL from baseline), Kaplan-Meier (product limit) survival curves were presented for each treatment group and a log-rank test were used to compare curves between treatment groups. The median time and 95% confidence interval were presented for each treatment group. Patients who did not experience the event by the end of the study (i.e., by Week 41) were censored at Week 41. Patients who withdrew or were discontinued prior to the Week 41 evaluation and who did not achieve a response at the time of withdrawal or discontinuation were censored at the time of the last known evaluation for that patient. In addition, the proportion of patients who responded versus non-responders were presented and compared between treatment groups using Fisher's Exact Test.

Safety Analyses: All patients who received at least one dose of study drug (or partial dose) were assessed for clinical safety and tolerability. No formal statistical tests were performed on the safety parameters. Vital signs, 12-lead ECG, clinical chemistry, hematology, and urinalysis safety monitoring were summarized. For categorical variables, such as AEs, the number and percentage of patients experiencing each AE were tabulated. AEs were summarized by severity of event. The number and percentage of patients experiencing drug related AEs and infusion-related AEs, as well as AEs that were not considered related to study drug will also be displayed. Clinical laboratory evaluations (hematology, serum chemistry, urinalysis, and determination of anti-velaglycerase alfa antibodies) were used to assess the safety of velaglycerase alfa.

Example 4: TKT034 Study (Multi-center open-label study in patients who transitioned from treatment with imiglucerase)

Summary

This example describes a global, open-label, 12-month study to examine the safety and efficacy of velaglycerase alfa in patients with type 1 Gaucher disease previously receiving imiglucerase. Patients aged ≥ 2 years received velaglycerase alfa at a dose equal to their prior imiglucerase dose, with infusions administered over 1 hour every other week.

Forty patients received velaglucerase alfa (18 male; 4 previously splenectomized; age range, 9–71 years). Median prior imiglucerase use was 67 months (range 22–192 months). Velaglucerase alfa doses were: 15–22.5U/kg (n=14) (“the 15U/kg group”), 22.5–37.5U/kg (n=12) (“the 30U/kg group”), 37.5–52.5U/kg (n=7) (“the 45U/kg group”), and >52.5U/kg (n=7) (“the 60U/kg group”). Velaglucerase alfa was generally well tolerated with most adverse events (AEs) of mild or moderate severity. Eleven patients (28%) experienced an AE considered possibly or probably related to study drug; the majority were considered infusion related. No patient experienced a life-threatening AE. One serious AE was considered probably related to treatment: one patient had a grade 2 hypersensitivity reaction during the first infusion, and chose to discontinue the study. This patient tested negative for IgE, IgM, IgG, IgA and neutralizing antibodies at the time of the infusion and 2 weeks later. No patients developed IgG antibodies to velaglucerase alfa. Hemoglobin concentration, platelet count, liver and spleen volume were sustained at therapeutic levels through 1 year.

In conclusion, adult and pediatric patients with type 1 Gaucher disease, previously treated with imiglucerase for ≥ 22 months, were successfully transitioned to velaglucerase alfa, with stability in clinical disease measures over 12 months.

Study Objectives

The primary objective of this study was to evaluate the safety of every other week dosing of velaglucerase alfa in patients with type 1 Gaucher disease who were previously treated with imiglucerase.

The secondary objectives were: to evaluate changes from Baseline in hemoglobin concentration after every other week dosing of velaglucerase alfa, to evaluate changes from Baseline in platelet count after every other week dosing of velaglucerase alfa, and to evaluate changes from Baseline in liver and spleen volume by abdominal MRI after every other week dosing of velaglucerase alfa.

The tertiary/exploratory objectives were: to evaluate changes from Baseline in levels of plasma chitotriosidase and Chemokine (C-C motif) ligand 18 (CCL18) after every other week dosing of velaglucerase alfa, to evaluate changes in skeletal age in patients between 2 and 17 years-old by radiography of the left hand and wrist after every

other week dosing of velaglucerase alfa, to evaluate changes in growth velocity and Tanner staging in patients between 2 and 17 years-old after every other week dosing of velaglucerase alfa, to establish a Baseline from which to monitor the long term effect of velaglucerase alfa therapy on Gaucher-related local and systemic bone disease in patients
5 ≥ 18 years-old, as measured by bone density (DXA) of the lumbar spine and femoral neck (including coronal imaging), serum alkaline phosphatase, N-telopeptide cross-links (NTx), and C-telopeptide cross links (CTx), and to establish a Baseline from which to evaluate bone disease in patients between 2 and 17 years-old by MRI of the lumbar spine and femoral neck.

Overall Study Design

This is a multicenter, Phase II/III, open-label study designed to evaluate the safety of velaglucerase alfa therapy for patients currently receiving imiglucerase therapy for type I Gaucher disease. 41 patients were enrolled to receive the same number of units of
15 velaglucerase alfa as their imiglucerase dose. Doses ranged between 15 U/kg and 60 U/kg. Patients had received the same dose of imiglucerase during the 6 months prior to study enrollment. The overall duration of the study for each patient was approximately 14 months (from Screening through the End of Study and/or follow-up, as appropriate).

The study was comprised of 5 phases as follows: Screening (Day -14 through Day
20 -4), Baseline (Day -3 through Day 0 (prior to first dose)), Treatment Phase: Week 1 (Day 1; first dose) through Week 51 (a total of 26 infusions were administered per patient), End of Study Visit: Week 53, Follow-up: 30 days after the final infusion (for patients who discontinued/withdrew prior to the Week 53 evaluation, or for patients who completed this study but did not elect to enroll in the subsequent long-term clinical
25 study).

Patients (or parent/legal guardian) who provided written informed consent underwent Screening evaluations within two weeks prior to their first dose to determine eligibility for enrollment. To determine study eligibility, a blood sample was collected from each patient during Screening to evaluate hemoglobin concentration and platelet
30 count.

An additional blood sample was collected during Screening for evaluation of hemoglobin concentration and platelet count for statistical analysis purposes.

Administration of the first dose of study drug was defined as Week 1 (Day 1). Velaglucerase alfa infusions were administered every other week for 12 months (51 weeks) for a total of 26 infusions. Patients received the same number of units of velaglucerase alfa as their imiglucerase dose. Doses ranged between 15 U/kg and 60 U/kg. Infusion time was 60 minutes (1 hour). Increased infusion durations (e.g., 2 hours) were documented in the source documentation and appropriate CRF. Infusions were not less than 1 hour in duration.

The first 3 velaglucerase alfa infusions for each patient were administered at the clinical site. Patients who did not experience a treatment-related serious adverse event or a velaglucerase alfa infusion-related adverse event might receive their subsequent infusions at home by qualified and trained medical personnel, per the discretion and direction of the Investigator. Patients who experienced an infusion-related adverse event might be re-evaluated at a later time point during the study for consideration to transition to home infusions. Patients receiving velaglucerase alfa as home therapy were required to return to the clinical site at Weeks 7, 13, 19, 25, 31, 37, 45, and 51 and 53.

The study completion visit is defined as Week 53. Patients were considered to have completed this study once they have 1) completed the 51-week treatment period, and 2) complete the study visits at Week 51 and Week 53.

Patients who completed this study were provided the opportunity to enroll in a subsequent long-term open-label clinical study. For patients who elect to enroll in the subsequent long-term open-label clinical study, certain assessments from the Week 51 and the Week 53 visits for this study (TKT034) were used as the Baseline assessments for that study; patients would receive their first infusion for the subsequent long-term open-label clinical study at the Week 53 visit, after they completed all of the assessments for that visit and provided written informed consent to participate in the subsequent long-term open-label clinical study. Therefore, it was intended that patients would receive continuous velaglucerase alfa treatment across the 2 studies. Patients who completed this study and did not elect to enroll in the subsequent long-term open-label clinical study

would have a safety assessment (for collection of adverse events and concomitant medications) by site visit or telephone 30 days after their last infusion.

Selection of Study Population

5 All enrolled patients who received at least 1 infusion (or partial infusion) were included in the ITT patient population.

Eligible participants were males or females age ≥ 2 years with diagnosed type 1 Gaucher disease (deficient glucocerebrosidase activity in leukocytes, or by genotype analysis), who had received consistent treatment with imiglucerase for a minimum of 30
10 consecutive months; one patient was allowed to participate having had 22 consecutive months of previous treatment with imiglucerase.

Participants were excluded if they had both hemoglobin concentration ≤ 10 g/dL and platelet count $\leq 80 \times 10^3$ platelets/mm³; had unstable hemoglobin concentration (exceeded a range of ± 1 g/dL of the screening value) or platelet count (exceeded $\pm 20\%$ of
15 the screening value) during the 6 months prior to screening; had (or were suspected of having) type 2 or 3 Gaucher disease; had experienced an anaphylactic shock to imiglucerase; had inconsistent treatment with imiglucerase or had received miglustat in the 6 months prior to study entry; or had radiologically-confirmed active, clinically significant spleen infarction or worsening bone necrosis within 12 months of screening.

20 Other exclusion criteria included treatment with any investigational drug or device within 30 days prior to study entry; positive test for HIV, hepatitis B or C; non-Gaucher disease-related anemia at screening; or any significant co-morbidity that could affect study data. Pregnant or lactating women were excluded and women of child-bearing potential were required to use a medically acceptable method of contraception at
25 all times.

Study Treatments

Treatment Assignment: Patients received velaglucerase alfa infusions every other week at the same number of units of as their imiglucerase dose. The patient's current
30 imiglucerase dose was recorded at Baseline. Velaglucerase alfa doses ranged between 15 U/kg and 60 U/kg.

Treatment Schedule: Patients received their first infusion on Week 1 (Day 1). All patients received velaglucerase alfa once every other week for 12 months (51 weeks); therefore, a total of 26 infusions are to be administered.

5 All doses of velaglucerase alfa were administered as continuous IV infusions at a maximum rate of 1 U/kg/minute. Infusion time was 60 minutes (1 hour). Increased infusion durations (e.g., 2 hours) were documented in the source documentation and appropriate CRF. Infusions might not be less than 1 hour in duration. Patients received their final imiglucerase dose a maximum of 30 days prior to study entry and a minimum
10 of 14 days prior to study entry.

Dose Calculation: A change in weight of $\geq 5\%$ from Baseline or the previously recorded weight used to calculate dose at Weeks 13, 25, or 37 required recalculation of the dose of study medication.

15 **Dose Adjustments:** Patients were monitored throughout the treatment period for changes in clinical parameters (i.e., hemoglobin concentration, platelet count, and liver and spleen volume). If a patient demonstrated a clinically significant change in these parameters the investigator evaluated the option of increasing the patient's dose by 15
20 U/kg. A dose adjustment was considered if two or more of the following four criteria were met and consistent over two consecutive evaluations: decrease from Baseline in hemoglobin concentration of $> 1\text{g/dL}$; a decrease from Baseline in platelet count of $> 20\%$; an increase in liver volume as indicated by organ palpation and confirmed to be $>15\%$ relative to Baseline as measured by MRI; and an increase in spleen volume as
25 indicated by organ palpation and confirmed to be $>15\%$ relative to Baseline as measured by MRI.

If the clinical parameter values did not return to Baseline levels within three months, the Investigator had the option of increasing the dose by increments of 15 U/kg. No dose increase was offered to patients receiving a dose of 60 U/kg, and no dose above
30 60 U/kg was allowed. If the patient failed to respond to the maximum dose of 60 U/kg,

the patient might be withdrawn if deemed appropriate based on the Investigator's clinical judgment.

Velaglucerase alfa Administration

5 General Instructions for velaglucerase alfa administration: Velaglucerase alfa was administered intravenously. Study drug infusions occurred on approximately the same day of the week but might occur every 14 days ($\pm$ 3 days) of the target day in order to facilitate patient scheduling. If at all possible, missed infusions should be avoided. If a patient was not dosed within 17-days from their scheduled dose, the patient would
10 receive the next infusion as soon as possible after approval for the patient to continue in the study. It might be acceptable to give the next infusion as early as 7 days after the previous infusion. Subsequent infusions would return to the original schedule.

15 Home Infusion Instructions for Velaglucerase alfa Administration: The first three velaglucerase alfa infusions were administered at the clinical site. After the first three doses, patients who had not experienced a treatment-related serious adverse event or an infusion-related adverse event might receive their subsequent infusions at home. Patients who had experienced an infusion-related adverse event might be re-evaluated at a later time point during the study for consideration to transition to home infusions. Patients
20 receiving velaglucerase alfa as home therapy were required to return to the clinical site at Weeks 7, 13, 19, 25, 31, 37, 45, 51, and 53.

In the home setting, vital signs and documentation of adverse events were collected at each visit

25 ***Management of Infusion-Related Adverse Events:*** An infusion-related adverse event was defined as an adverse event that 1) begins either during or within 12 hours after the start of the infusion, and 2) is judged as possibly or probably related to study drug.

30 ***Description of Study Drug:*** Velaglucerase alfa is a lyophilized product that was supplied and shipped by a qualified distributor to the clinical study site to be stored at 2 to 8°C.

Gaucher Disease Specific Treatment History: At Screening, all Gaucher disease-specific treatments, including the patient's current imiglucerase dose, were recorded. The patient's initial velaglucerase alfa dose was based on the current imiglucerase dose recorded.

Historical Hemoglobin and Platelet Values: All evaluations of hemoglobin concentration and platelet count within the 30 months prior to study entry were collected and reviewed to determine patient eligibility.

Gaucher Disease and Chitotriosidase Genotyping: At Screening only, all patients had a blood sample collected for Gaucher disease and plasma chitotriosidase genotyping.

Vital Signs: Vital signs parameters recorded included pulse, blood pressure, respiration rate, and temperature.

The following schedule was followed for recording vital signs at all infusion visits: start of infusion (within 10 minutes prior to starting the infusion; during infusion (30 minutes (± 5 minutes))); after infusion (within 5 minutes after the infusion is completed, 30 minutes (± 5 minutes) after completing the infusion, and 60 minutes (± 5 minutes) after completing the infusion)

Physical Examinations: Physical examinations were performed at the Baseline visits, and at Weeks 13, 25, 37, 51 and 53. Physical examinations included the following: general appearance, endocrine, head and neck, cardiovascular, eyes, abdomen, ears, genitourinary, nose, skin, throat, musculoskeletal, chest and lungs, and neurological. Any abnormal change in physical findings was recorded as an adverse event on the appropriate CRF page(s).

Height and Weight: Height and weight were recorded at the Baseline visit, and at Weeks 13, 25, 37 and 51. Growth velocity was calculated using height and weight measurements, and correlated with Tanner staging.

5 **12-Lead Electrocardiograms:** A 12-Lead ECG was performed at the Baseline visit, and at Weeks 13, 25, 37 and 51. Each 12-lead ECG included assessment of PR, QRS, QT, and QTc intervals, and heart rate.

10 **Clinical Laboratory Testing:** Blood and urine samples were collected as described below for the following evaluations.

15 Hematology: Blood samples were collected for hematology testing at the Screening and Baseline visits, and at Weeks 7, 13, 19, 25, 31, 37, 45, 51 and 53. The following hematology parameters were evaluated: complete blood count (CBC) with differential, activated partial thromboplastin time (aPPT), reticulocyte count (performed by the site's local laboratory), platelet count, and prothrombin time (PT). At Screening, Baseline, and at every study visit (except at the Week 1 visit), blood samples were collected to measure hemoglobin concentration and platelet count.

20 An additional blood sample was collected during Screening to measure hemoglobin and platelet count for statistical analysis purposes.

25 Serum Chemistry: Blood samples were collected for serum chemistry testing at the Baseline visit, and at Weeks 13, 25, 37, 51 and 53.

30 The following serum chemistry parameters were evaluated: sodium, total bilirubin, potassium, alkaline phosphatase*, glucose, alanine aminotransferase, total calcium, aspartate aminotransferase, total protein, lactate dehydrogenase, albumin, Gamma-glutamyl transferase, creatinine, creatinine phosphokinase, urea nitrogen, CTx, NTx* (* results were used for assessments of bone biomarkers). Patients who at Screening had folic acid and/or vitamin B₁₂ deficiency-related anemia, and so did not meet study entry criteria were considered a screen failure. These patients might be treated for their folic acid and/or vitamin B₁₂ deficiency-related anemia for up to 12 weeks at the Investigator's discretion, according to the clinical site's standard practice. Such patients

might be re-screened for this study after they completed the folic acid and/or vitamin B₁₂ treatment regimen.

Urinalysis: Urine samples were collected for urinalysis at the Baseline visit, and
5 at Weeks 13, 25, 37, 51 and 53. The following urinalysis parameters were evaluated: pH, microscopic evaluation, and macroscopic evaluation.

Serum Anti-imiglucerase Antibody Determination: All patients had a blood
sample at Baseline only for determination of serum anti-imiglucerase antibodies. Patients
10 were eligible for enrollment in this study regardless of their anti-imiglucerase antibody status. Patients who were anti-imiglucerase antibody positive would be allowed to enter this study. These blood samples were evaluated to determine the presence of anti-imiglucerase antibodies.

Serum Anti-velaglucerase alfa Antibody Determination: Blood samples were
15 collected for determination of anti-velaglucerase alfa antibodies at the Baseline visit, and at Weeks 7, 13, 19, 25, 31, 37, 45 and 51. Blood samples collected for anti-velaglucerase alfa antibody determination were evaluated. These samples were screened using an enzyme-linked immunosorbence assay (ELISA).

Adverse Events: Adverse events were monitored throughout the study from the
20 time the patient provides signed informed consent through 30 days after their last infusion for patients who completed the study and did not elect to enroll in the subsequent long-term open-label clinical study, or for patients who discontinued or
25 withdrew from the study prior to the Week 53 visit. For patients who completed this study and elected to enroll in the subsequent long-term open-label clinical study, adverse events were monitored from informed consent through the Week 53 visit of this study (TKT034).

Prior and Concomitant Illnesses: Additional illnesses present at Baseline were
30 regarded as concomitant illnesses and were documented on the appropriate medical

history pages of the CRF. Illnesses first occurring or detected during the study, or worsening of a concomitant illness during the study, were regarded as AEs and were documented as such in the CRF.

5 *Study Procedures for Efficacy Evaluations*

Hemoglobin concentration: Hemoglobin concentration was measured at the time points described herein. The change from Baseline to 12 months in hemoglobin concentration was a secondary endpoint of this study.

10 **Platelet count:** Platelet count was measured at the time points described herein. The change from Baseline to 12 months in platelet count was a secondary endpoint of this study.

Liver and Spleen Volumes Measured by Abdominal MRI: Patients underwent
15 quantitative abdominal MRI of the liver and spleen at Baseline, Week 25, and Week 51. The change from Baseline to 12 months in liver and spleen volume was a secondary endpoint of this study.

Plasma Chitotriosidase and CCL19 Levels: Blood samples were collected for the
20 evaluation of plasma chitotriosidase and CCL18 levels at the Baseline visit, and at Weeks 13, 25, 37, 51 and 53. The change from Baseline to 12 months in chitotriosidase and CCL18 levels was a tertiary endpoint of this study.

Growth Velocity and Tanner Staging: For patients 2 to 17 years-old, growth was
25 assessed at the time points defined herein. Growth velocity was calculated using height and weight measurements recorded at regular time points during this study, and correlated with Tanner staging. The change from Baseline to 12 months in growth velocity and Tanner staging was a tertiary endpoint of this study.

30 **Skeletal Growth:** Patients between 2 and 17 years-old underwent radiography of the left hand and wrist at Baseline and Week 51 for evaluation of skeletal age. The

change from Baseline to 12 months in skeletal growth in patients 2 to 17 years of age was a tertiary endpoint of this study.

Additional Study Procedures

5 Bone Biomarkers: At Baseline only, patients ≥ 18 years-old underwent DXA of the lumbar spine and femoral neck, including coronal imaging, to determine Gaucher-related local and systemic bone disease. Bone loss and demineralization were also evaluated by measuring serum alkaline phosphatase, NTx, and CTx. Results for these parameters were obtained from blood samples collected for clinical laboratory testing at
10 Baseline only.

For patients 2 to 17 years-old, MRI of the femoral neck and lumbar spine were obtained at Baseline (at the same time patients undergo MRI of the liver and spleen). It was not expected that any treatment effect would be apparent for these parameters during this study, therefore, measurements were collected at Baseline only to establish a
15 reference point from which to monitor these biomarkers during the subsequent long-term open-label clinical study.

Adverse Events

20 ***Adverse Event Definition:*** An adverse event (AE) is any noxious, pathologic, or unintended change in anatomical, physiologic, or metabolic function as indicated by physical signs, symptoms, and/or laboratory changes occurring in any phase of a clinical study, and whether or not considered study drug-related. This includes an exacerbation of a pre-existing condition. Adverse events were collected from informed consent until 30 days after the last dose of study drug and/or until the event had been resolved/stabilized
25 or an outcome was reached, whichever came first. For patients who discontinued or were withdrawn prior to the Week 53 visit, AEs were followed up to 30 days after their last infusion of velaglucerase alfa.

AEs include: worsening (change in nature, severity, or frequency) of conditions present at the onset of the study; intercurrent illnesses; drug interactions; events related to
30 or possibly related to concomitant medications; abnormal laboratory values (this includes significant shifts from Baseline within the range of normal that the Investigator considers

to be clinically important); clinically significant abnormalities in physical examination, vital signs, weight, and ECG.

In addition, AEs might also include unexpected laboratory values that become significantly out of range and determined to be clinically significant by the Investigator.

5 In the event of an unexpected out-of-range value, the laboratory test would be repeated until it returns to normal or can be explained and the patient's safety is not at risk.

Infusion-Related Adverse Event Definition: An infusion-related adverse event was defined as an adverse event that 1) begins either during or within 12 hours after the
10 start of the infusion, and 2) is judged as possibly or probably related to study drug. Other AEs which occurred prior to the infusion, along with AEs associated with protocol-defined testing and assessments (e.g., laboratory testing, ECGs, and physical examinations) which were performed prior to the infusion, was not be defined as infusion-related adverse events. Infusion-related adverse events were managed as
15 defined above.

Serious Adverse Event Definition: A serious AE (SAE) is any AE occurring at any dose that results in any of the following outcomes: death, is life-threatening, requires inpatient hospitalization, requires prolongation of existing hospitalization, a persistent or
20 significant disability/incapacity, and a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

25 A life-threatening AE is defined as an AE that placed the patient, in the view of the initial reporter, at immediate risk of death from the AE as it occurred (i.e., it does not include an AE that, had it occurred in a more severe form, might have caused death).

Classification of Adverse Events and Serious Adverse Events: The National
30 Cancer Institute Common Toxicity Criteria (NCI CTC) Version 3.0 grading scale was referenced when assessing the severity of an AE. If an AE was not described in the NCI

CTC, the severity was recorded based on the scale below. The severity of all AEs/SAEs were recorded on the appropriate CRF page as Grade 1, 2, 3 or 4 corresponding, respectively, to a severity of mild, moderate, severe, or life-threatening. Grade 1 (mild) is defined as no limitation of usual activities. Grade 2 (moderate) is defined as some
5 limitation of usual activities; Grade 3 (severe) is defined as inability to carry out usual activities; and Grade 4 (life-threatening) is defined as immediate risk of death.

Relationship of an adverse event or serious adverse event to blinded study medication was determined by the Investigator based on the following definitions. “Not related” is defined as unrelated to study drug. “Possibly related” is defined as a clinical
10 event/laboratory abnormality with a reasonable time sequence to administration of study drug, but which could also be explained by concurrent disease or other drugs/chemicals. “Probably related” is defined as a clinical event/laboratory abnormality with a reasonable time sequence to administration of study drug, unlikely to be attributable to concurrent disease or other drugs and chemicals and which follows a clinically reasonable response
15 on dechallenge. The association of the clinical event/laboratory abnormality must also have some biologic plausibility, at least on theoretical grounds.

Clarification between Serious and Severe: The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe
20 myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious,” which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) and causality serve as a guide for defining regulatory reporting obligations.

Adverse Event Monitoring and Period of Observation: For the purposes of this study, the period of observation extended from the time the patient provides signed informed consent until the patient’s final evaluation of the study. For safety purposes, the final evaluation was defined as the post-study safety evaluation performed approximately
30 30 days after the last infusion for patients who completed the study and did not elect to enroll in the subsequent long-term open-label clinical study, or for patients who

discontinued or withdrew from the study prior to Week 53. For patients who elected to enroll in the subsequent long-term open-label clinical study, adverse events were monitored until Week 53; adverse events that had not resolved as of the Week 53 visit for this study were recorded in the patient's medical history for the subsequent long-term open-label clinical study.

Statistical Methods

General Statistical Methodology: The intent-to-treat (ITT) patient population was defined as all enrolled patients who received at least one infusion (full or partial infusion). Statistical data analyses were performed on the ITT population. Continuous data collected at Baseline and at subsequent study visits were summarized using descriptive statistics (n, mean, median, minimum, maximum, and standard deviation). Categorical data were summarized as frequencies and percentages. Descriptive statistics were presented for all patients in the ITT population according to demographic and Baseline characteristics.

Analysis of the secondary endpoints (i.e., clinical parameters) was based on a non-inferiority hypothesis as described below.

Sample Size Justification: This was a safety trial, and it was difficult to identify a single primary safety outcome variable for the study. However, the sample size selected which was based on the efficacy parameters was suitable for the evaluation of less common adverse effects.

The inclusion of at least 26 patients provided basic information on safety and tolerability. From the TKT025 study, the average change at 6 months across patients does not indicate any worsening of adverse events (AEs) from Baseline. Some patients will have their AEs resolved, some patients a worsening of their AEs. Assuming the natural variability of the patients, then the chance of seeing a worsening for a single patient is 11%. This 11% failure rate is for one patient. The likelihood that all 26 patients in the trial will not have an SAE is equal to the likelihood of patient 1 not having an SAE multiplied by the likelihood of patient 2 not having an SAE multiplied by (. . . etc . . .) the likelihood of patient 26 not having an SAE. That is 95% likelihood that at least one

patient out of 26 will show a worsening of an AE from Baseline [$1 - (.89)^{26} = 0.95$]. In other words, for a sample size of 26, the probability of observing at least one event will be 0.95, when the probability of the event is 0.11. Or, when no events are observed, to obtain an upper bound of 0.11 on the 95% confidence interval to the probability of a rare event, would require a sample size of 26.

The null hypothesis is that the mean changes from Baseline (i.e., the end of imiglucerase treatment) to Month 12 for each of the selected clinical parameters (hemoglobin concentration, platelet count, liver and spleen volumes) are within the pre-specified clinically significant values. Clinically significant changes from Baseline to Week 53 for hemoglobin was defined as a change of no less than 1 gm/dL, and a 20% change in platelet count. For normalized liver and spleen volume, the changes from Baseline to Week 53 were defined as being no more than 15% increase. The sample size estimate of 26 patients was based on a paired t-test of means, with a standard deviation of 0.671 with a two-sided alpha level of 0.05, and 80% power.

Primary Analysis: All patients who received at least one full or partial dose of study drug were assessed for clinical safety and tolerability. Vital signs, clinical chemistry and hematology that were collected for safety monitoring were listed for each patient and abnormal values were flagged. For categorical variables such as AEs, the number and percentage of patients experiencing each AE were tabulated. AEs were summarized by severity of event. The number and percentage of patients experiencing drug related AEs and AEs that were not considered related to study drug were also displayed. Infusion-related adverse events reactions and rates of anti-velaglucerase alfa antibody formation were also summarized.

The primary clinical variable was to evaluate the safety of velaglucerase alfa administered every other week to patients with type I Gaucher disease who were clinically stable on imiglucerase. Safety was evaluated by assessing vital signs and documenting adverse events (by type, frequency, and severity) at each study visit, as well as by performance of physical examinations and changes in laboratory assessments at required visits.

All AEs were coded using MedDRA Coding Dictionary. AE summaries in general were based on all AEs occurring after the patient's first infusion of study drug (treatment-emergent).

Secondary Analyses: Secondary endpoints of this study are: change from Baseline to 12 months in hemoglobin concentration; change from Baseline to 12 months in platelet count; change from Baseline to 12 months in spleen volume by abdominal MRI (evaluated as % change) (Spleen volumes were normalized by body weight); and change from Baseline to 12 months in liver volume by abdominal MRI (evaluated as % change) (Liver volumes were normalized by body weight).

For each clinical activity parameter, the alternative hypothesis is that the mean change from Baseline (i.e., the end of imiglucerase treatment) to Month 12 was within the specified clinically significant levels for the parameters to be evaluated (where the population mean change from Baseline for hemoglobin is within 1 g/dL, the platelet count is within 20%, and the liver and spleen volumes are within 15%. This was evaluated using a 2-sided 90% confidence interval for the true difference from Baseline for these clinical parameters. For example, efficacy of velaglucerase alfa was concluded if the confidence interval for the change from Baseline of hemoglobin was within the interval -1 to 1 g/dL.

The Sponsor's expectation is that the mean hemoglobin concentration was essentially constant over the 12-month period. For example, instead of using a 90% confidence interval for secondary efficacy analysis for hemoglobin, the following pair of statistical hypothesis tests, each at an alpha level of 0.05, could be used.

$$H_{01}: \mu_d \geq 1 \text{ Vs } H_{11}: \mu_d < 1$$

$$H_{02}: \mu_d \leq -1 \text{ Vs } H_{21}: \mu_d > -1$$

By rejecting the first null hypothesis (H_{01}) in favor of the first alternative hypothesis (H_{11}), one concludes at the 0.05 significance level that the treatment mean change from Baseline for hemoglobin is less than 1 g/dL higher than the Baseline value. By rejecting the second null hypothesis (H_{02}) in favor of the second alternative hypothesis (H_{21}), one concludes at the 0.05 significance level that the treatment mean change from Baseline for hemoglobin is greater than 1 g/dL lower than the Baseline

value. Because H_{01} and H_{02} cannot be simultaneously true, the overall Type I error rate is 0.05 for the above pair of hypothesis tests. Therefore, by rejecting both null hypotheses in favor of the alternative hypotheses, one concludes at the 0.05 significance level that the treatment (velaglucerase alfa) hemoglobin concentration is within the interval –
5 1 to 1 g/dL.

The sponsor considers the confidence interval method to be easier to interpret than the corresponding method using hypothesis tests. Therefore the confidence interval method will be used for the secondary inference.

10 **Tertiary Analyses:** The tertiary endpoints for this study are: change from Baseline to 12 months in plasma chitotriosidase and CCL18 levels; change from Baseline to 12 months in skeletal age in patients 2 to 17 years old; and change from Baseline to 12 months in growth velocity and Tanner staging.

The tertiary endpoints were summarized using descriptive statistics (mean,
15 median, standard deviation, minimum and maximum) at each time point. For endpoints where data were collected at Baseline and other time points during the study, the within group changes were examined.

Analysis of Subgroups: Additional analyses were conducted specifically for
20 patients between 2 to 17 years old. Also, consideration was given in the analysis to disease severity with regard to hemoglobin Baseline values.

Results

40 patients were included in the intent-to-treat (ITT) analysis (**Table 19**), and 38
25 patients (93%) completed the study. One patient discontinued before receiving study drug; and two patients in the 15 U/kg group discontinued, one due to an anaphylactoid reaction during her first infusion with velaglucerase alfa, and one at Week 31 because of a perceived lack of improvement in Gaucher-related symptoms.

Patients received velaglucerase alfa at the same number of units to their prior
30 imiglucerase regimen. Median prior imiglucerase use was 67 months (range 22—192 months). Velaglucerase alfa doses were grouped into four ranges: ≤ 22.5 U/kg (n=14),

22.5–37.5 U/kg (n=12), 37.5–52.5 U/kg (n=7), and >52.5 U/kg (n=7). Investigators had the option to increase the velaglucerase alfa dose (to a maximum of 60 U/kg every other week) if a patient demonstrated a clinically significant change in hemoglobin or platelet counts. No dose adjustments were made during the study.

5

Table 19 TKT034 Patient characteristics at baseline

	ITT population (n=40)
Age, mean (range)	36 years (9–71 years); 25% <18 years
Gender, n (%)	18 (45%) male / 22 (55%) female
Clinical parameters, median (range)	
Hemoglobin	13.8 g/dL (10.4–16.5 g/dL)
Platelet count	162 x 10 ⁹ /L (29–399 x 10 ⁹ /L)
Liver volume*	0.8 MN (0.6–1.6 MN)
Spleen volume**	2.5 MN (1.0–16.0 MN)
Biomarkers, median	
Chitotriosidase	3071.3 nmol/mL/h
C-C motif ligand 18 chemokine (CCL18)	325.0 ng/mL
Prior imiglucerase use, median (range)	67 months (22–192 months)
Anti-imiglucerase antibody positive prior to receiving velaglucerase alfa, n (%)	3 (8%)

* A normal liver volume is 2.5% of body weight.

**In 36 patients with spleen intact; four patients had undergone a splenectomy prior to enrollment. A normal spleen volume is 0.2% of body weight. MN=multiples of normal.

Clinical parameters were sustained at therapeutic levels through 1 year (Table 20).

5

Table 20

	n	Baseline median	Mean change or % change from baseline to month 12	90% CI	Clinically significant cutoffs
Hemoglobin concentration (g/dL)	40	10.8	-0.1	-0.3, 0.1	-1, 1
Platelet count ($\times 10^9/L$)	40	162	7.0%	0.5%, 13.5%	-20%, 20%
Normalized liver volume (% of body weight)	40	1.9	0.0%	-2.6%, 2.6%	-15%, 15%
Normalized spleen volume (% of body weight)	36	0.5	-5.6%	-10.8%, -0.4%	-15%, 15%

Velaglucerase alfa was generally well tolerated, with most adverse events (AEs) of mild or moderate severity (Table 21). The most frequently reported AEs were nasopharyngitis (8/40 patients), arthralgia (9/40 patients), and headache (12/40 patients). Overall, 11 of 40 patients (28%) experienced an AE considered possibly or probably related to study drug; the majority of these events were considered infusion related. No patient experienced a life-threatening AE. One severe adverse event was considered probably related to treatment and occurred in a patient who had a severe hypersensitivity reaction. This patient tested negative for all 4 isotypes (IgE, IgM, IgG, IgA), including neutralizing antibodies, both at the time of the infusion and 2 weeks later. One patient (in the 15 U/kg group) experienced an anaphylactoid reaction that led to discontinuation; no other patients discontinued due to AEs. No patients developed IgG antibodies to velaglucerase alfa, including three patients who tested positive for anti-imiglucerase alfa antibodies at screening.

Table 21 TKT034 Safety summary

	Patients, n (%)				
	Total (n=40)	15 U/kg (n=15)	30 U/kg (n=12)	45 U/kg (n=6)	60 U/kg (n=7)
Experienced ≥ 1 treatment-emergent AE*	34 (85)	12 (80)	11 (92)	5 (83)	6 (86)
Experienced ≥ 1 drug-related AE	11 (28)	6 (40)	3 (25)	1 (17)	1 (14)
Experienced ≥ 1 infusion-related AE [†]	9 (23)	6 (40)	2 (17)	0	1 (14)
Experienced ≥ 1 severe AE	5 (13)	0	2 (17)	1 (17)	2 (29)
Experienced ≥ 1 life-threatening AE	0	0	0	0	0
Experienced ≥ 1 serious AE	4 (10)	1 (7)	1 (8)	2 (33)	0
Discontinued due to an AE	1 (3)	1 (7)	0	0	0
Deaths	0	0	0	0	0
Developed anti-velaglucerase alfa antibodies	0	0	0	0	0

*A treatment-emergent AEs was defined AEs that occurred on or after the day of the first infusion until 30 days after the patient's last infusion.

[†]An infusion-related AE was defined as an AE that 1) began either during or within 12 hours after the start of the infusion, and 2) was judged as possibly or probably related to study drug.

The first three infusions for each patient were administered at the clinical site, after which patients who had not experienced a drug-related serious AE or an infusion-related AE were eligible to receive subsequent infusions at home. During the study, 25 (63%) of 40 eligible patients received home therapy at least once, ten patients (67%) in the 15 U/kg group, six (50%) in the 30 U/kg group, five (83%) in the 45 U/kg group, and four (57%) in the 60 U/kg group.

For hemoglobin concentration, the mean change from Baseline was -0.1 g/dL, with a 90% confidence interval of -0.3 to 0.1 g/dL, within the predefined efficacy criterion of ± 1 g/dL. For platelet counts, the percent change from Baseline was +7.0%, with a 90% confidence interval of 0.5 to 13.5%, within the predefined efficacy criterion of $\pm 20\%$. For liver volume, the percent change from Baseline was -0.0%, with a 90%

confidence interval of -2.6 to 2.6% within the predefined efficacy criterion of $\pm 15\%$. For spleen volume, the percent change from Baseline was -5.6%, with a 90% confidence interval of -10.8 to -0.4% within the predefined efficacy criterion of $\pm 15\%$. Hemoglobin concentration, platelet counts, and liver and spleen volume were sustained at therapeutic levels through 1 year of velaglucerase alfa treatment, as demonstrated by pre-specified efficacy criteria for clinically significant change. Mean change in hemoglobin and mean percent change in platelet count and organ volumes are shown in **FIGS. 15-18**. For each parameter, similar results were seen across the four dose groups.

The percent change in plasma chitotriosidase and plasma CCL18 are shown in **FIGS. 19 and 20**. Levels of both biomarkers were sustained and possibly reduced over the 12-month treatment period.

Example 5: HGT-GCB-058 Study

Summary

HGT-GCB-058 is a multicenter, open-label treatment study to observe the safety of velaglucerase alfa in patients with Gaucher disease type 1 who were newly diagnosed (treatment naïve) or transitioned from imiglucerase to velaglucerase alfa. The study design was for male or female patients two years old or older. Velaglucerase was administered at a dose of 15-60 U/kg every other week (EOW) by 1-hour intravenous (IV) infusion- Patients received the same number of units of velaglucerase alfa as their prior imiglucerase dose (patients receiving <15 U/kg imiglucerase EOW received 15 U/kg velaglucerase alfa). The rate of infusion was a maximum of 1 U/kg/minute.

Patients and methods

HGT-GCB-058 was initiated to provide an alternative treatment option for patients who would otherwise have limited or no access to imiglucerase due to supply constraints. The primary endpoint was to observe the safety of velaglucerase alfa.

For HGT-GCB-058, within 3 months of the first site initiated, 20 clinical sites across the US were enrolling patients. Between September 1, 2009 and January 31, 2010 more than 150 patients enrolled onto HGT-GCB-058 and received at least one infusion of

velaglucerase alfa. Only 3 patients were treatment naïve; all others were previously treated with imiglucerase.

Preliminary safety results

5 Discontinued: <10%; Withdrawal of consent & other (<10%); AE experience including SAE (<2%).

Treatment emergent adverse events (TE-AE):

10 Treatment naïve patients (n=3): No serious AEs; no severe AEs; two patients experienced moderate AEs: headache (moderate) and back pain (moderate) (infusion related – possibly related)

15 Previously treated with imiglucerase (n>150): 35.8% at least one TE-AE; 18.2% at least one possibly/probably related TE-AE; 13.8% at least one infusion related reaction; <1% - serious AE (a 69-year-old female experienced a severe cerebrovascular accident requiring hospitalization) not related; 3.1% - at least one severe AE: arthralgia (not related), fatigue (probably related), bone pain (not related), pain in extremity (not related), leucopenia (possibly related), cerebrovascular accident (not related).

Example 6: Comparative Study

20 The objective of this example is to compare the efficacy of Ceredase®, Cerezyme®, velaglucerase alfa, Genz-112638, and Zavesca® in treating type 1 Gaucher disease. Hemoglobin concentration, platelet count, liver volume, and spleen volumes were measured after 6, 9, or 12 months of treatment.

Doses: Enzyme replacement therapy (ERT): 60U/kg EOW; Genz112638: 50 & 100 mg BID; Zavesca: 100 mg TID

25 **Ratio of Males to Females:** Cerezyme (8 to 7); Ceredase (3 to 2); Velaglucerase Ph I/II TKT025 study (5 to 7); Velaglucerase-TKT032 study-45U/kg dose (8 to 5); Velaglucerase-TKT032 study-60U/kg dose (7 to 5); Zavesca (1 to 1); Genz-112638 (3 to 4)

Inclusion criteria:

30 Cerezyme & Ceredase: anemia & splenomegaly

 Velaglucerase TKT025: anemia and thrombocytopenia

Genz-112638: anemia, thromb, & splenomegaly

Zavesca dose: organomegaly & <100/nL Pl or <11.5 Hb

Velaglucerase HGT-GCB-039 and TKT032: anemia and 1 other parameter manifestation

5 Baseline comparison in naïve patients is shown in **Table 22**.

Table 22

	Hemoglobin (g/dL)	Platelets (cells / nL)	Liver Vol. MN (L)	Spleen Vol. MN (L)
Ceredase®	10.8	71	1.83	24
Cerezyme®	10.7	72	1.65	19
Velaglucerase (Ph I/II TKT025)	11.6	57	1.95 (2.4L)	19 (2.1L)
Velaglucerase (TKT032- 45U/kg dose)	10.9	58	3.50 (% of body weight)	2.90 (% of body weight)
Velaglucerase (TKT032) 60U/kg dose	10.83	66	3.60 (% of body weight)	2.90 (% of body weight)
Genz-112638	11	70	1.7	19
Zavesca®	11.9	77	(2.4L)	(1.6L)

MN=Multiple of Normal

10 Comparative results from patients treated with Ceredase®, Cerezyme®, velaglucerase alfa, or Genz-112638 for six months are shown in **Table 23**.

Table 23

	Mean Spleen Volume Reduction (%)	Mean Liver Volume Reduction (%)	Mean Platelet Increase (%) [actual]	Mean Hemoglobin Increase (g/dL)
Ceredase®¹ 6mo	32.1 ± 8.8	11.4 ± 9.4	34 [16 /nL]	1.60
Cerezyme®¹ 6mo	37.3 ± 13.6	13.4 ± 13.1	22 [23 /nL]	1.82
velaglucerase² 6mo (n=11)	41.3 ± 9.8	14.7 ± 12.0	38 [23.4 ± 24.6 /nL]	1.92 ± 0.82
Genz-112638³ 6mo (n=17-21)	27	7	18 [12.5 /nL ⁴]	0.9

1 Cerezyme and Ceredase data as reported by Grabowski *et al.* (1995) *Ann. Intern. Med.* **122**, 33-39.

-Mean increase in platelet count in cells/nL for Cerezyme and Ceredase are from the Cerezyme SBA.

2 velaglucerase alfa Phase I/II results

3 Oppenheimer Analyst Report: May 8, 2008 from Genzyme Analyst Day 2008

4 Calculated by multiplying baseline by percent change

ERT administered at 60U/kg EOW, Genz112638 administered at 50 & 100mg

BID

Comparative results from patients treated with Ceredase®, Cerezyme®, velaglucerase alfa, or Genz-112638 for nine and twelve months are shown in **Table 24**.

Table 24

	Mean Spleen Volume Reduction (%)	Mean Liver Volume Reduction (%)	Mean Platelet Increase (%) [actual]	Mean Hemoglobin Increase (g/dL)
Ceredase¹ 9mo (n=15)	42.2 ± 6.9	16.4 ± 8.4	53.2 [25.1 /nL]	2.28

Cerezyme¹ 9mo (n=15)	47.1 ± 13.7	21.4 ± 10.8	43.5 [30.9 /nL]	2.54
velaglucerase² 9mo (n=11)	49.5 ± 12.6	18.2 ± 8.2	67.6 [40.6 ± 30.7 /nL]	2.24 ± 0.89
Velaglucerase³ 1yr (n=10)	not available	not available	95.3 ± 21.2 [54.9 ± 11.1]	2.35 ± 0.3
Velaglucerase⁴ 1yr (n=12)	50.4 ± 5.3	17.0 ± 4.5	65.9 ± 16.9 [50.88 ± 12.2]	2.43 ± 0.3
Velaglucerase⁵ 1yr (n=10)			40.92	2.44
Genz-1126383⁵ 1yr (n=11-13)	40	17	40 [26 /nL4]	1.6

1 Cerezyme and Ceredase 9-Mo data as reported by Grabowski *et al.* (1995) *Ann. Intern. Med.* **122**, 33-39. Dose was 60U/kg every other week (EOW).

-Mean increase in platelet count in cells/nL calculated from raw data

2 velaglucerase alfa 9-Mo data from TKT025 (Ph I/II) – 60U/kg dose EOW

5 3 velaglucerase alfa 12-Mo data from TKT025Extension – 60U/kg dose EOW

4 velaglucerase alfa 12 Mo data from TKT032 – 60U/kg dose dose EOW

5 velaglucerase afa 12 Mo data from TKT032 – 45U/kg dose EOW

3 WORLD meeting presentation, Feb. 20, 2009 – administered at 50 & 100 mg
 BID

10 **Conclusions:** this study shows that velaglucerase alfa was at least as effective as Ceredase®, Cerezyme®, Genz-112638, and Zavesca® in treating type 1 Gaucher disease based on the measurement of hemoglobin concentration, platelet count, liver volume, and spleen volume, 6, 9, or 12 months after the treatment was initiated.

15 Example 7: Anti-Drug Antibody (ADA) Assays

Summary

Development of antibodies to therapeutic proteins can impact patient safety, efficacy and drug pharmacokinetics. A panel of anti-drug antibody (ADA) and

neutralizing antibody (NAb) assays were developed and validated in order to evaluate and compare antibody response in patients receiving velaglucerase alfa or imiglucerase in one of three velaglucerase alfa Phase III studies.

Assessment of potential immunogenicity of a biological therapy such as enzyme replacement therapy (ERT) was performed by the following steps:

1. Screen for antibody to the ERT
 - i. Allow for false positives
 - ii. Broad specificity for all isotypes
 - iii. Tolerant to presence of drug
2. Confirmatory step
 - i. Rule out false positives
 - ii. Isotype specific
3. Titer step
 - i. Relative concentration
4. Test for neutralizing antibody
 - i. In vitro activity
 - ii. In vitro cellular uptake

This assessment can be performed for any ERT.

An example of such an assessment is shown in **FIG. 21**.

Immunoassay methods

Anti-velaglucerase alfa and anti-imiglucerase antibodies were evaluated identically using bridge immunoassays and immunoglobulin (Ig) subclass-specific indirect immunoassays, all based on an electrochemiluminescent platform, as well as RIP assays. The bridge electrochemiluminescent immunoassay detected all immunoglobulin subclasses and was considered the antibody screening assay. The Ig subclass electrochemiluminescent immunoassays were confirmatory assays for the presence of IgA, IgM and IgE antibodies, while the RIP assay was confirmatory for the presence of IgG antibodies. The antibody screening assays and IgG assays were calibrated, quantitative and utilized human antibody positive controls. The IgA, IgM and IgE assays were semi-quantitative and utilized hybrid (human-sheep) positive controls.

All anti-velaglycerase alfa and anti-imiglycerase immunoassays were identical, including positive cut off criteria, except that either velaglycerase alfa or imiglycerase were used to interrogate the sample. These assays are high throughput, provide increased surface area for detection, allow use of high serum sample concentration with minimum non-specific binding and detect all antibody subclasses.

Antibody screening assays

As shown in **FIG. 22**, anti-drug antibody screening can be performed using an electrochemiluminescence (ECL) immunoassay.

Anti-velaglycerase alfa (anti-imiglycerase) antibodies were detected using immobilized biotin-conjugated velaglycerase alfa (or imiglycerase) on streptavidin-coated microwell plates. Immobilized biotinylated velaglycerase alfa (or imiglycerase) captured anti-velaglycerase alfa (anti-imiglycerase) antibodies present in patients' sera and unbound proteins were removed by washing. Ruthenium complex-labeled velaglycerase alfa (or imiglycerase) was added to each microwell resulting in the formation of a complex with the bound anti-velaglycerase alfa (anti-imiglycerase) antibodies. This was followed by a second wash step, in which unbound labeled proteins were removed.

Labeled molecules bound near the microwell surface emit light in a process triggered by the electrochemiluminescent reaction, measured by an MSD SECTOR Imager 2400 instrument (http://www.mesoscale.com/CatalogSystemWeb/WebRoot/products/imager_2400.aspx).

A mouse monoclonal antibody with cross-reactivity to velaglycerase alfa and imiglycerase was used as a calibrator within each assay plate and a human anti-imiglycerase antibody cross-reactive with velaglycerase alfa was use as positive assay control. The concentration of anti-velaglycerase alfa (anti-imiglycerase) antibodies in test samples was estimated by interpolating the unknown's measured electrochemiluminescent signal on the calibration curve.

A minimum of 67 Gaucher patient baselines were tested in order to set the antibody positive cut points for these assays. The test design included at least three analysts testing replicate samples using different plate lot numbers over a period of at

least 14 days. At least three different microwell plate lots were used. Two available MSD instruments were used randomly for a total of 1269 determinations for each assay. The assay cut point for anti-velaglucerase alfa (anti-imiglucerase) antibodies was established as the mean plus 1.645 standard deviation of electrochemiluminescent values obtained as recommended in Mire-Sluis, AR *et al. Journal of Immunological Methods* 289 (2004), pp 1-16. The assay sensitivity was estimated to be 33.4 and 65.6 ng/mL for anti-velaglucerase alfa and anti-imiglucerase antibodies, respectively.

The screening characteristics for this assay (using velaglucerase as the drug) are shown in **Table 25**.

The specification for each parameter shows highly sensitive and reproducible ADA screening assays were validated for evaluating antibody response in patients receiving velaglucerase alfa or imiglucerase.

Table 25

Parameter	Specification
Imprecision, % RSD	
Intra assay	≤6.2
Inter assay	≤8.5
Accuracy, %	93.5 – 107.7
Sensitivity, 1 ng/mL	100
LOD, 2 ng/mL	5
LOQ, ng/mL	15
Positive controls	Human & sheep ADA
Calibrator	Mouse monoclonal ADA

ADA = anti-drug antibody; LOD = limits of detection; LOQ = limit of quantification; RSD = relative standard deviation

The screening assay dose response curve for velaglucerase is shown in **FIG. 23**.

An example of the affinity and binding kinetics of a monoclonal ADA on the BIACORE® platform are shown in **Table 26**. Similar ligand affinity and binding kinetics were observed for the ADA assay calibrator.

5

Table 26

Ligand	ka (1/Ms)	kd (1/s)	KD (M)
velagucerase alfa	9.2E+05	2.7E-04	3.0E-10
imiglucerase	2.8E+06	1.0E-04	3.7E-10
Biotinylated velaglucerase alfa	4.0E+05	2.2E-04	5.4E-10
Biotinylated imiglucerase	2.0E+06	9.5E-04	4.8E-10

Radioimmunoprecipitation assay

If anti-drug antibody is detected in a sample, confirmatory assays to determine the Ig isotype of the antibody can be performed. Immunoglobulin G (IgG) antibodies were detected using radioimmunoprecipitation. A radioimmunoprecipitation (RIP) assay is shown in **FIG. 24**.

In the radioimmunoprecipitation assay, anti-velaglucerase alfa (anti-imiglucerase) IgG antibodies present in patient serum bound ¹²⁵I-velaglucerase alfa (or imiglucerase) in solution phase and formed antigen/antibody complexes that were captured using Protein G mini-columns. The mini-columns were washed to remove free label and quantified directly in a gamma counter. The radioactive counts retained in the mini-column were proportional to the concentration of anti-velaglucerase alfa (anti-imiglucerase) IgG antibodies in the test sample. The concentration of anti-velaglucerase alfa (anti-imiglucerase) IgG antibodies in test samples was estimated from a calibration curve using

the same monoclonal antibody calibrator discussed above. The same human antibody positive control described above was used in this assay.

The least squares line fit to the high purity, monoclonal antibody based calibration curve, using well characterized known concentrations of antibody, provided a reliable and consistent method for calculating uncertainty in assay determinations. This tool allows for normalization of the cut point for inter-assay changes in counts that may occur from reagent radiolabel decay, radioautolysis and/or assay handling variability as well as allowing for changes in non specific binding and for changing assay readouts that may occur over time.

A total of 59 Gaucher patient baselines were tested in order to set the antibody positive cut point for this assay. The assay cut points for anti-velaglycerase alfa (imiglucerase) IgG were established as described above and recommended in Mire-Sluis et al². Assay sensitivity was estimated to be 28.3 and 64.5 ng/mL for the anti-velaglycerase alfa IgG and anti-imiglucerase IgG assays, respectively.

An example of the results obtained with such an assay using velaglycerase as the drug is shown in **Table 27**.

Highly sensitive and reproducible IgG ADA confirmatory assays were validated for evaluating antibody response in patients receiving velaglycerase alfa or imiglucerase.

Table 27

Parameter	Specification
Imprecision, % RSD	
Intra assay	≤8.7
Inter assay	≤12.0
Accuracy, %	90.5 – 132.8
Sensitivity, 1 ng/mL	80
LOD, 2 ng/mL	4
LOQ, ng/mL	13
Positive controls	Human & sheep ADA
Calibrator	Mouse monoclonal ADA

ADA = anti-drug antibody; LOD = limits of detection; LOQ = limit of quantification; RSD = relative standard deviation

An example of a dose response curve obtained for a RIP assay using velaglucerase is shown in **FIG. 25**.

Indirect electrochemiluminescent immunoassays

Parallel to screening for IgG antibodies, assays are performed to screen for the presence of IgE antibodies. Assays can also be performed to detect the presence of IgA and IgM antibodies.

IgA, IgM, and IgE ADA were detected using ECL assays. An example of such an assay is shown in **FIG. 26**.

Anti-velaglucerase alfa (imiglucerase) antibodies were analyzed for their Ig subclass using indirect electrochemiluminescent immunoassays. Antibodies were detected in serum by immobilizing biotinylated velaglucerase alfa (imiglucerase) on streptavidin-coated microwell plates. Diluted serum samples were added to the immobilized velaglucerase alfa (imiglucerase), which captured any anti-velaglucerase alfa (anti-imiglucerase) antibodies present in the sample. After sample incubation, the microwells were washed to remove unbound proteins. Next, ruthenium complex-labeled anti-human secondary antibodies against IgA, IgM or IgE were added separately and further incubated resulting in the formation of an Ig class-specific complex with any bound anti-velaglucerase alfa or imiglucerase antibodies. This was followed by another wash step, in which unbound labeled secondary antibody was removed. The labeled molecules bound near the microwell surface then emit light as described above.

Artificial antibody positive controls were prepared for these assays since anti-velaglucerase alfa (anti-imiglucerase) IgA, IgM or IgE antibodies were not available. Human IgA-, IgM- and IgE-antibody hybrids were synthesized by chemically cross-linking purified, non-specific human IgA, IgM or IgE fragments to an antibody raised in sheep hyperimmunized with velaglucerase alfa and cross-reactive with imiglucerase. The IgA-, IgM- and IgE-antibody hybrids therefore bound to velaglucerase alfa (or imiglucerase) through the sheep antibody domain, and were detected using ruthenium

complex-tagged anti-human secondary antibodies against the human IgA, IgM or IgE domains, respectively.

The assay cut points for anti-velaglucerase alfa (imiglucerase) IgA, IgM and IgE were established as described above and recommended in Mire-Sluis et al². Assay sensitivity was estimated to be 10.6 and 11.0 ng/mL for the anti-velaglucerase alfa IgE and anti-imiglucerase IgE assays, respectively. For anti-velaglucerase alfa (imiglucerase) IgA and IgM antibodies, the assay positive cut points had to be met in addition to a ratio greater than or equal to 2.0 of the time point signal to the pre-infusion baseline signal³.

For these assays, hybrid positive controls can be used. For example:

- Sheep ADA IgG, and human IgA, IgM and IgE are treated to yield pyridylthiol-activated proteins (see Gu M. L., Feng S. L., and Glenn J. K. Development of an animal-human antibody complex for use as a control in ELISA. *J. Pharmaceutical and Biomedical Analysis*, 32 (2003), 523-529)

- Activated IgA, IgM and IgE are reduced and desalted
- Activated IgG is mixed with reduced IgA, IgM or IgE
- The formed hybrid antibodies were characterized by size exclusion

chromatography and ECL ELISA

- Human/sheep molar ratios of 5 for IgA and IgM, and 2 for IgE were obtained

An example of IgA, IgM and IgE confirmatory assay characteristics (when velaglucerase was used as the drug) are shown in **Table 28**.

Highly sensitive and reproducible IgA, IgM, and IgE ADA isotyping assays were validated for evaluating antibody response in patients receiving velaglucerase alfa or imiglucerase.

Table 28

Parameter ⁴	IgA Assay Specification	IgM Assay Specification	IgE Assay Specification
Imprecision, % RSD			
Intra assay	≤3.1	≤6.2	≤6.6
Inter assay	≤4.8	≤3.7	≤13.3

Specificity	α -chain Fc	μ -chain Fc	ϵ -chain Fc
Linearity, ¹ ng/mL	156 – 2000	156 – 2500	156 – 10000
LOD, ² ECL counts	260	460	240
Positive controls ³	Hybrid ADA	Hybrid ADA	Hybrid ADA

¹based on hybrid control response; ²positive cut point; ratio of time point to baseline must be ≥ 2.0 ; ³human/sheep ADA hybrid controls; ⁴highly sensitive and reproducible IgA, IgM and IgE ADA isotyping assays were validated for evaluating antibody response in patients receiving velaglucerase alfa or imiglucerase

ADA = anti-drug antibody; LOD = limits of detection; LOQ = limit of quantification; RSD = relative standard deviation

Antibody inhibition of *in vitro* enzymatic activity

Inhibition of *in vitro* enzymatic activity by anti-velaglucerase alfa (imiglucerase) antibodies was tested using an assay that detects and quantifies antibodies that inhibit velaglucerase alfa (imiglucerase) activity. The method is based on a colorimetric activity assay that measures the ability of velaglucerase alfa (imiglucerase) to hydrolyze the synthetic substrate 4-nitrophenyl- β -D-glucopyranoside to p-nitrophenol and D-glucopyranoside.

Anti-velaglucerase alfa (imiglucerase) antibody positive serum samples were pre-incubated with a fixed amount of velaglucerase alfa or imiglucerase, respectively for 30 minutes at 37°C. Sheep polyclonal antibodies known to inhibit velaglucerase alfa and imiglucerase *in vitro* activity were used as positive controls. The 4-nitrophenyl- β -D-glucopyranoside substrate solution was then added and incubated with the serum sample/enzyme mixture for one hour at 37°C. The enzymatic reaction was stopped by addition of glycine/sodium carbonate buffer (pH 10.7) and the product (p-nitrophenol) was measured at the absorbance wavelength of 405 nm. One unit of velaglucerase alfa (imiglucerase) activity was defined as the amount of enzyme required to hydrolyze one μ mole of the substrate 4-nitrophenyl- β -D-glucopyranoside in one minute at 37°C.

Enzymatic activity was quantified by comparison of the released p-nitrophenol in test samples and assay controls to a p-nitrophenol calibration curve measured in the same assay plate. Results of the test samples were expressed relative to the activity of velaglucerase alfa (imiglucerase) measured in the absence of serum sample and reported as % inhibition.

The assay cut point was determined from individual healthy human donor sera (N = 52) and enzyme replacement therapy-naïve Gaucher patients (N = 35). The cut point for the velaglucerase alfa and imiglucerase neutralizing antibody assays was defined as inhibition >20.0% based on these 87 samples. Therefore, a patient sample was considered to be negative for inhibitory antibodies if the level of inhibition observed was ≤20.0% and to be positive if inhibition >20.0%.

In vitro cell-based assay

An in vitro cell-based assay was used to assess anti-drug antibodies to determine if the antibodies were neutralizing.

Table 29 shows the specification of assay characteristics for neutralizing levels of anti-drug antibody (velaglucerase was used as the drug).

Table 29

Parameter	Specification
Imprecision, % RSD	
Intra assay	≤12.9
Inter assay	≤6.1
Accuracy, %	86.7 – 92.7
Linearity, mU/mL	0.3 – 33.3
Positive cut point, ¹ % inhibition	>20
LOD, mU/mL	0.3
LOQ, mU/mL	1.0

Positive controls	Human & sheep ADA

¹n=104 NHS and 70 ERT-naïve Gaucher sera

LOD = limits of detection; LOQ = limit of quantification; RSD = relative standard deviation

5

With respect to neutralizing antibodies in ERT such as ERT for Gaucher disease, points to consider include:

- Receptor-mediated cell uptake is critical for in vivo function of therapeutic agent
- Receptor binding interference by ADA can hinder enzyme trafficking
- 10 • Inhibition of cell uptake is an important tool to evaluate successful ERT since
 - It closely mimics the mechanism by which NAb may exert an effect in vivo, and
 - NAb could reduce or abolish the biological activity of the therapeutic agent
- 15 • Cell-based assays provide the most appropriate biological model for assessment of NAb

Samples from patients undergoing ERT in clinical trials for Gaucher disease were evaluated for neutralizing antibodies to velaglucerase or imiglucerase. Results are shown in Table 30.

20

Table 30

Patient ID	Treatment	Inhibition of imiglucerase uptake, % mean				Inhibition of velaglucerase alfa uptake, % mean			
		Visit Week				Visit Week			
		0	13	19	41	0	13	19	41
032-191-0002	velaglucerase	NS ²				NS			
039-009-0001	imiglucerase								
039-194-0001	imiglucerase								
039-194-0002	imiglucerase								
039-167-0001	imiglucerase	NT ³	38	38	33	NT	4	5	5
034-027-0002	imiglucerase ¹	NS	NT ³			NS	NT		

034-154-0001	imiglucerase ¹	5		10	
034-164-0001	imiglucerase ¹	38		10	
A marked cell-based inhibition of imiglucerase uptake was observed in two ADA-positive patients					
Cell-based inhibition of velaglucerase alfa uptake was either negligible or not observed in any ADA-positive patient tested					

¹previously receiving imiglucerase treatment and switched to velaglucerase alfa at trial inception; ²not significant (less than the limit of quantification); ³not tested as yet; time point negative for anti-velaglucerase alfa antibodies

5

In vitro cell-based assay I

Major objectives: Experiments were conducted to determine the ability of human antibodies reactive with velaglucerase alfa and/or imiglucerase to inhibit (block or neutralize) CD206-mediated uptake of the recombinant enzymes by a human cell line (HT1080) engineered to express CD206, and to compare in this respect anti-drug antibodies (ADA) produced in response to imiglucerase to those produced in response to velaglucerase alfa. The hypothesis is that antigenic differences exist between velaglucerase alfa and imiglucerase; these epitopes will differentiate velaglucerase alfa from imiglucerase with respect to the functional effect of ADA on cell binding, cell internalization, and/or intracellular trafficking of the therapeutic agent.

15

Materials and equipment:

Critical Materials

1. Cell line MRC1-18 is derived from the line HT1080 and is stably-transfected with human CD206 (the macrophage mannose receptor; MMR, also referred to as MRC1, mannose receptor C type 1). HT1080(saf) cells were transfected with an expression vector carrying the gene encoding the MMR (that was isolated from a human liver cDNA library) by electroporation and immediately plated into 96-well plates. Stable clones were selected using media containing 0.4 mg/mL G418. MRC1 expression was analyzed using FITC anti-MRC1 staining and analysis by fluorescence shift. Expression of MMR on MRC1-18 was confirmed by surface staining with anti-MMR Ab. Additionally, MRC1-

25

18 has been verified by immunostaining and flow cytometry to be negative for expression of Fc (gamma) receptors.

2. Alexa FLUOR® 488 conjugated velaglucerase alfa and imiglucerase: velaglucerase alfa or imiglucerase was conjugated with Alexa FLUOR® 488 using the
5 Alexa FLUOR® 488 protein labeling kit following the manufacturer's protocol (Molecular Probes, catalog # A 10235).

3. Imiglucerase or velaglucerase alfa ADA-positive patient sera from clinical trials TKT-032, TKT-034, and HGT-GCB-039 (see **Table 31** for sample IDs)

4. Assay Positive Control (PC): 250 µg/mL purified polyclonal sheep anti-
10 velaglucerase alfa antibody (G140) in Normal Human Serum (NHS; BRH127439)

5. Negative control samples: human serum samples from normal healthy donors (Bioreclamation, Catalog #HMSM, BRH127438, BRH127439), or baseline serum samples from patients enrolled in clinical trial TKT-032 (N=25).

6. Mannan: Sigma Catalog # M7054

15 7. D-Mannose-6-phosphate: Sigma Catalog# M3655

8. Growth medium: 50% CD-CHO (Invitrogen catalog # 10743) and 50% CD-293 (Invitrogen catalog# 11913) supplemented with 4 mM L-glutamine (Invitrogen Catalog# 25230) and 0.4 mg/mL Geneticin (G418, Invitrogen Catalog # 11811-031).

9. 0.05% trypsin-EDTA: Invitrogen catalog# 25300

20 10. Wash buffer: PBS/0.5%BSA

11. BD Cytometer setup and tracking beads: BD Bioscience catalog# 641319

Partial List of Equipment

1. 37°C Incubator with 5% CO₂: Forma Scientific Model 3033
- 25 2. Centrifuge: Thermo Scientific Model Sorvall Legend T +
3. Cell counter: Mexcelom Bioscience LLC, Model Cellometer Auto T 4
4. Flow cytometer: BD Bioscience, FACSCanto II

Methods: Patient sera that tested positive for anti-drug antibodies by antibody
30 screening and confirmatory assay were further examined by an *in vitro* cell uptake assay, utilizing an HT1080 cell line engineered to express the human macrophage mannose

receptor (MMR). Briefly, MRC1-18 cells were maintained in CD media supplemented with 0.5 mg/mL G418. For each assay, 1.5×10^5 cells/well in CD media with G418 were added in a flat bottom 96-well plate, and pre-incubated with 1:20 diluted test patient serum samples, 1:20 diluted normal human serum (NHS), or assay positive controls (G140 antibody, 5 mg/mL mannan) in 1:20 diluted NHS at 37°C for 15 minutes. Then 5 nM Alexa FLUOR® 488 labeled velaglucerase alfa or imiglucerase was added, and incubated at 37°C for an additional 2 hours. A calibration curve of Alexa FLUOR® 488 labeled velaglucerase alfa or imiglucerase was included in each experiment by incubation of MRC1-18 cells with Alexa FLUOR® 488 labeled velaglucerase alfa or imiglucerase (0 - 10 nM) in 1:20 diluted NHS at 37°C for 2h.

After a 2-hour incubation, media was removed by centrifugation, cells were treated with trypsin-EDTA for 3 minutes to remove surface-bound velaglucerase alfa or imiglucerase, then neutralized by addition of an equal volume of media with 10% FBS. Cells were washed with PBS/0.5% BSA once, resuspended in PBS/0.5% BSA and analyzed by BD FACS Canto II with the fixed instrument setting. The Canto II instrument was set up with the BD Cytometer setup and tracking beads before each analysis. The results were analyzed with FlowJo software, and the mean fluorescence intensity (MFI) for each sample recorded. The adjusted MFI was calculated by subtraction of the MFI of the background sample (cells with 0 nM drug) from the MFI of each sample. Inhibition of velaglucerase alfa and imiglucerase uptake by patient samples was determined relative to a normal human serum sample (NHS) or the patient's own naive baseline sample, when available. Percent inhibition can be calculated using the following equation:

$$\% \text{ inhibition} = 1 - (\text{Adj. MFI of test sample} / \text{Adj. MFI of patient baseline or NHS}) \times 100$$

Results:

Assay Development:

The following initial results were obtained upon development of this assay (data not shown):

- The internalization of velaglucerase alfa and imiglucerase by the MRC1-18 cell line is dose dependent.

- The internalization of velaglucerase alfa and imiglucerase by the MRC1-18 cell line is mediated by the mannose receptor, since the internalization was inhibited by mannan (5 mg/mL, >89%), but not by M6P (5 mM).

- G140 antibody spiked in 1:20 diluted NHS (normal human serum) inhibits velaglucerase alfa and imiglucerase uptake by the MRC1-18 cell line. The inhibition is dose dependent.

- Twelve lots of NHS tested at 1:20 dilution did not inhibit velaglucerase alfa uptake by the MRC1- 18 cell line.

Assay Variability:

Twenty-five individual naïve Gaucher serum samples from a clinical study were tested over three days (N = 75) to establish the baseline effect on the uptake of velaglucerase alfa or imiglucerase into the MRC1-18 cell line.

The average baseline serum effect on the uptake of velaglucerase alfa is similar to the baseline serum effect on the uptake of imiglucerase. However, the variability, calculated as the CV%, is much greater for imiglucerase than for velaglucerase alfa (28% vs 15%) (data not shown).

ADA-Positive Patient Serum Samples Results and Reproducibility of the Assay:

The inhibition of velaglucerase alfa and imiglucerase uptake by patient serum samples previously determined to have antibodies against imiglucerase or velaglucerase alfa was tested. Each serum sample was tested in parallel for its ability to block imiglucerase uptake as well as velaglucerase alfa uptake, regardless of which enzyme preparation initially elicited the production of the antibodies. **Table 31** lists the patient serum samples and the protein treatment received (only one antibody positive patient, ENU, received treatment with velaglucerase alfa).

Table 31: ADA-Positive Patient Serum Samples

Patient	Patient Initials	Treatment Received
032-191-0002	ENU	Velaglucerase alfa
039-009-0001	RW	imiglucerase

039-194-0001	GAJ	imiglucerase
039-194-0002	AVL	imiglucerase
039-167-0001	KM	imiglucerase
034-027-0002	SB	imiglucerase
034-154-0001	JMS	imiglucerase
034-164-0001	MPQ	imiglucerase

Samples from patients ENU, RW, GAJ, AVL, and SB showed no inhibition of either imiglucerase or velaglucerase alfa uptake (data not shown). The reproducibility of inhibition of velaglucerase alfa and imiglucerase uptake by patient samples JMS, KM, MPQ, and SB (as a negative control) was determined over three days relative to a normal human serum sample or the patient's own naïve baseline sample, when available (**Table 32**).

Patient KM (039-167-0001) was naïve at baseline and was then treated with imiglucerase in a subsequent study. Samples from all three visits showed a significant inhibition of imiglucerase uptake (24 - 52% inhibition relative to its baseline), relative to the equivalent inhibition of velaglucerase alfa uptake (0 - 15%, which is within the range of variability of the assay).

Patients SB (034-027-0002), JMS (034-154-0001), and MPQ (034-164-0001) were previously treated with imiglucerase. Serum from patient SB showed no inhibition of either imiglucerase uptake (0%) or of velaglucerase alfa uptake (0%) relative to a normal human serum sample. The inhibition of imiglucerase uptake (0 - 14%) by serum from patient JMS was consistent with the inhibition observed of velaglucerase alfa uptake (0 - 20%). The inhibition of imiglucerase uptake (32 - 45%) by serum from patient MPQ was significantly greater than the equivalent inhibition of velaglucerase alfa uptake (0 - 15%, which is within the range of variability of the assay).

These preliminary results suggest that neutralizing antibodies present in the serum from patients KM and MPQ inhibit imiglucerase uptake but not velaglucerase alfa uptake into cells.

Table 32: Inhibition of Imiglucerase or Velaglucerase alfa Uptake by ADA-Positive Patient Samples

Patient #	Initials	Visit	Treatment	Inhibition of imiglucerase Uptake				Inhibition of Velaglucerase alfa Uptake
				Day 1	Day 2	Day 3	Mean	
039-167-0001	KM	Week 13	naive at baseline, then treated with imiglucerase	38%	24%	52%	38%	0-8%
		Week 19		29%	45%	40%	38%	
		Early termination		28%	38%	33%	33%	
034-027-0002	SB	Baseline visit	Previously treated with imiglucerase	0%	0%	0%	0%	0-14%
034-154-0001	JMS	Baseline visit	Previously treated with imiglucerase	14%	0%	2%	5%	0-20%
034-164-0001	MPQ	Baseline visit	Previously treated with imiglucerase	45%	32%	40%	38%	0-15%

5 Inhibition of uptake of imiglucerase or velaglucerase alfa by the mannose inhibitor mannan and by polyclonal sheep anti-velaglucerase alfa antibody G140 (positive control) was also determined over three days (**Table 33**). Mannan inhibits the uptake of both imiglucerase and velaglucerase alfa into MRC1-18 cells through the mannose receptor. The G140 antibody inhibits the uptake of both imiglucerase and
 10 velaglucerase alfa into MRC1-18 cells by an equivalent amount.

Table 33: Inhibition of Imiglucerase or Velaglucerase alfa Uptake by Positive Controls

		Inhibition of Imiglucerase Uptake		Inhibition of Velaglucerase alfa Uptake	
		Mean	CV%	Mean	CV%
G140	5 µg/mL	52%	37%	42%	14%

G140	10 µg/mL	78%	11%	71%	7%
Mannan	5 mg/mL	92%	3%	91%	3%

Conclusions: Velaglucerase alfa and imiglucerase differ structurally, with regard to both protein sequence (e.g., R495H mutation in imiglucerase) and carbohydrate structure. Experiments were performed to address the hypotheses that antigenic differences exist between velaglucerase alfa and imiglucerase, and that these epitopes differentiate velaglucerase alfa from imiglucerase with respect to the ability of anti-drug antibodies (ADA) to block cell binding and/or cell internalization of each therapeutic. Experiments were conducted using the recently developed HT1080 cell line in which uptake of therapeutic is facilitated principally by the MMR, and is minimally confounded by other known cell uptake mechanisms. The inhibition of imiglucerase or velaglucerase alfa uptake by patient serum samples was measured relative to each other. The data show that out of a total of 7 serum samples taken from patients that produced ADA in response to imiglucerase treatment, and 1 serum sample taken from a patient that produced ADA in response to velaglucerase alfa treatment, 2 sera (2/7=29%) from patients that produced ADA in response to imiglucerase caused 24% to 52% inhibition of cell uptake of imiglucerase, but only 0-15% inhibition of cell uptake of velaglucerase alfa which is within assay variability. In comparison, only one patient has been identified to date that produced antibody in response velaglucerase alfa treatment; this serum did not inhibit uptake.

Each serum sample was tested in parallel for its ability to block imiglucerase uptake and/or velaglucerase alfa uptake, regardless of which enzyme preparation initially elicited the production of the antibodies. Such comparisons of the inhibition of velaglucerase alfa uptake, versus inhibition of imiglucerase uptake, allow for crude "mapping" of epitopes to biologically-relevant portions of each therapeutic (with the caveat that the antisera tested herein are polyclonal, and thus each antiserum likely contains a mixture of antigen specificities, perhaps recognizing multiple epitopes). With these precautions in mind, the inhibition of uptake observed with anti-imiglucerase antisera seems to be restricted to imiglucerase since the same antisera do not inhibit internalization of velaglucerase alfa tested in parallel. These data suggest that some ADA

produced in response to imiglucerase inhibit cellular internalization *via* epitopes that are uniquely exposed in imiglucerase. Furthermore, the difference in variability observed of the serum effect on imiglucerase vs. velaglucerase alfa uptake can perhaps be due to a greater homogeneity in the velaglucerase alfa preparation than in the imiglucerase preparation in terms of post translational modifications.

Inhibition of enzyme uptake by anti-velaglucerase alfa (imiglucerase) antibodies was tested using a cell-based assay that detects and quantifies antibodies that interfere with macrophage mannose receptor (MMR)-mediated velaglucerase alfa (imiglucerase) uptake. The method is based on quantification of fluorescently-labeled velaglucerase alfa (imiglucerase) which is internalized by MRC1-18 cells under defined conditions. MRC1-18 is a cell line engineered at Shire HGT from HT1080 cells that were stably-transfected with the human macrophage mannose receptor C, type 1.

Briefly, anti-velaglucerase alfa (imiglucerase) antibody positive serum samples were pre-incubated with MRC1-18 cells in culture media in flat-bottom, 96-well plates at 37°C for 15 minutes. Pooled normal human serum (NHS) was used as negative control. Sheep polyclonal antibodies known to inhibit velaglucerase alfa and imiglucerase uptake as well as mannan, the MMR-specific ligand (Sung SJ *et al. J. Cell Biol.* 1983; 96:160–166) were used as positive controls to block enzyme uptake.

Following preincubation, Alexa Fluor-488-labeled velaglucerase alfa or imiglucerase was added, and incubated at 37°C for an additional 2 hours. A calibration curve consisting of incubation of Alexa Fluor-488-labeled enzyme with MRC1-18 cells was included in each experiment. After incubation, media was removed by centrifugation, cells were treated with trypsin-EDTA for 3 minutes to remove surface-bound enzyme, and finally the reaction pH was returned to neutral with by addition of an equal volume of culture media. Cells were washed once and resuspended using phosphate buffered saline containing 0.5% BSA. Cells were analyzed using a Becton Dickinson FACS Canto II instrument calibrated before each analysis using the instrument's cytometer setup and tracking beads.

The results were analyzed with the instrument's FlowJo software, and the mean fluorescence intensity (MFI) for each sample was recorded. The adjusted MFI was calculated by subtracting the background MFI from sample wells containing no enzyme

from each unknown and control sample MFI. Inhibition of enzyme uptake by patient serum samples was estimated relative to the NHS control according to the following equation:

$$\% \text{ Inhibition} = [1 - [\text{Adjusted MFI of test sample} / \text{Adjusted MFI of NHS}]] \times 100$$

The assay cut point was determined by analysis of 25 individual sera collected from treatment-naïve Gaucher patients. Each serum was tested on four separate days for a total of 100 values and the positive cut point was defined as inhibition greater than the mean of these values plus 1.645 standard deviations.

In vitro cell-based assay II

This assay was developed to compare uptake of velaglucerase alfa to imiglucerase.

Methods: For Design of Experiments (DOE) assays, general factorial design was assisted by Statease DESIGN EXPERT™ software. DOE utilized macrophages derived from phorbol myristate acetate (PMA) induced U937 cells, and were conducted in the presence of 5 mM mannose-6-phosphate (M6P). For internalization comparisons, U937-derived macrophages were incubated for 3 hours with GCB at pH 7.5 with 10 mM calcium. Internalized drug was measured by an activity assay with a synthetic substrate (4-MU-glc) that fluoresces upon cleavage.

Results: Comparison in U937 cells of the internalization rates of velaglucerase alfa and imiglucerase showed that velaglucerase alfa is internalized up to 2.5-fold more efficiently than imiglucerase. This differentiation in cellular internalization was also observed using the MMR-expressing murine cell line J774. Under specific assay conditions, the addition of calcium mildly inhibited the cellular uptake of imiglucerase while it enhanced the uptake of velaglucerase alfa. The internalization of both enzymes could be inhibited by addition of mannan to the culture medium, although the inhibition of velaglucerase alfa uptake by J774 cells was more complete than that of imiglucerase. DOE assays revealed that: i) the interaction of calcium with pH greatly impacts uptake;

and ii) bioassay sample comparisons required the presence of calcium, consistent with the known calcium-dependence of the MMR. The presence of mannose-6-phosphate (M6P) in DOE experiments ensured that the M6P receptor on U937 cells did not contribute to the measured internalization.

5 These data suggest that velaglucerase alfa is internalized more efficiently than imiglucerase. While both enzymes are primarily internalized via the MMR, a small portion, greater for imiglucerase than for velaglucerase alfa, is internalized by an alternative mechanism. These data may prove valuable in differentiating velaglucerase alfa, imiglucerase, and other future therapies.

10 *Summary*

From these studies, a summary of antibody detection methods is as follows:

- Equivalent assays validated for velaglucerase alfa and imiglucerase ADA as per ICH and FDA guidelines. See, e.g.,
15 <http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM128049.pdf>
 and <http://www.ich.org/LOB/media/MEDIA417.pdf>
 - Screening
 - Confirmatory
 - Titer
 - 20 • Isotype
 - Neutralizing
- Masked patient specimens evaluated for velaglucerase alfa and imiglucerase ADA in parallel
- Controls and calibrators covered a broad range of antibody affinities
- 25 • Developed isotype-specific hybrid controls when human positive sera not found

Immunogenicity Status of Patients in Phase III Studies:

- TKT032: patients randomized to velaglucerase alfa 60 U/kg or 45 U/kg EOW
 - One patient ADA-negative at baseline developed NAb in response to
30 velaglucerase alfa

- TKT034: patients previously stable on imiglucerase switched to velaglucerase alfa 15–60 U/kg
 - Three patients anti-imiglucerase positive at baseline, were anti-velaglucerase negative through the 12-month treatment.
- 5 • HGT-GCB-039: patients randomized to velaglucerase alfa 60 U/kg or imiglucerase 60 U/kg EOW
 - Four patients ADA-negative at baseline, seroconverted in response to imiglucerase
 - Of these, one developed NAb reactive against both imiglucerase and velaglucerase alfa, while three developed non-inhibitory IgG ADA
 - 10 reactive against only imiglucerase

The patient seroconversion summary is shown in **Table 34**. Of the 99 patients who were treated, 82 received velaglucerase alfa and 17 received imiglucerase. One of 82 patients receiving velaglucerase alfa and four of 17 patients receiving imiglucerase developed antibodies during the trials.

Table 34

Study	Months	Treatments	U/kg EOW	n	Seroconversion
TKT032					
	12 months	velaglucerase alfa	60	12	0/12 patients
		velaglucerase alfa	45	13	1/13 patients
TKT034					
	12 months	velaglucerase alfa	15–60	40	To date: 0 patients (3 patients imiglucerase antibody-positive at baseline)
HGT-GCB-039					
	9 months	velaglucerase alfa	60	17	0/17 patients
		imiglucerase	60	17	4/17 patients

The clinical implications of the studies discussed in this example are as follows:

- Highly sensitive and equivalent methods were developed, optimized and validated to directly assess and compare patient antibody response to velaglucerase alfa and imiglucerase treatments
- Results show seroconversion in 1% of patients treated with velaglucerase alfa and 23% of patients who were treated with imiglucerase, suggesting significant antigenic differences between velaglucerase alfa and imiglucerase
- In addition, there was marked cell uptake inhibition by ADA of imiglucerase but not velaglucerase alfa

Example 8: Effect of Moisture Content on the Stability of a Lyophilized Velaglucerase Product

Purpose: To assess the effect of moisture content on the stability of a lyophilized velaglucerase product.

Methods: The protein was formulated into a sucrose-containing solution and lyophilized using a FTS lab-scale lyophilizer (Lyostar II). The lyophilized vials were removed at intervals after primary drying with a sample thief to yield samples with varying moisture content. The secondary structure of the lyophilized protein was examined by FT-IR. The thermal stability of these samples was characterized by physical appearance, moisture content, size exclusion and reversed phase HPLC, and oxidation by peptide mapping methods.

Upon reconstitution with Sterile Water for Injection, the velaglucerase product contains approximately 2.5 mg/mL (40 U/mL) of velaglucerase alfa, 50 mg/mL sucrose, 12.9 mg/mL sodium citrate dihydrate, 1.3 mg/mL citric acid monohydrate and 0.11 mg/mL polysorbate 20.

Results: A moisture content range of 1.3% to 6.2% was achieved in the lyophilized product. Cake collapse was observed at accelerated temperature (40°C) in drug product with high moisture content ($\geq 5\%$). However, chemical stability testing results for the reconstituted solution demonstrate that the samples with higher moisture content ($\geq 3\%$) showed significantly less degradation than the drier samples by both SE-

HPLC and RP-HPLC methods, as well as showing lower amounts of oxidation. This stability trend correlates with changes observed in the secondary structure by FT-IR of the dried product.

5 Conclusion: Higher moisture content resulted in better chemical stability of a lyophilized protein. This stability trend is explained by fewer changes to the secondary structure in the solid phase with higher moisture content. A choice of proper moisture content in the lyophilized product needs to balance both structural stability of the cake and chemical stability of the protein.

10 **Example 9: Analysis of Therapeutic Goals for Velaglucerase alfa**

Therapeutic goals have been described to monitor achievement, maintenance and continuity of therapeutic response in patients with type 1 Gaucher disease receiving ERT (Pastores G *et al.*, (2004) *Seminars in Hematology*, 41 (suppl 5): 4–14)

15 To benchmark the impact of velaglucerase alfa treatment against therapeutic goals for clinical parameters of type 1 Gaucher disease (anemia, thrombocytopenia, hepatomegaly, and splenomegaly), the proportion of patients at goal for anemia, thrombocytopenia, hepatomegaly and splenomegaly at baseline was compared with the proportion achieving each of these goals at 9 months or 1 year.

20 No data imputation was utilized. Only patients who have data for each goal at both time points are included. For hematologic parameters, Baseline was the average of the screening and baseline values; 1 year therapeutic goal values were the average of Week 51 and Week 53 values for TKT032 and TKT034, and the average of Week 39 and Week 41 values for HGT-GCB-039. For organ volumes, Week 41 value was applied to 1

25 year therapeutic goal criteria in HGT-GCB-039.

Therapeutic goals for TKT032, HGT-GCB-039, HGT-GCB-039 patients with intact spleen, HGT-GCB-039 splenectomized patients, TKT034, and TKT025 are described in **Tables 35-40**.

Table 35

Therapeutic goals	velaglucerase alfa 60 U/kg N=12	velaglucerase alfa 45 U/kg N=13	velaglucerase alfa Overall N=25

	Baseline	1 Year	Baseline	1 Year	Baseline	1 Year
Hemoglobin concentration	2/12 (17%)	11/12 (92%)	3/13 (23%)	12/13 (92%)	5/25 (20%)	23/25 (92%)
Platelet count	2/11 (18%)	6/11 (55%)	4/13 (31%)	9/13 (69%)	6/24 (25%)	15/24 (63%)
Liver	5/11 (45%)	11/11 (100%)	7/12 (58%)	8/12 (67%)	12/23 (52%)	19/23 (83%)
Spleen	3/11 (27%)	11/11 (100%)	4/12 (33%)	11/12 (92%)	7/23 (30%)	22/23 (96%)
≥3 goals	1/11 (9%)	10/11 (91%)	5/12 (42%)	9/12 (75%)	6/23 (26%)	19/23 (83%)
All 4 goals	1/10 (10%)	6/10 (60%)	0/12 (0%)	7/12 (58%)	1/22 (5%)	13/22 (59%)

Table 36

Therapeutic goals	velaglucerase alfa 60 U/kg N=17		imiglucerase 60 U/kg N=17	
	Baseline	9 Months	Baseline	9 Months
Hemoglobin concentration	10/16 (63%)	15/16 (94%)	4/15 (27%)	12/15 (80%)
Platelet count	8/16 (50%)	16/16 (100%)	9/15 (60%)	15/15 (100%)
Liver	8/16 (50%)	15/16 (94%)	4/16 (25%)	16/16 (100%)
Spleen	1/6 (17%)	6/6 (100%)	3/6 (50%)	6/6 (100%)
≥3 goals	1/16 (6%)	14/16 (88%)	1/15 (7%)	12/15 (80%)
All 4 goals	0/6 (0%)	6/6 (100%)	0/6 (0%)	6/6 (100%)

Table 37

Therapeutic goals	velaglucerase alfa 60 U/kg N=7		imiglucerase 60 U/kg N=7	
	Baseline	9 Months	Baseline	9 Months
Hemoglobin concentration	5/7 (71%)	7/7 (100%)	2/6 (33%)	6/6 (100%)
Platelet count	0/7 (0%)	7/7 (100%)	0/6 (0%)	6/6 (100%)
Liver	6/7 (86%)	7/7 (100%)	1/6 (17%)	6/6 (100%)
Spleen	1/6 (17%)	6/6 (100%)	3/6 (50%)	6/6 (100%)
≥3 goals	1/7 (14%)	7/7 (100%)	1/6 (17%)	6/6 (100%)

All 4 goals	0/6 (0%)	6/6 (100%)	0/6 (0%)	6/6 (100%)

Table 38

Therapeutic goals	velaglucerase alfa 60 U/kg N=10		imiglucerase 60 U/kg N=10	
	Baseline	9 Months	Baseline	9 Months
Hemoglobin concentration	5/9 (56%)	8/9 (89%)	2/9 (22%)	6/9 (67%)
Platelet count	8/9 (89%)	9/9 (100%)	9/9 (100%)	9/9 (100%)
Liver	2/9 (22%)	8/9 (89%)	3/10 (30%)	10/10 (100%)
≥2 goals	6/9 (67%)	9/9 (100%)	5/9 (56%)	9/9 (100%)
All 3 goals	0/9 (0%)	7/9 (78%)	0/9 (0%)	6/9 (67%)

Table 39

Therapeutic goals	velaglucerase alfa overall N=40	
	Baseline	1 Year
Hemoglobin concentration	37/38 (97%)	37/38 (97%)
Platelet count	29/36 (81%)	29/36 (81%)
Liver	37/37 (100%)	37/37 (100%)
Spleen	30/34 (88%)	31/34 (91%)
≥3 goals	32/37 (86%)	33/37 (89%)
All 4 goals	26/32 (81%)	26/32 (81%)

5

Table 40

Therapeutic goals	TKT025 velaglucerase alfa 60 U/kg N=12		TKT032 velaglucerase alfa 60 U/kg N=12	
	Baseline	Month 9	Baseline	Year 1
Hemoglobin concentration	5/10 (50%)	10/10 (100%)	2/12 (17%)	11/12 (92%)
Platelet count	0/10 (0%)	7/10 (70%)	2/11 (18%)	6/11 (55%)

Liver	5/10 (50%)	7/10 (70%)	5/11 (45%)	11/11 (100%)
Spleen	0/10 (0%)	9/10 (90%)	3/11 (27%)	11/11 (100%)
≥3 goals	0/10 (10%)	8/10 (80%)	1/11 (9%)	10/11 (91%)
All 4 goals	0/10 (10%)	5/10 (50%)	1/10 (10%)	6/10 (60%)

Example 10. Efficacy and Safety Study of Velaglucerase Alfa in Children and Adolescents with Type 3 Gaucher Disease.

Gaucher disease is a rare lysosomal storage disorder caused by the deficiency of the enzyme glucocerebrosidase (GCB). Gaucher disease has been classified into 3 clinical subtypes based on the presence or absence of neurological symptoms and the severity of neurological symptoms. Patients with type 2 Gaucher disease present with acute neurological deterioration, and those with type 3 disease typically display a more sub acute neurological course; type 1 Gaucher disease, the most common form accounting for more than 90% of all cases, does not involve the central nervous system.

Velaglucerase alfa is an approved enzyme replacement therapy (ERT) for pediatric and adult patients with type 1 Gaucher disease. ERT has been proven to reduce organomegaly, improve hematological parameters and positively impact health-related quality of life. ERT has not been shown cross the blood brain barrier and as a result has shown limited ability to improve the neurological (Central Nervous System; CNS) manifestations associated with Gaucher disease.

This study will provide a basis for exploring the efficacy and safety of velaglucerase alfa in patients with type 3 Gaucher disease. The study will be an interventional multi-center, open label, efficacy and safety study of velaglucerase alfa enzyme replacement therapy in children and adolescents with type 3 Gaucher disease. Primary outcome measures will include; change from baseline in hemoglobin concentration; change from baseline in platelet count; change from baseline in normalized liver volumes measured using magnetic resonance imaging (MRI); change from baseline in normalized spleen volumes measured using magnetic resonance imaging (MRI); and change from baseline in neurological symptoms. Safety assessments will include the assessment of adverse events and infusion related adverse events; serious

adverse events; clinical laboratory values; urinalysis; vital signs; 12-lead electrocardiogram (ECG) recordings; physical examination; and anti-velaglycerase alfa antibody formation. The experimental arm of the study will receive velaglycerase alfa by intravenous infusion, every other week.

5 For inclusion in the study each patient must meet all of the following criteria; a confirmed diagnosis of type 3 Gaucher disease; ≥ 2 and <18 years of age at the time of enrollment; either naive to treatment or has not received treatment (investigational or approved) for Gaucher disease within 12 months prior to study entry; the patient must have Gaucher disease-related anemia, defined as a hemoglobin concentration below the
10 lower limit of normal for age and sex. The patient must also meet one or more of the following criteria; at least moderate splenomegaly (2 to 3 cm below the left costal margin) by palpation; Gaucher disease-related thrombocytopenia, defined as a platelet count $<120 \times 10,000$ platelets/cubic mm; a Gaucher disease-related readily palpable enlarged liver. Patients who have undergone splenectomy may still be eligible to
15 participate in the study. Female patients of child-bearing potential must agree to use a medically acceptable method of contraception at all times during the study. Pregnancy testing will be performed at the time of enrollment and as required throughout participation in the study. Male patients must agree to use a medically acceptable method of contraception at all times during the study and report a partner's pregnancy to the
20 Investigator. The patient's parent(s) or the patient's legally authorized representative(s) must provide written informed consent that has been approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC).

Patients who meet any of the following criteria will be excluded from this study; the patient is suspected of having type 2 or type I Gaucher disease; the patient is <2 years
25 of age; the patient has experienced a severe (Grade 3 or higher) infusion-related hypersensitivity reaction (anaphylactic or anaphylactoid reaction) to any enzyme replacement therapy for Gaucher disease (approved or investigational); the patient has received any non-Gaucher disease-related treatment with an investigational drug within 30 days prior to study entry; the patient is a pregnant and/or lactating female.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

What is claimed:

1. A method of treating a subject with Gaucher Type III disease, the method
5 comprising

administering a glucocerebrosidase enzyme replacement therapy by intravenous
infusion to the subject over a period of less than 2 hours, to thereby treat the subject.

2. The method of claim 1, wherein the glucocerebrosidase enzyme replacement
10 therapy is administered over a period of 90 minutes or less.

3. The method of claim 1, wherein the glucocerebrosidase enzyme replacement
therapy is administered over a period of 60 minutes or less.

4. The method of claim 1, further comprising administering a second
15 glucocerebrosidase enzyme replacement therapy by intravenous infusion to the subject
over a period of less than 2 hours.

5. The method of claim 1, wherein the glucocerebrosidase enzyme replacement
20 therapy is selected from the group consisting of: velaglucerase, imiglucerase and uplyso.

6. The method of claim 1, wherein the glucocerebrosidase enzyme replacement
therapy is administered at a dose of 15 to 60 U/kg.

7. A method for identifying a subject as suitable for treatment with a
25 glucocerebrosidase enzyme replacement therapy, comprising:

determining if neutralizing antibodies to a glucocerebrosidase enzyme
replacement therapy are present in a sample from the subject to obtain a measured value
of antibody to the therapy, wherein the subject is currently being administered a first
30 glucocerebrosidase enzyme replacement therapy or has previously received a first
glucocerebrosidase enzyme replacement therapy, and

comparing the measured value of antibody to the therapy to a standard,
wherein if the measured antibody value is greater than the standard, the subject is
identified as having antibodies to the glucocerebrosidase enzyme replacement therapy
and as a candidate for glucocerebrosidase enzyme replacement therapy with
5 velaglucerase, and if the measured antibody value is not greater than the standard,
identifying the subject as a candidate for glucocerebrosidase enzyme replacement therapy
with the first glucocerebrosidase enzyme replacement therapy or an alternative
glucocerebrosidase enzyme replacement therapy.

10 8. The method of claim 7, wherein the first glucocerebrosidase enzyme
replacement therapy is imiglucerase or uplyso.

9. The method of claim 7, wherein the sample is a blood or serum sample.

15 10. The method of claim 9, wherein the blood or serum sample has been
modified.

11. The method of claim 10, wherein the sample has been contacted with an
analytical reagent or substrate.

20 12. The method of claim 10, wherein the sample is a concentrated portion of a
blood or serum sample.

25 13. The method of claim 7, wherein the subject is identified as a candidate for
glucocerebrosidase enzyme replacement therapy with velaglucerase, and the method
further comprises administering velaglucerase to the subject.

30 14. A method for treating a subject with Gaucher Type III disease that is
currently taking or has previously received a glucocerebrosidase enzyme replacement
therapy, comprising:

selecting a subject on the basis that the subject has tested positive for the production of antibodies to the therapy the subject is currently taking or has previously taken for Gaucher disease, and
administering velaglucerase to the subject.

5

15. The method of claim 14, wherein the subject tested positive for the production of IgE antibodies to the therapy the subject is currently taking or has previously taken for Gaucher disease.

10

16. The method of claim 14, wherein the subject tested positive for the production of IgM antibodies to the therapy the subject is currently taking or has previously taken for Gaucher disease.

15

17. The method of claim 14, wherein the subject tested positive for the production of IgG antibodies to the therapy the subject is currently taking or has previously taken for Gaucher disease.

20

18. The method of claim 14, wherein the therapy the subject is currently taking or has previously taken for Gaucher disease is imiglucerase.

19. The method of claim 14, wherein the therapy the subject is currently taking or has previously taken for Gaucher disease is uplyso.

25

20. The method of claim 14, wherein velaglucerase is administered at a dose of 15 to 60 U/kg.

21. The method of claim 14, wherein the velaglucerase is administered to the subject by intravenous infusion over 90 minutes or less.

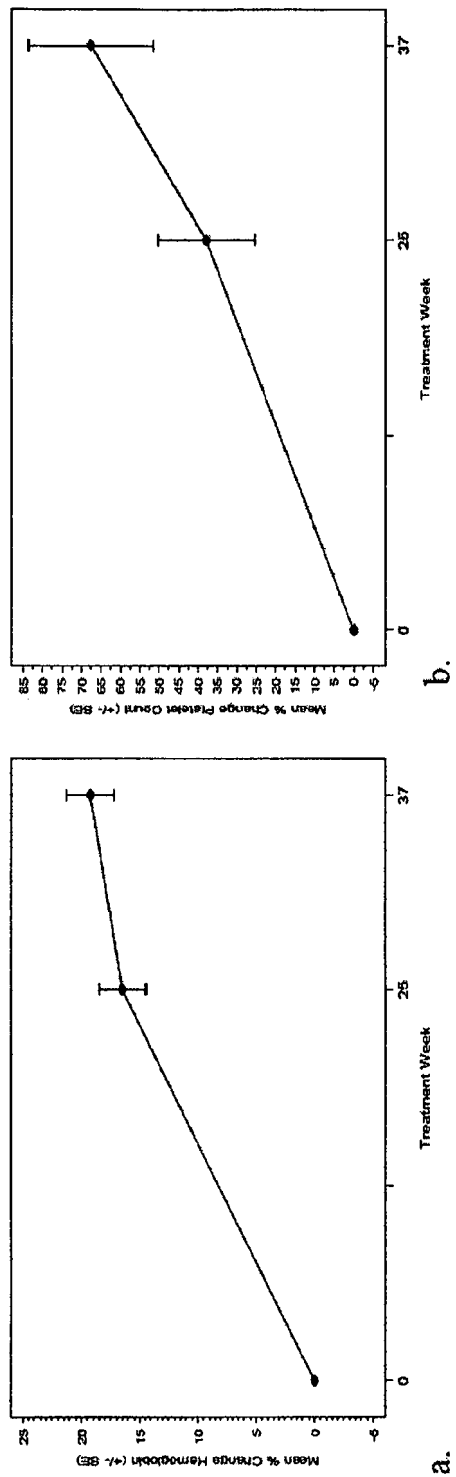


FIG. 1

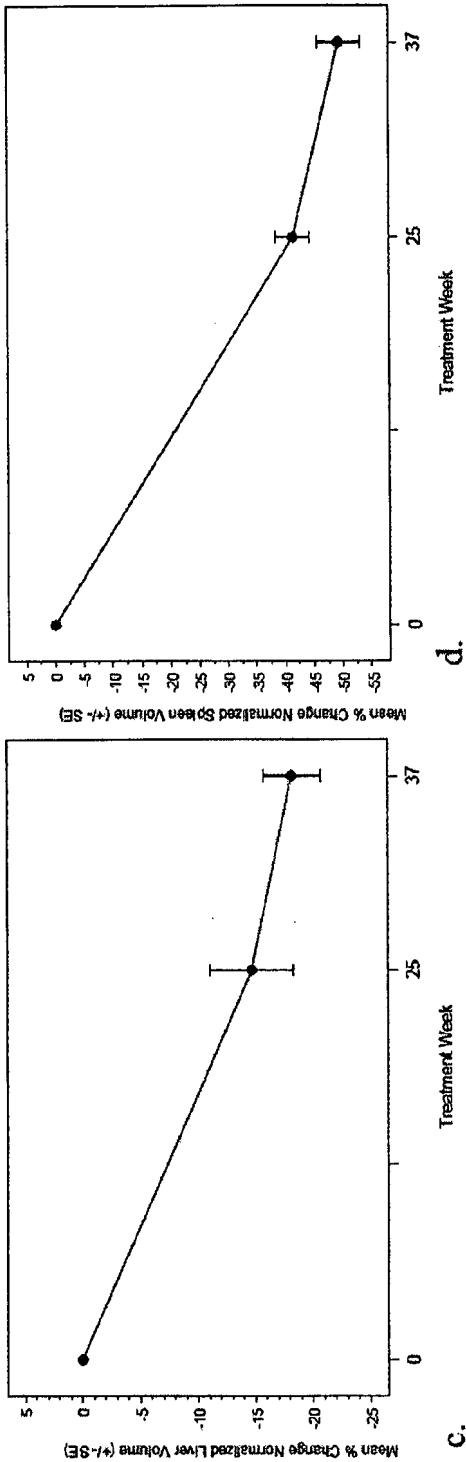


FIG. 1 CONT.

3/28

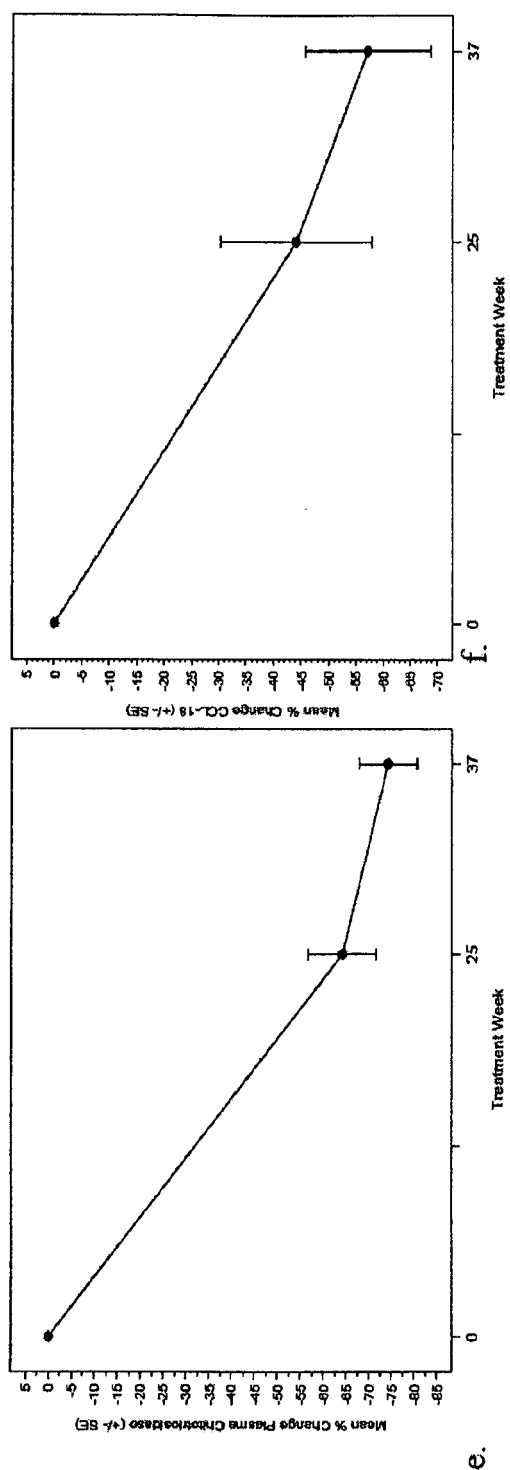


FIG. 1 CONT.

4/28

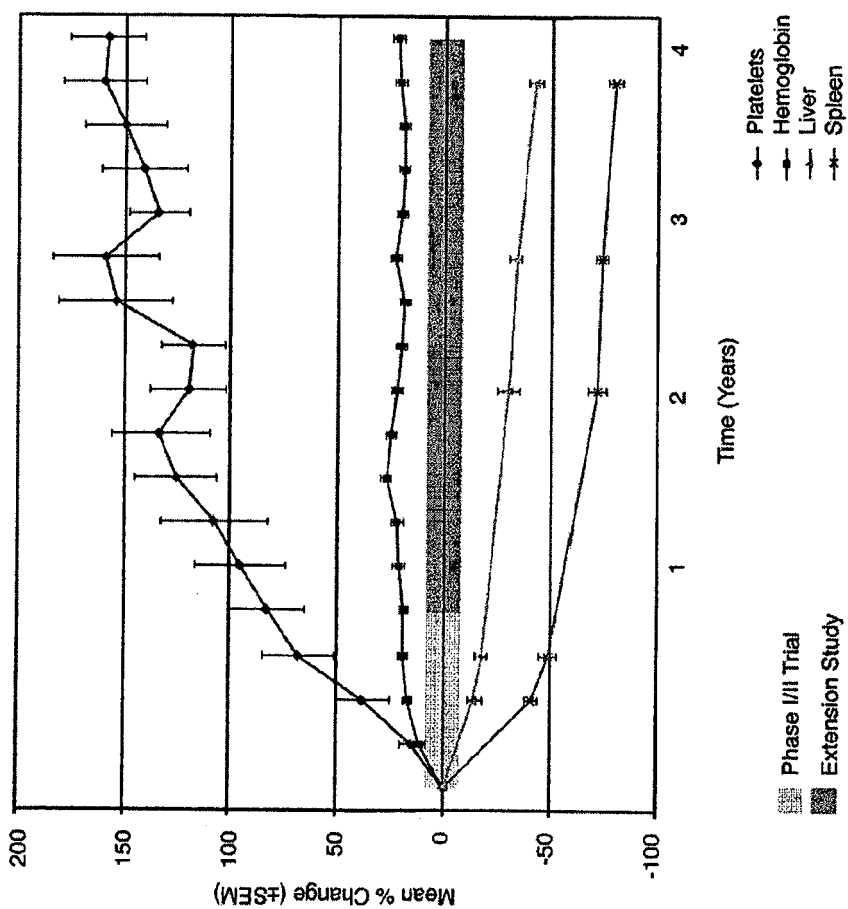
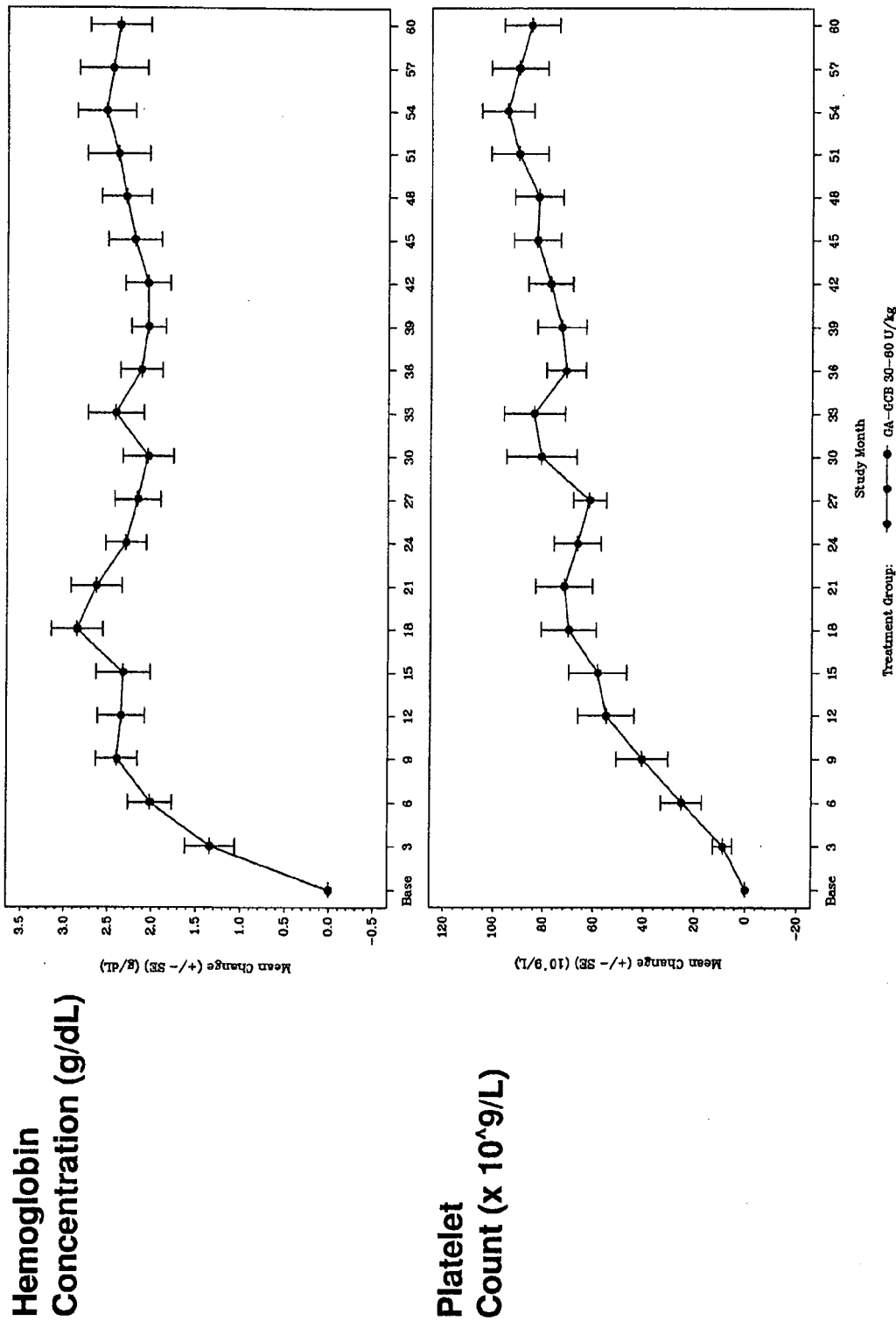
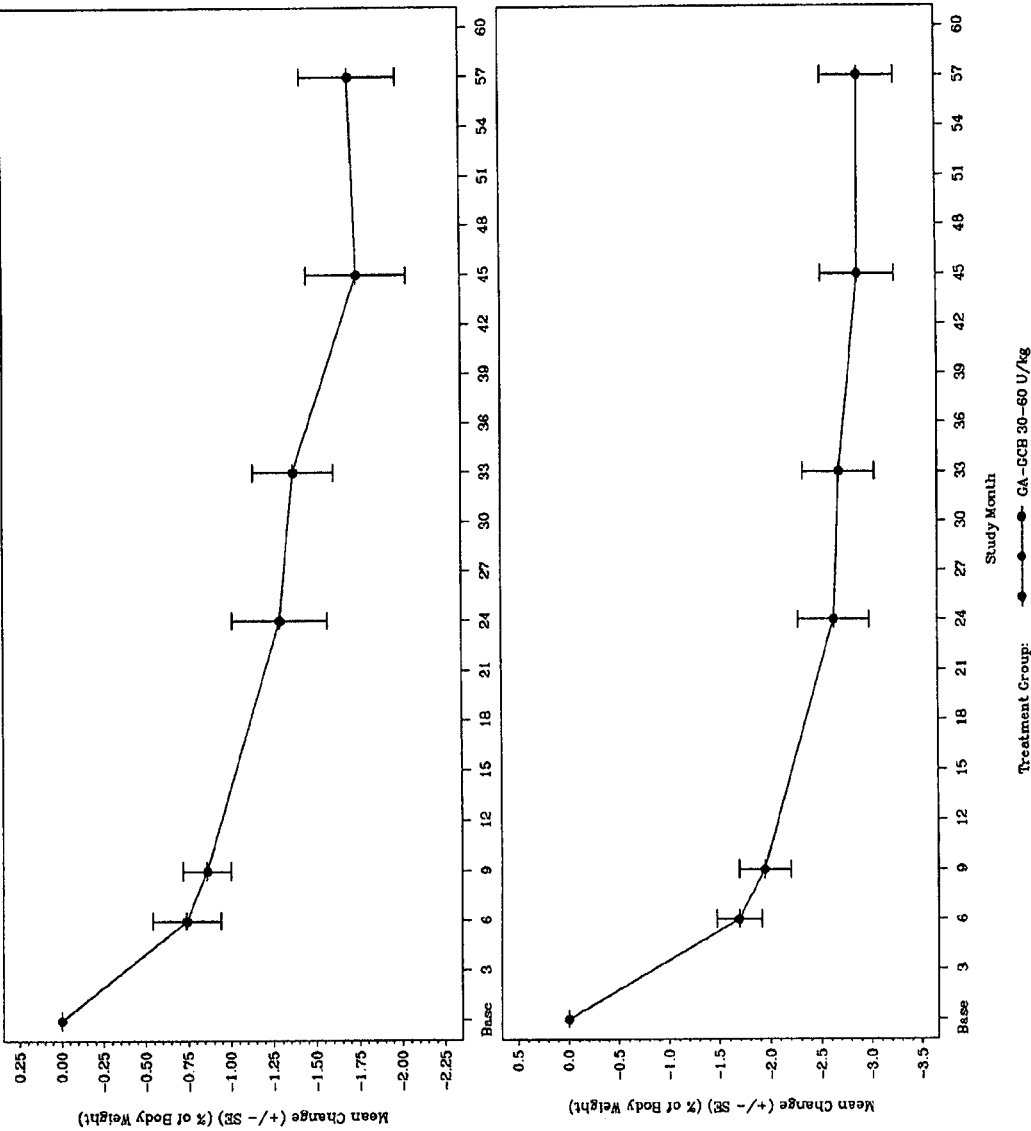


FIG. 2



Note: Imputation was applied to missing data.

FIG. 3



Note: Imputation was applied to missing data.

FIG. 4

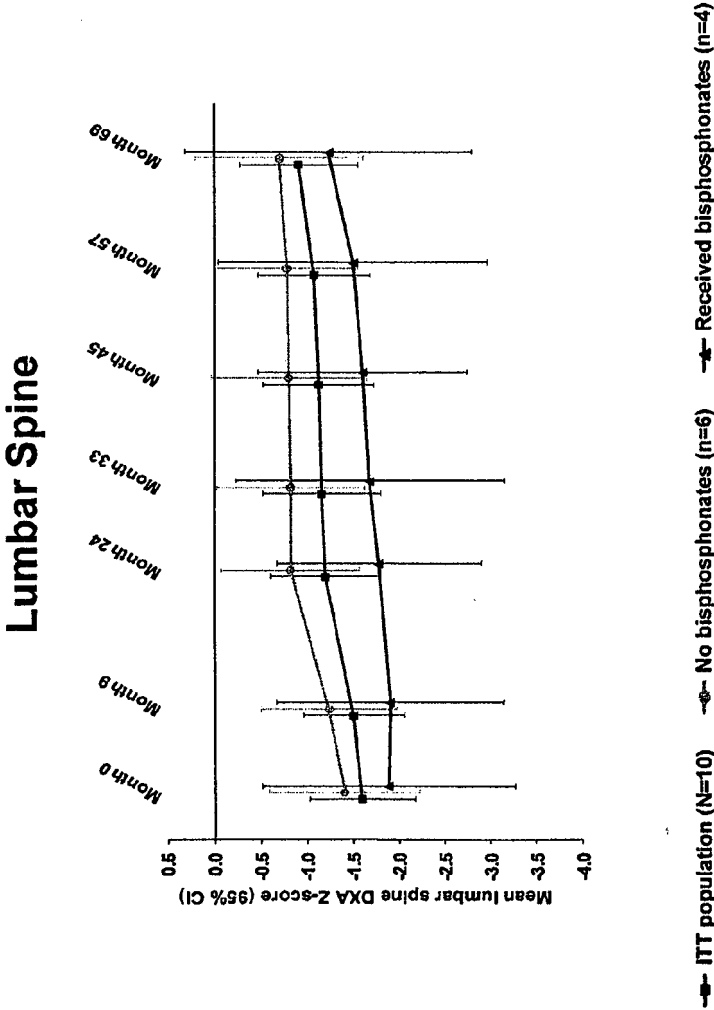


FIG. 5

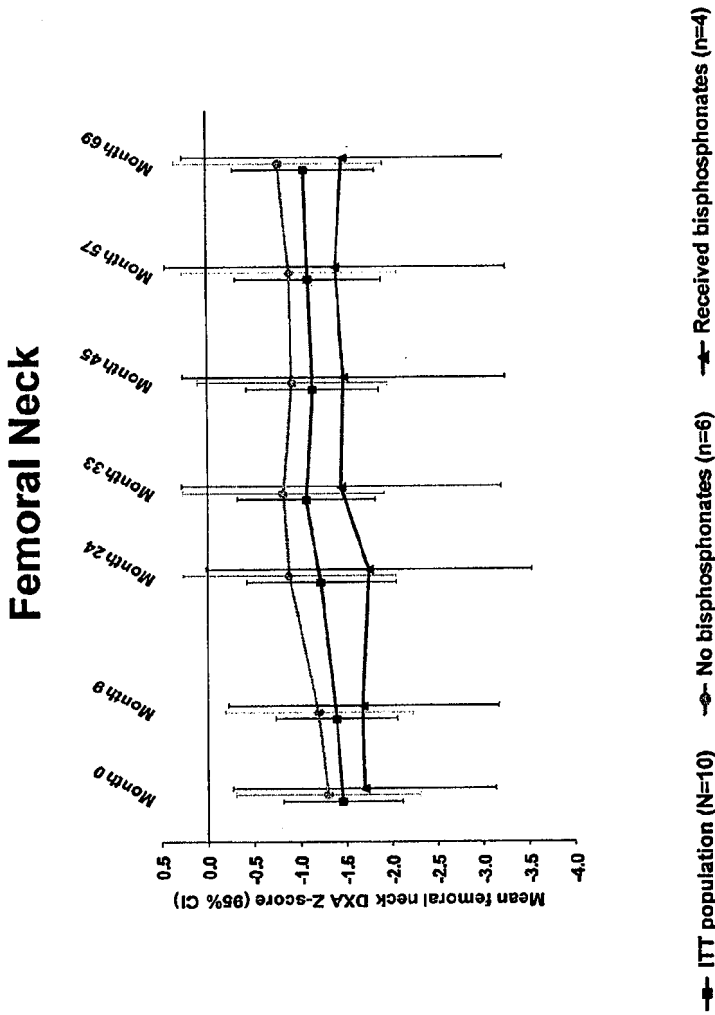


FIG. 6

9/28

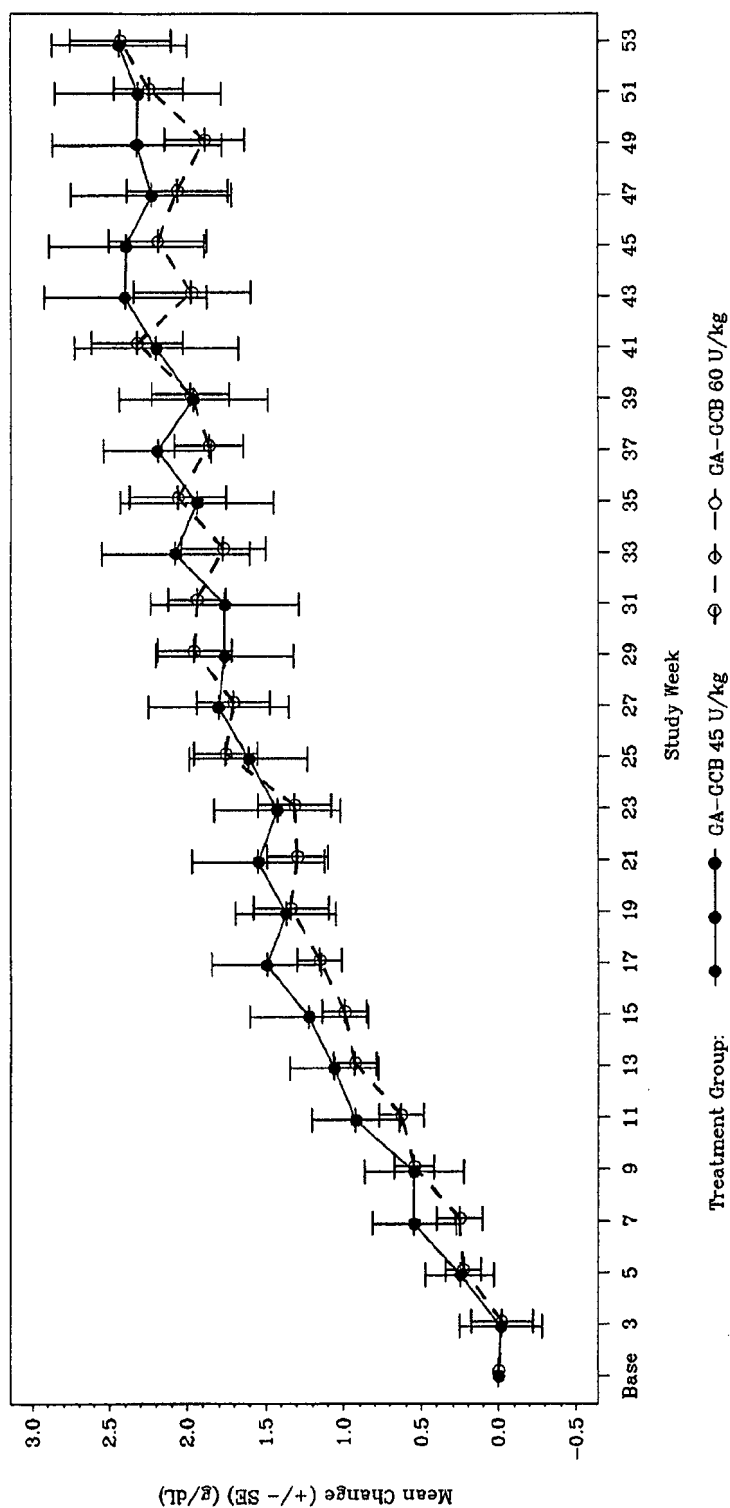


FIG. 7

10/28

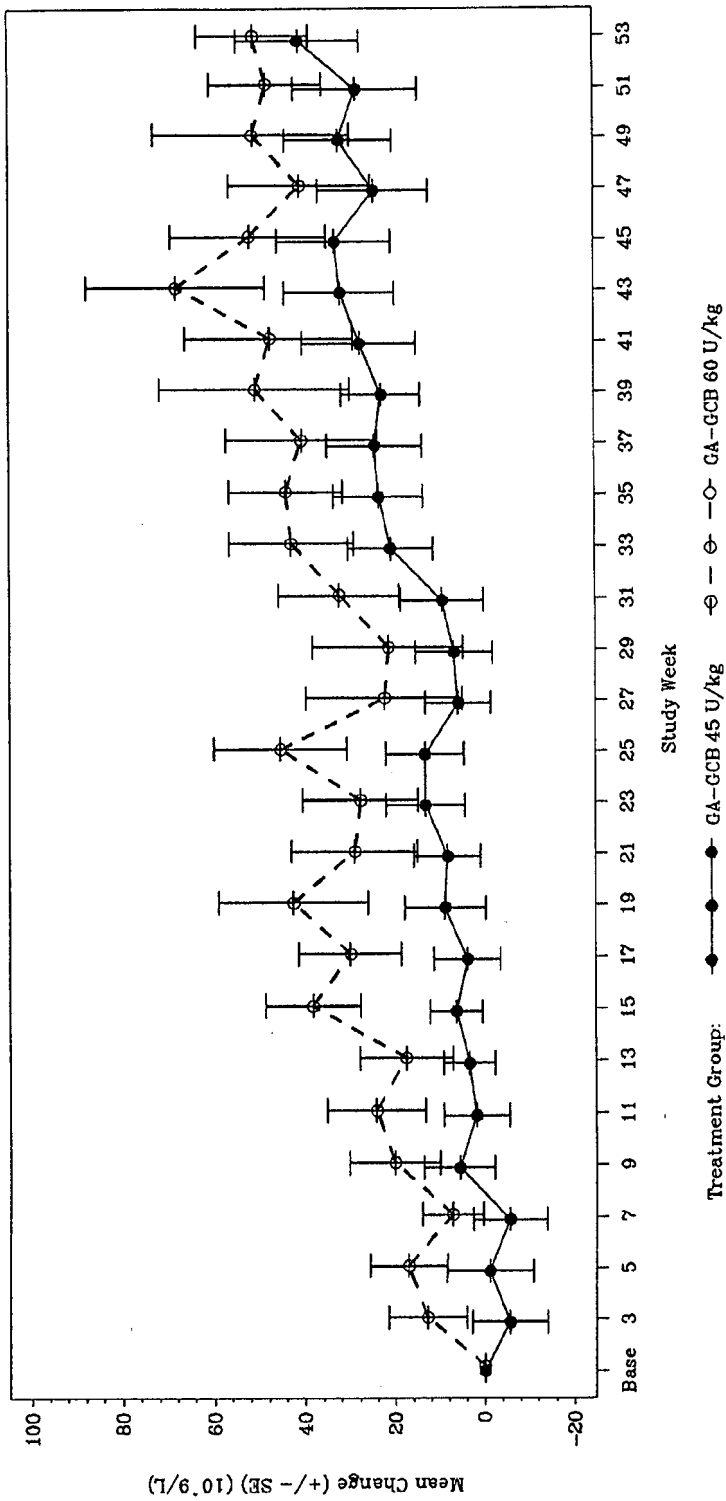


FIG. 8

11/28

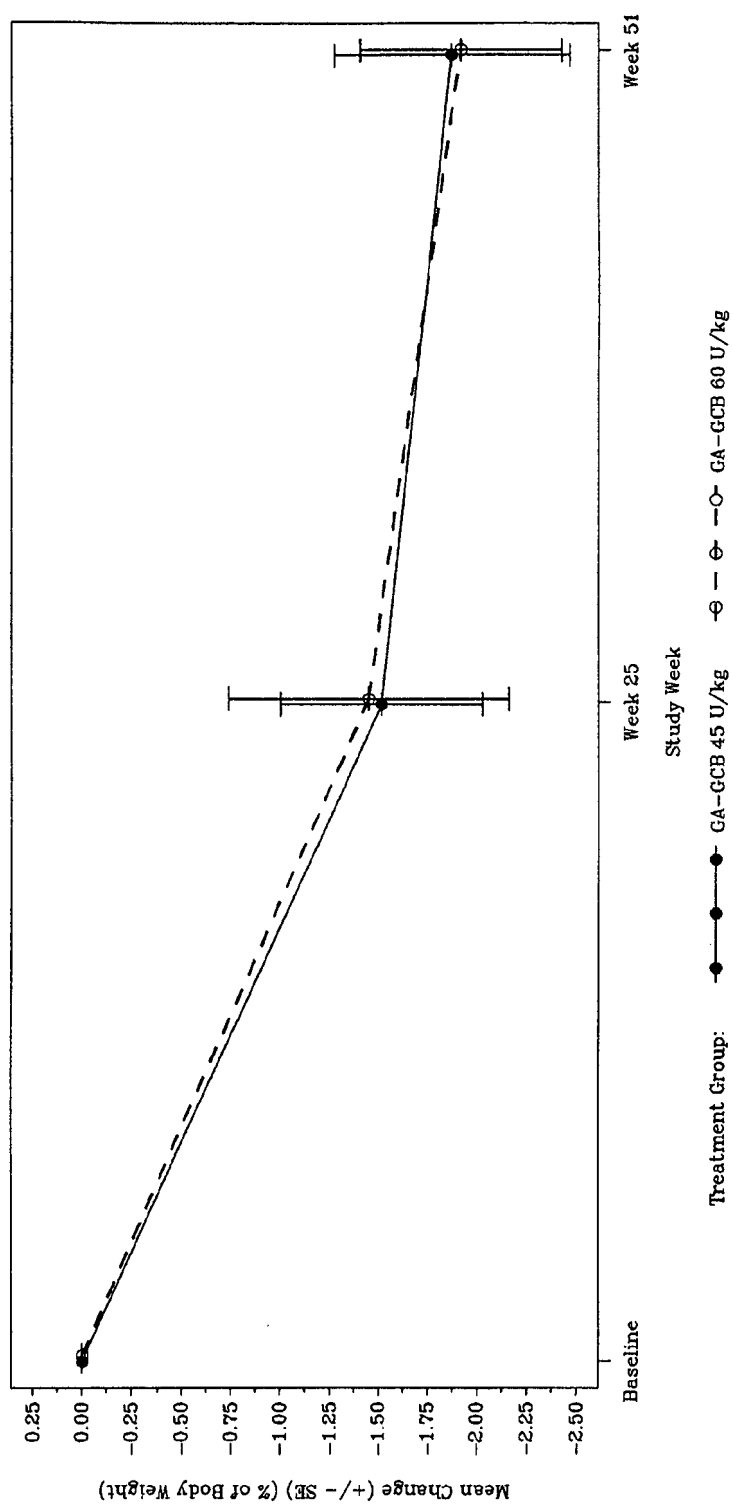


FIG. 9

12/28

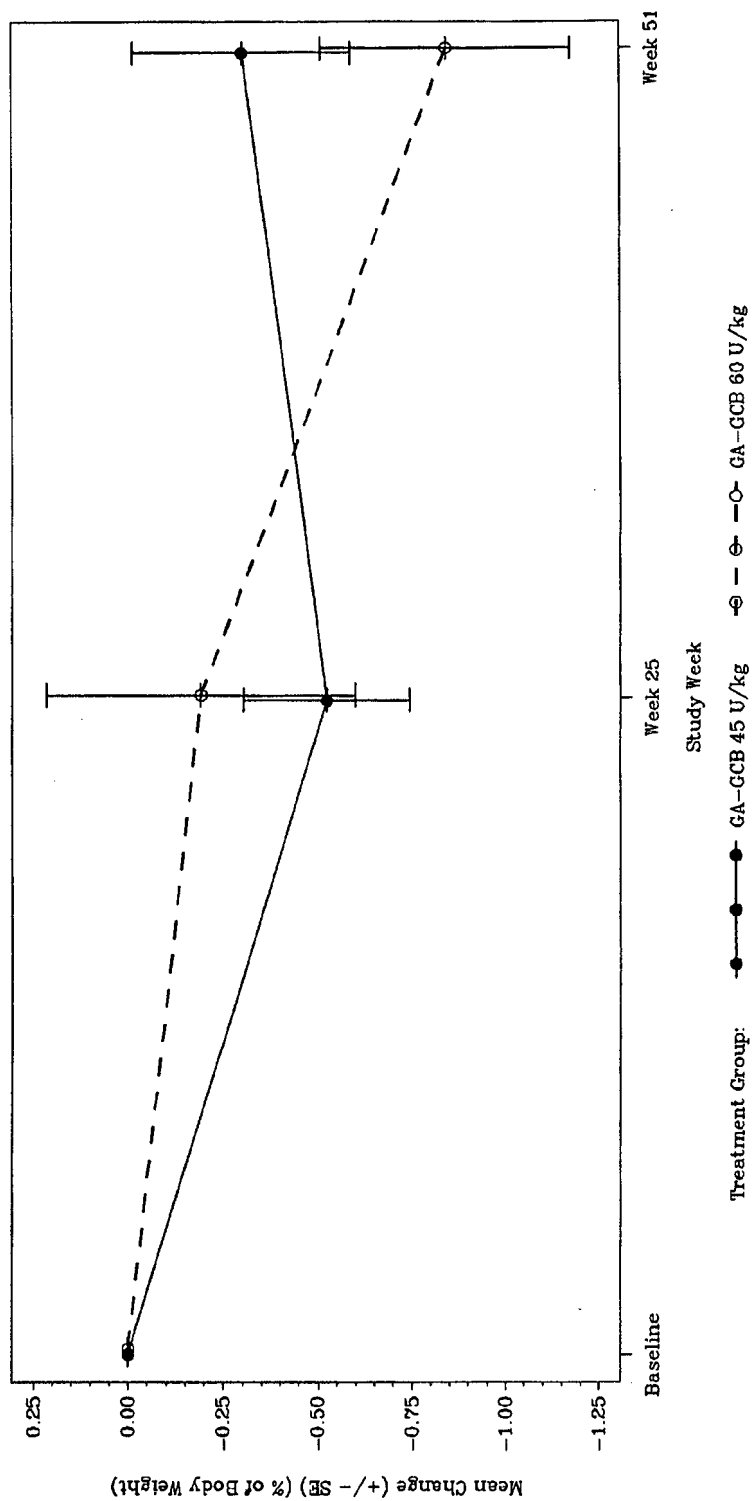


FIG. 10

13/28

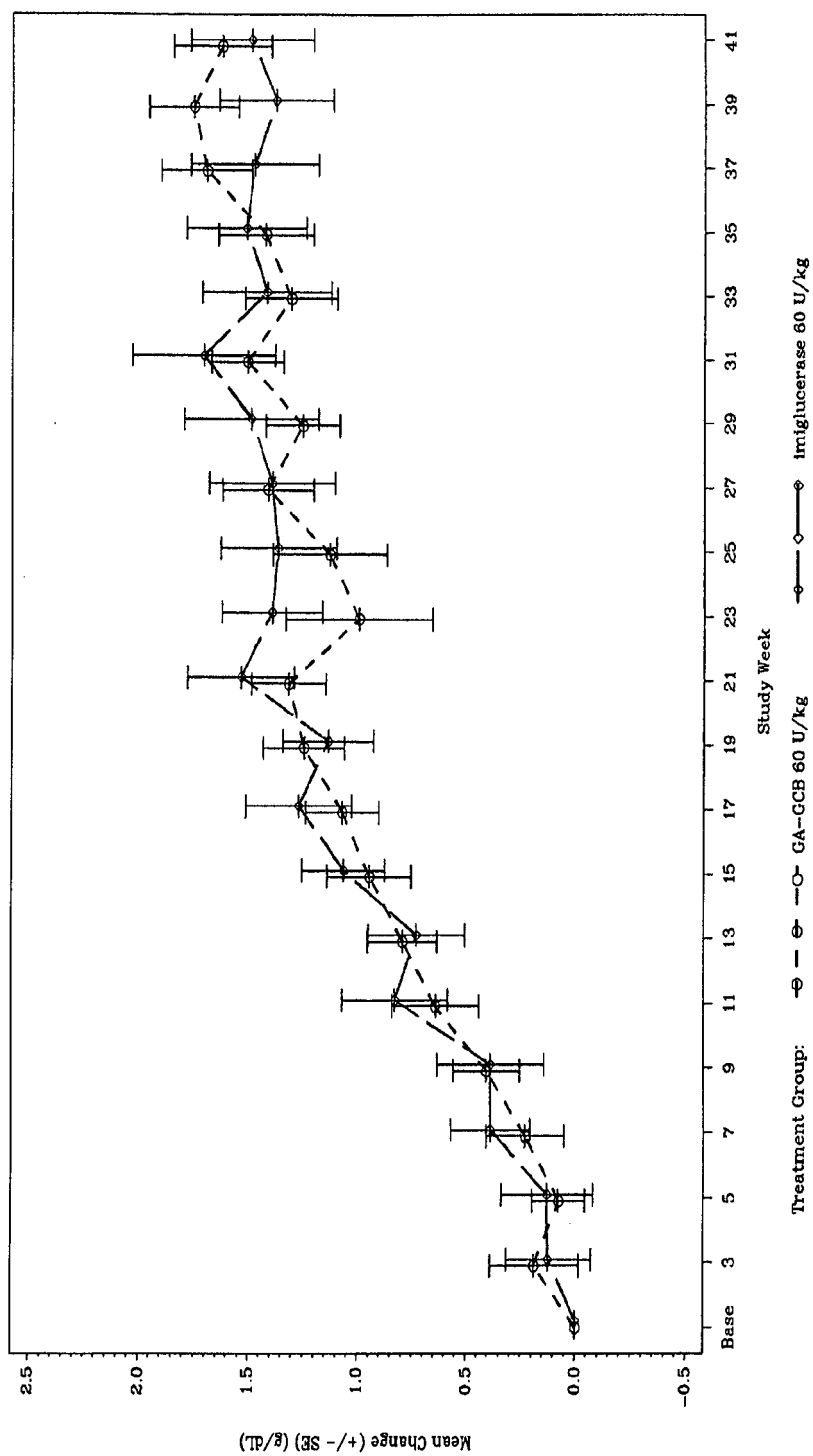


FIG. 11

14/28

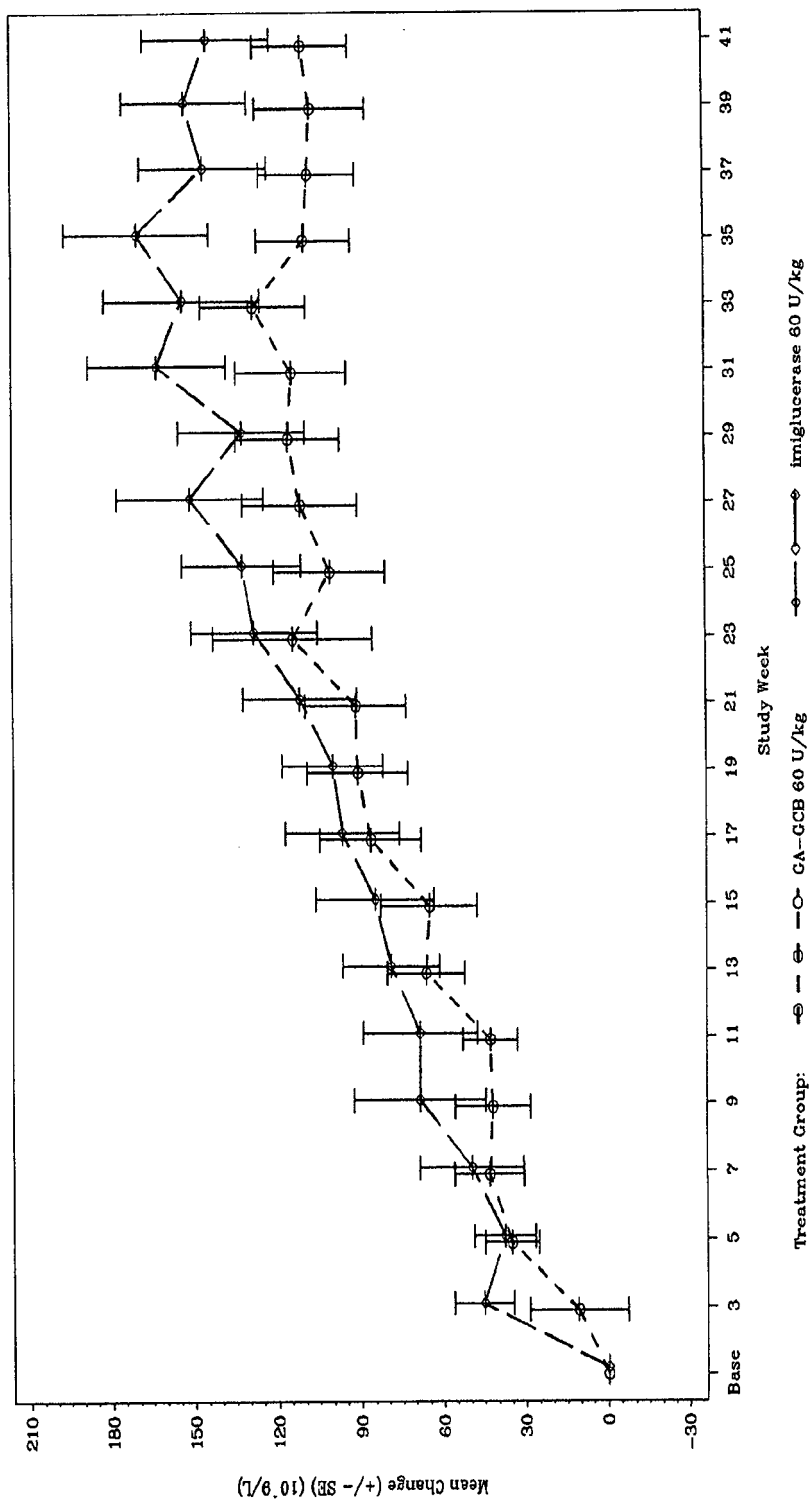


FIG. 12

15/28

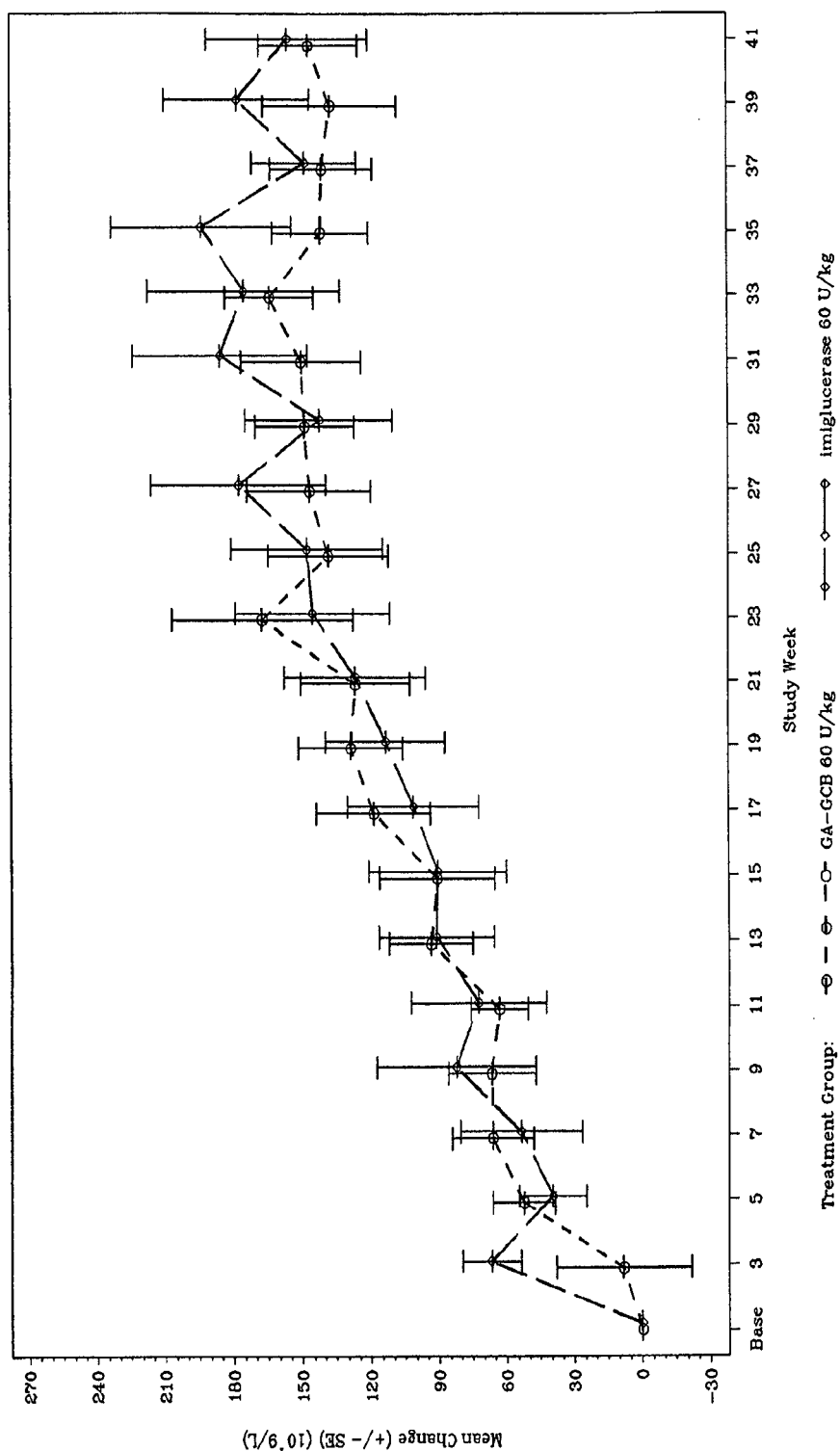


FIG. 13

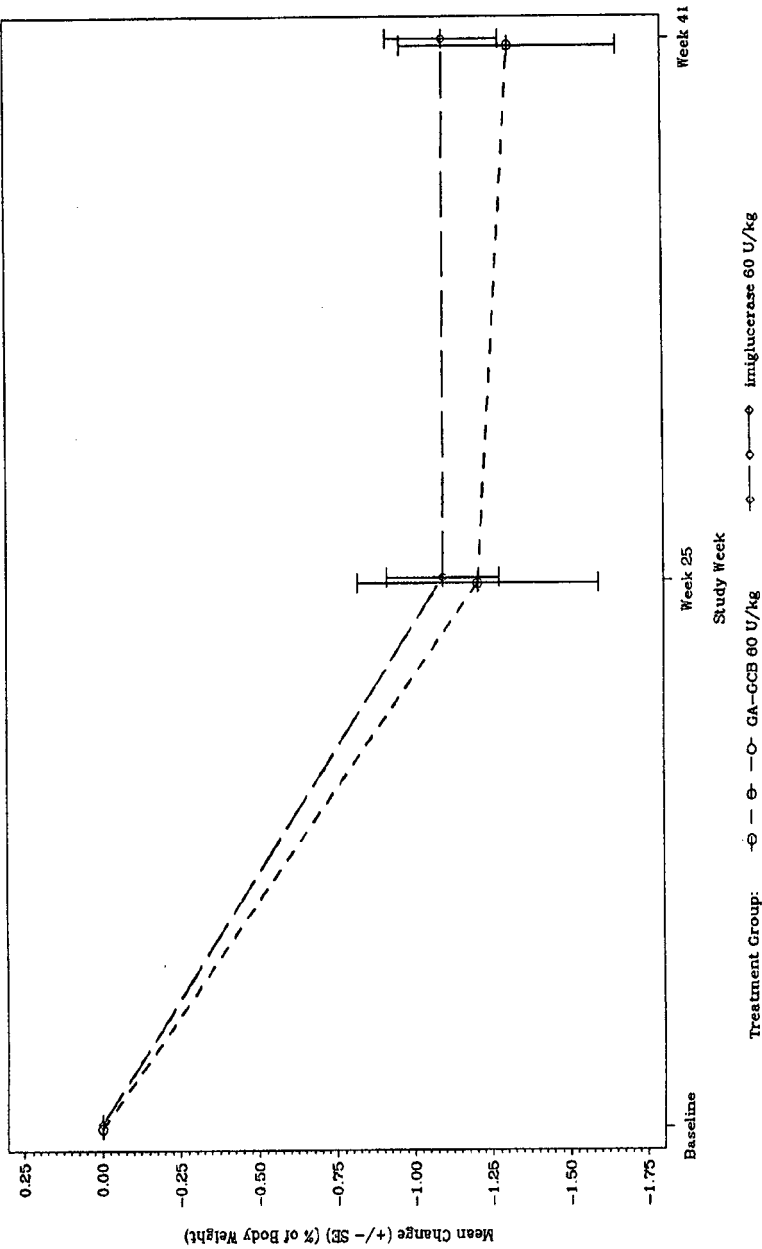


FIG. 14

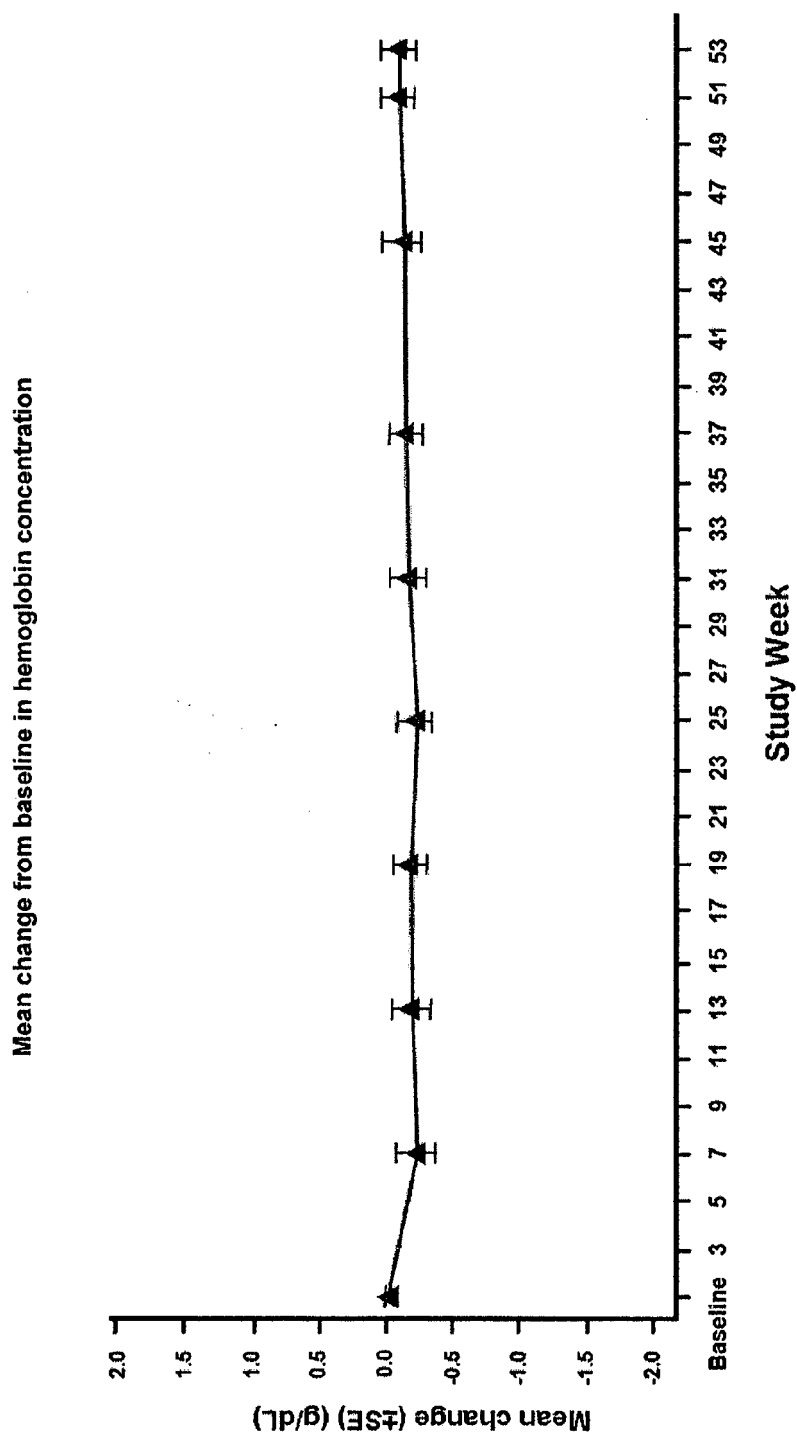


FIG. 15

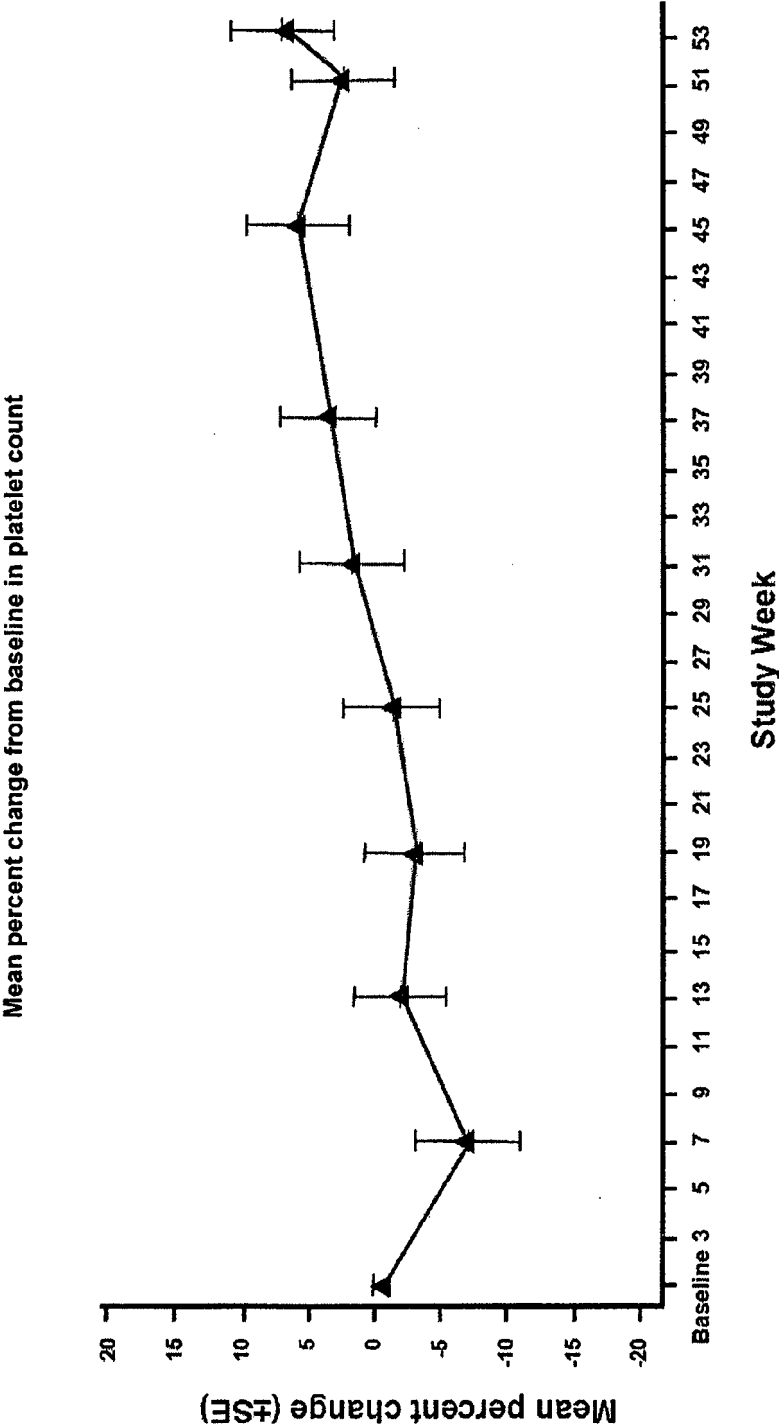


FIG. 16

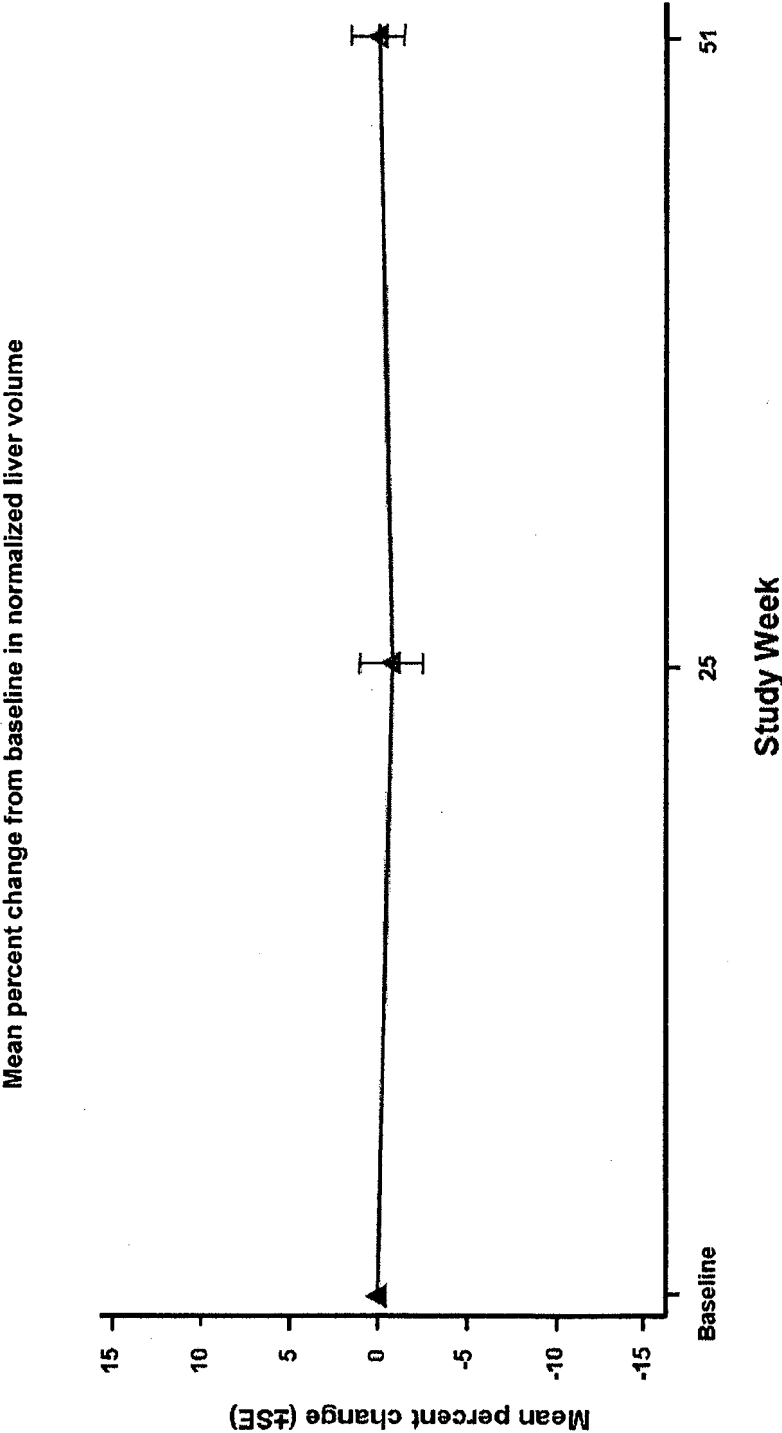


FIG. 17

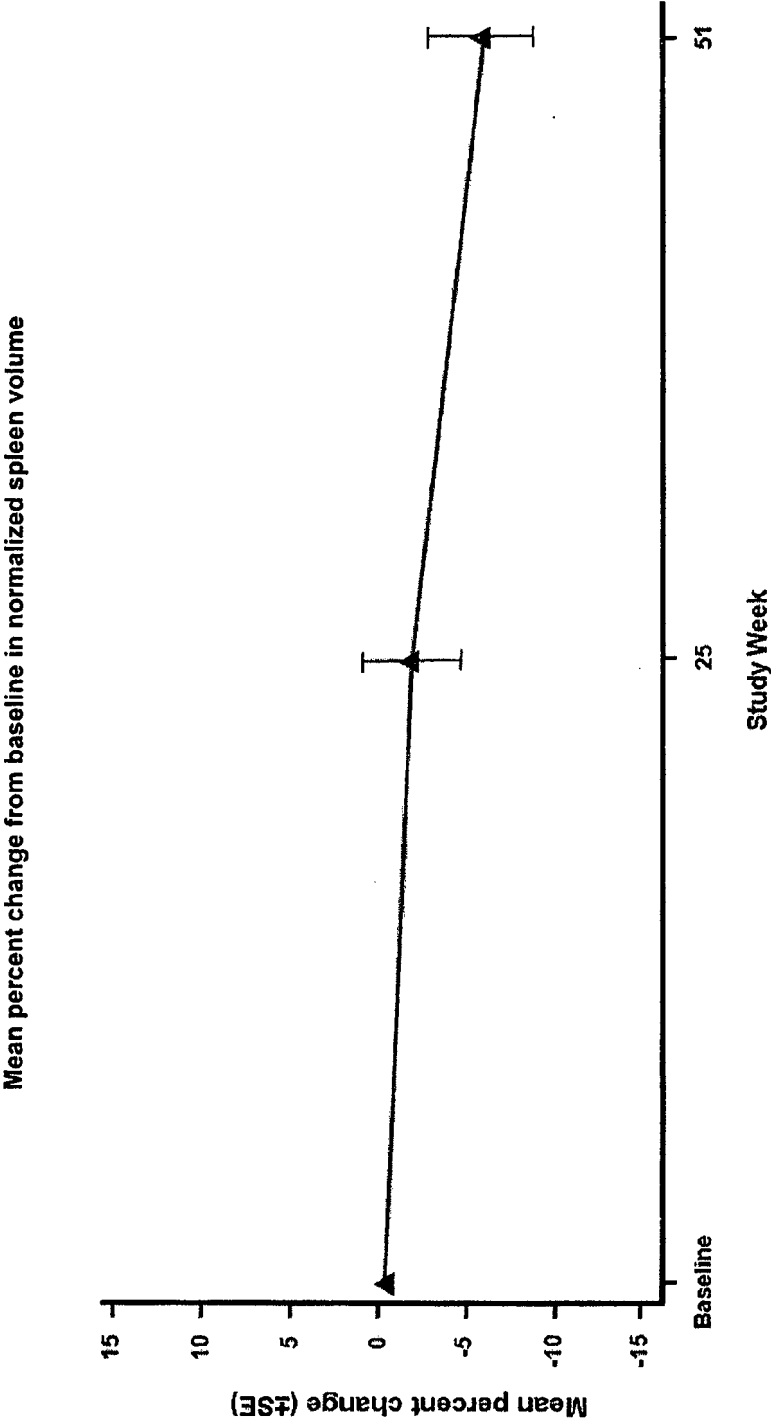


FIG. 18

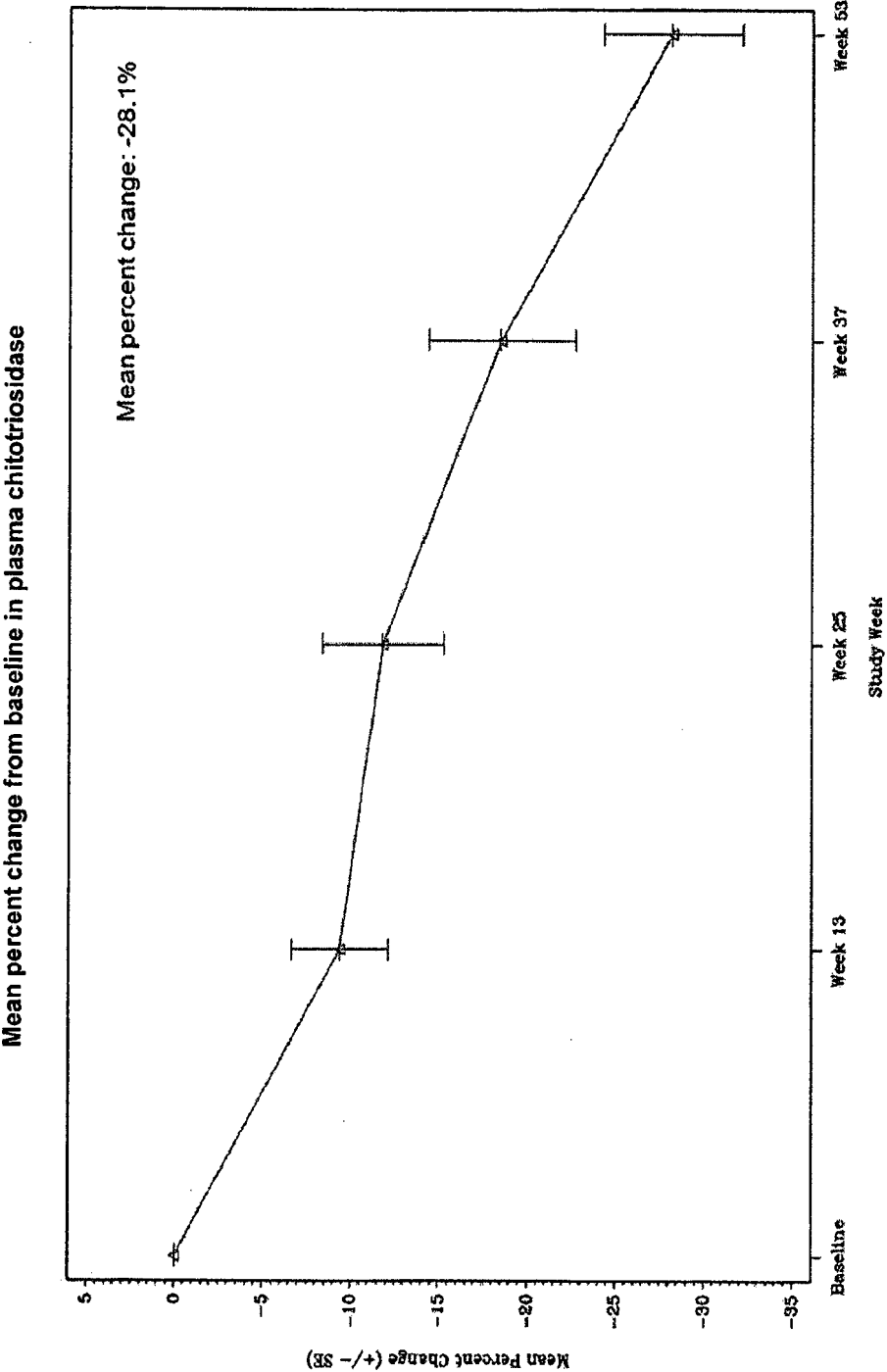


FIG. 19

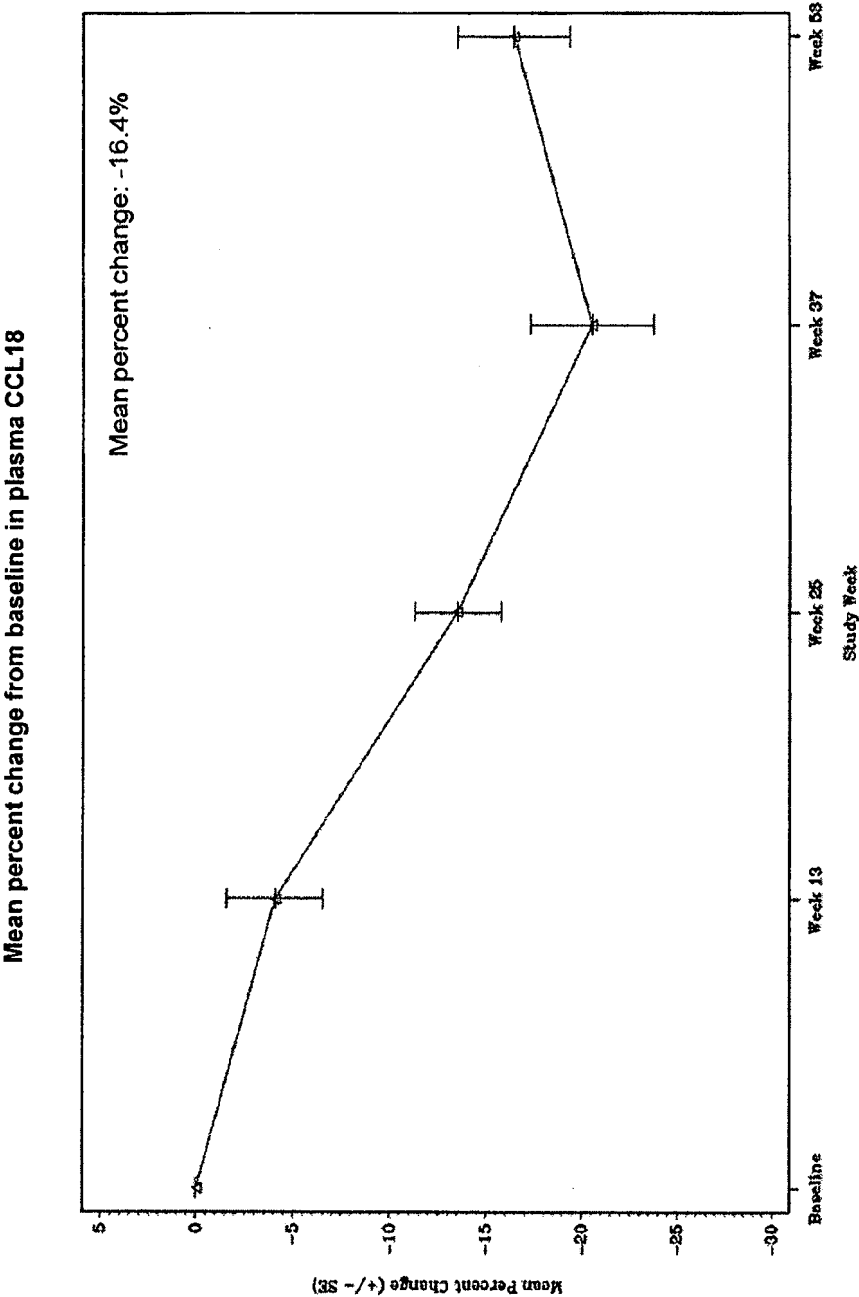
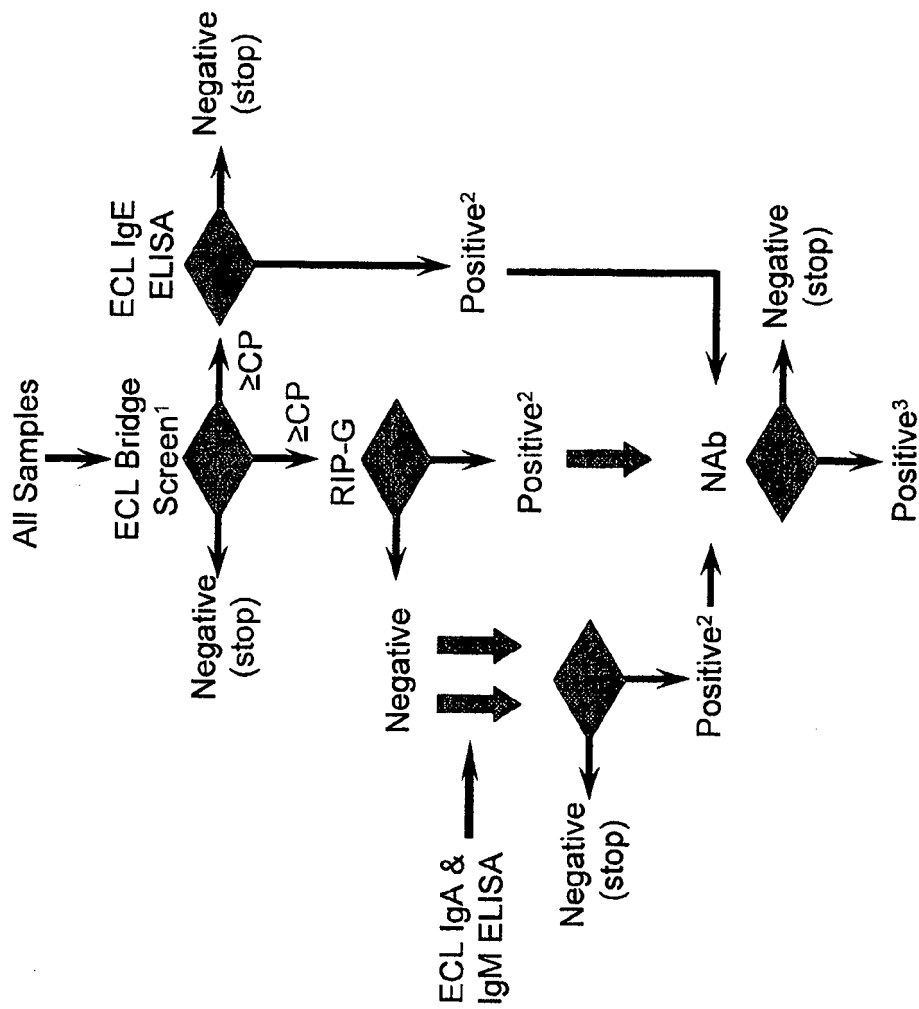


FIG. 20

Immunogenicity Evaluation of Patients in Velaglucerase alfa Clinical Studies



¹Positive cut point (CP) in ng/mL; ²Report titer; ³Report %NAb

FIG. 21

Anti-Drug Antibody Screening by Electro-chemiluminescence (ECL) Immunoassay

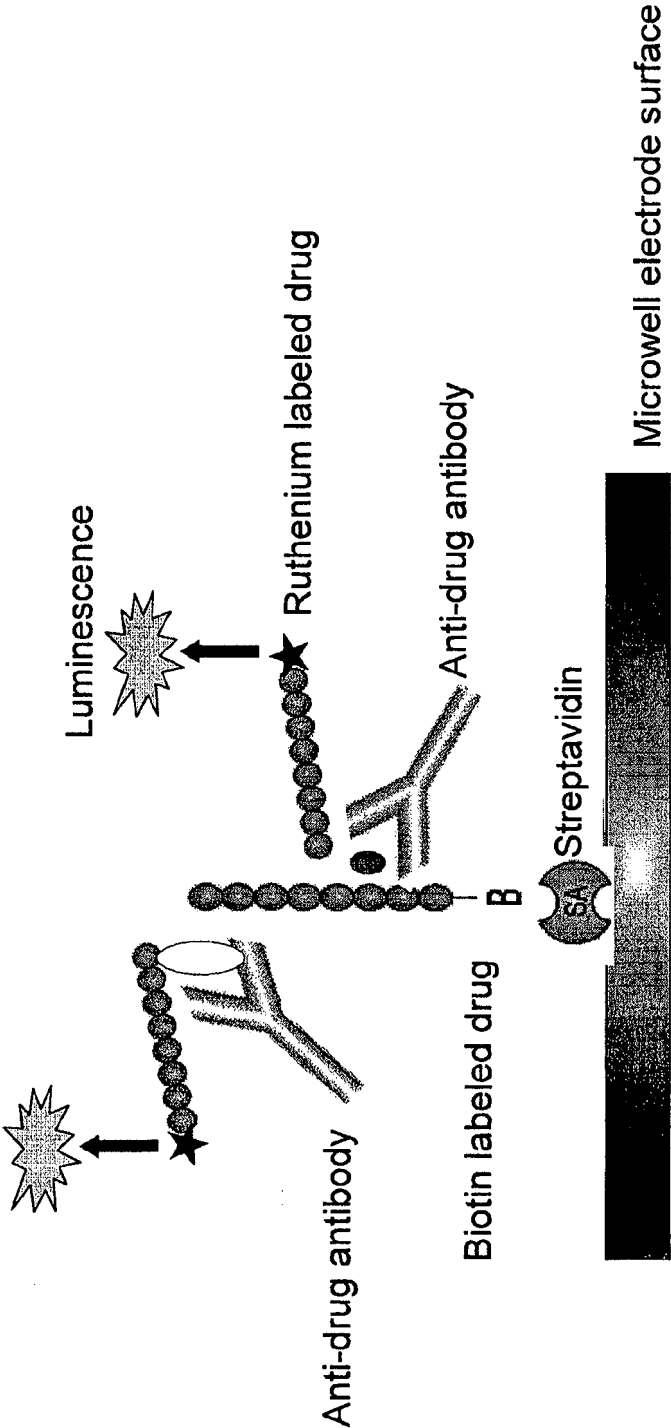
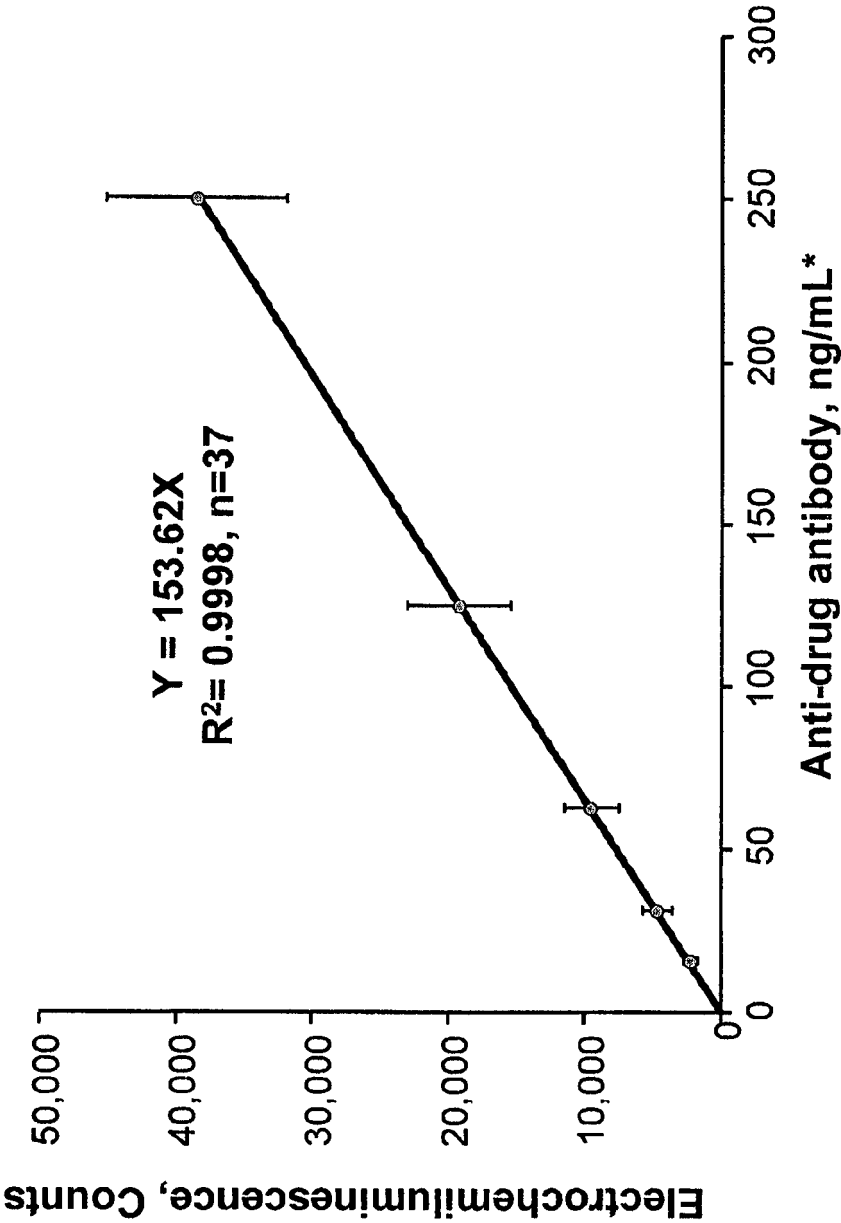


FIG. 22

Screening Assay Dose Response Curve



*equivalents relative to a mouse monoclonal IgG ADA

FIG. 23

IgG ADA Confirmatory Assay

Radioimmunoprecipitation (RIP) Assay

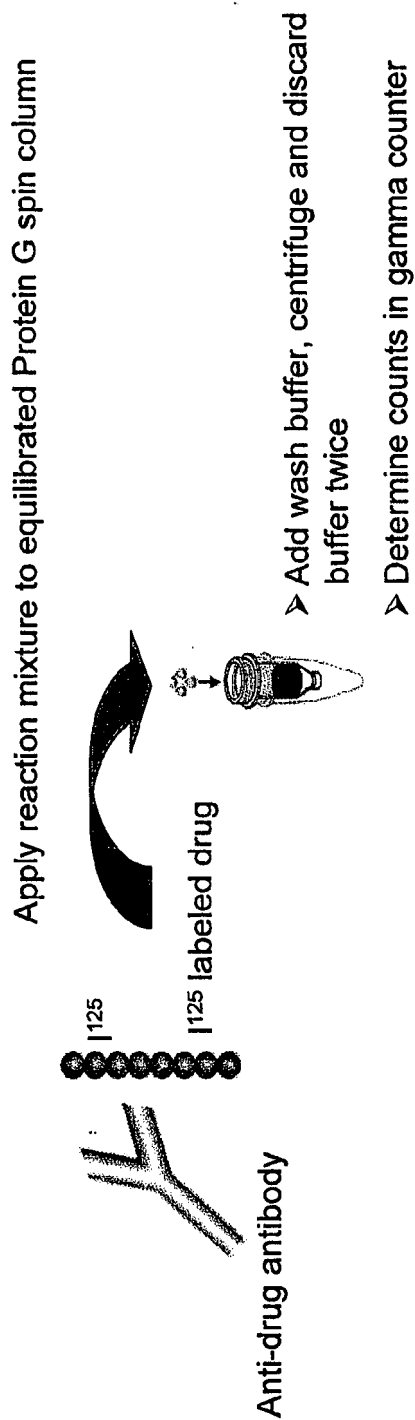
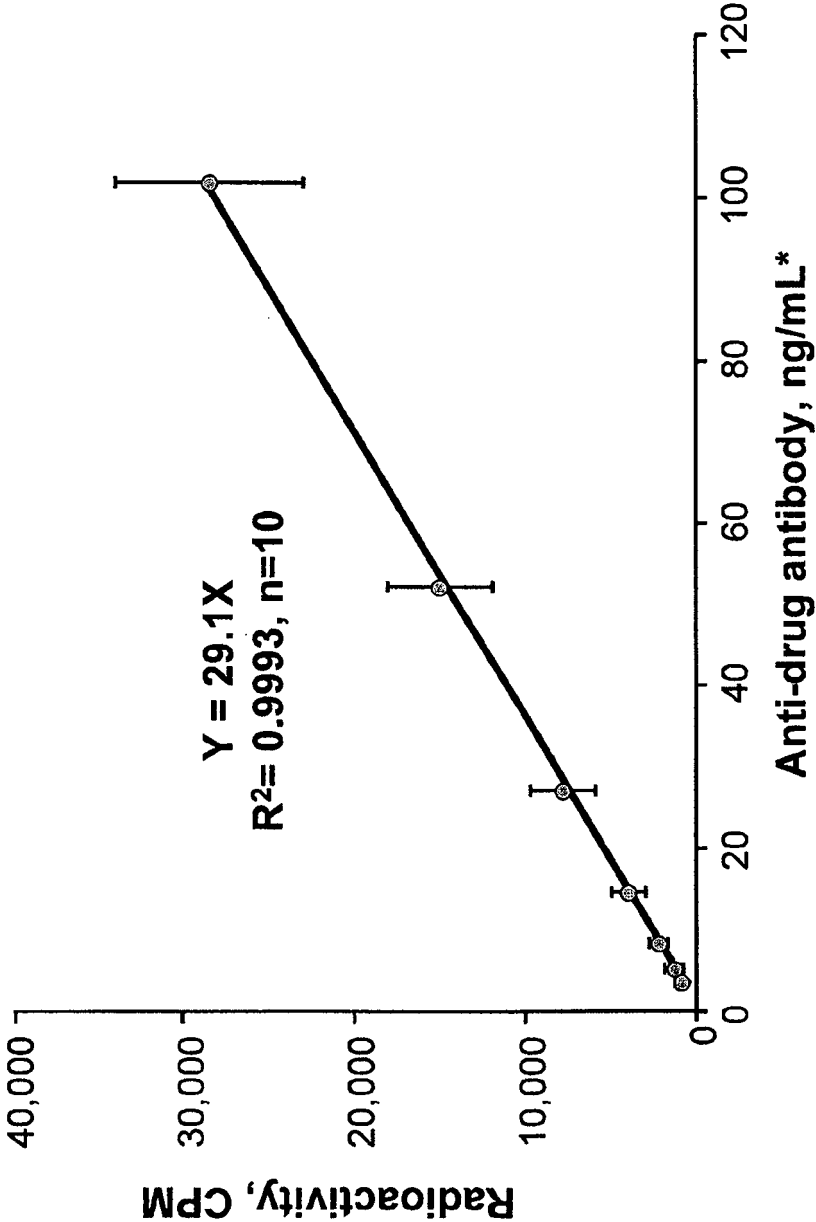


FIG. 24

RIP Assay Dose Response Curve



*equivalents relative to a mouse monoclonal IgG ADA

FIG. 25

IgA, IgM and IgE ADA Confirmatory Assays

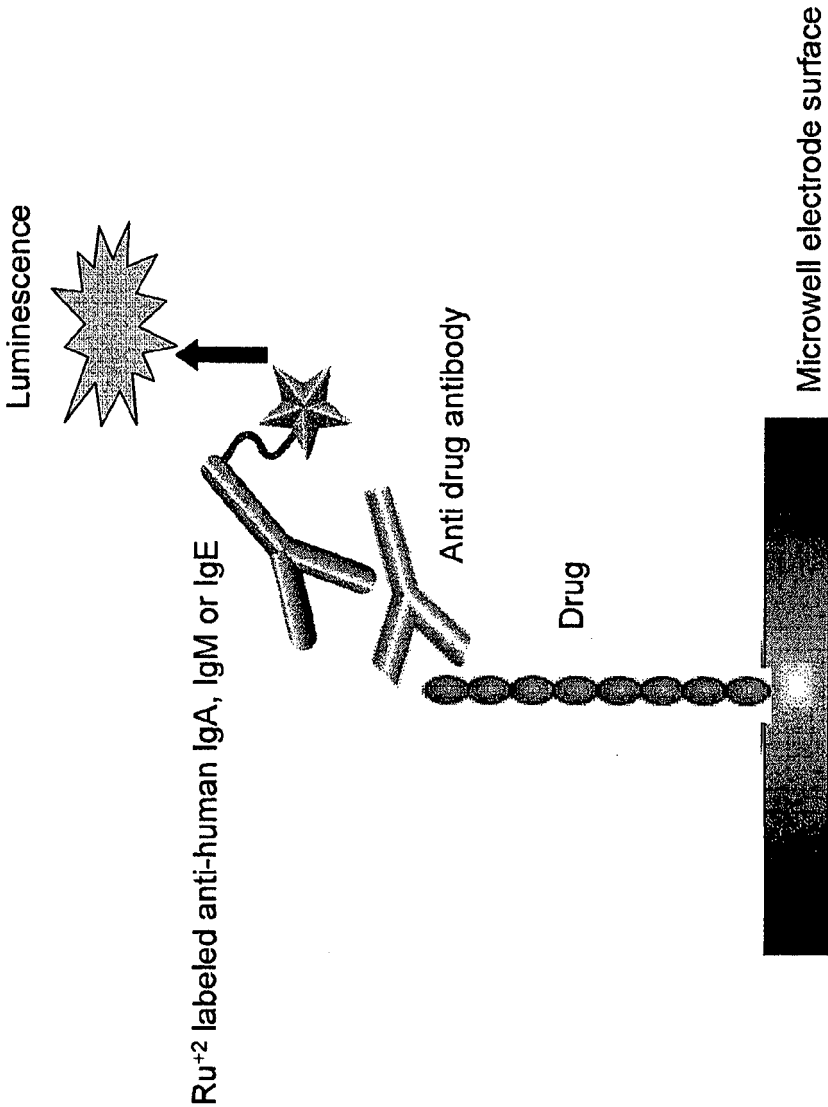


FIG. 26

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/28608

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/46; A61K 38/47 (2013.01)

USPC - 424/94.61; 435/7.92

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)
USPC 424/94.61; 435/7.92Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC: A61K 38/46; A61K 38/47 (See keywords below).

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

PatBase; Google Patents; Google Scholar; Gaucher disease; Type III; type 3; glucocerebrosidase; replacement therapy; Intravenous; infusion; minutes; hours; dose; dosage; velaglucerase; imiglucerase; uplyso; serum; modif*; IgE; IgM; IgG; analytical reagent; substrate; select*; subject; candidate; patient.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0027254 A1 (DANIEL et al.) 3 February 2011 (03.02.2011); Abstract; para [0005]-[0009], [0017]-[0031], [0336]-[0340].	1-21
A	US 2007/0031945 A1 (DANIEL et al.) 8 February 2007 (08.02.2007); entire document.	1-21
A	WO 2011/107992 A2 (RUDERFER et al.) 9 September 2011 (09.09.2011); entire document.	1-21
A	WO 2012/012461 A2 (RUIZ et al.) 26 January 2012 (26.01.2012); entire document.	1-21



Further documents are listed in the continuation of Box C.



* Special categories of cited documents:

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

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

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

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

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

"T"

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

"X"

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

"Y"

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

"&"

document member of the same patent family

Date of the actual completion of the international search

13 April 2013 (13.04.2013)

Date of mailing of the international search report

14 MAY 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:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

(19) 中华人民共和国国家知识产权局



(12) 发明专利申请

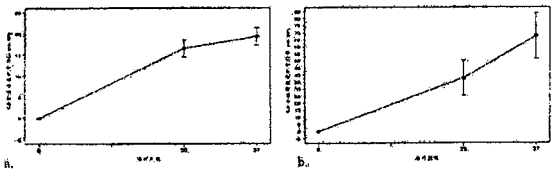
(10) 申请公布号 CN 104519905 A
(43) 申请公布日 2015. 04. 15

(21) 申请号 201380019869. 4
(22) 申请日 2013. 03. 01
(30) 优先权数据
61/606, 089 2012. 03. 02 US
(85) PCT国际申请进入国家阶段日
2014. 10. 14
(86) PCT国际申请的申请数据
PCT/US2013/028608 2013. 03. 01
(87) PCT国际申请的公布数据
W02013/130963 EN 2013. 09. 06
(71) 申请人 夏尔人类遗传性治疗公司
地址 美国马萨诸塞州
(72) 发明人 E · 克罗姆贝兹 K · 比兰基
G · M · 科恩
(74) 专利代理机构 中国国际贸易促进委员会专
利商标事务所 11038
代理人 刘晓东
(51) Int. Cl.
A61K 38/46(2006. 01)
A61K 38/47(2006. 01)

权利要求书2页 说明书106页 附图27页

(54) 发明名称
用于治疗 III 型戈谢病的组合物和方法

(57) 摘要
本发明描述了用于治疗戈谢病的方法和组合物。



1. 一种治疗患有 III 型戈谢病的受试者的方法,所述方法包括以下步骤:
通过在不到 2 小时的时间内向所述受试者静脉内输注来施用葡糖脑苷脂酶替代疗法,由此治疗所述受试者。
2. 根据权利要求 1 所述的方法,其中所述葡糖脑苷脂酶替代疗法在 90 分钟或更短的时间内施用。
3. 根据权利要求 1 所述的方法,其中所述葡糖脑苷脂酶替代疗法在 60 分钟或更短的时间内施用。
4. 根据权利要求 1 所述的方法,其进一步包括通过在不到 2 小时的时间内向所述受试者静脉内输注来施用第二葡糖脑苷脂酶替代疗法。
5. 根据权利要求 1 所述的方法,其中所述葡糖脑苷脂酶替代疗法选自由以下组成的组:维拉苷酶、伊米苷酶和 uplyso。
6. 根据权利要求 1 所述的方法,其中所述葡糖脑苷脂酶替代疗法以 15-60U/kg 的剂量施用。
7. 一种鉴定适合于用葡糖脑苷脂酶替代疗法治疗的受试者的方法,其包括以下步骤:
测定葡糖脑苷脂酶替代疗法的中和抗体是否存在于来自所述受试者的样品中以获得所述疗法的抗体的测量值,其中所述受试者当前正被施用第一葡糖脑苷脂酶替代疗法或其先前已接受第一葡糖脑苷脂酶替代疗法,和
将所述疗法的所述抗体测量值与标准比较,
其中,如果所述抗体测量值大于所述标准,则将所述受试者鉴定为具有所述葡糖脑苷脂酶替代疗法的抗体且为使用维拉苷酶的葡糖脑苷脂酶替代疗法的候选者,且如果所述抗体测量值不大于所述标准,则将所述受试者鉴定为使用所述第一葡糖脑苷脂酶替代疗法或备选葡糖脑苷脂酶替代疗法的葡糖脑苷脂酶替代疗法的候选者。
8. 根据权利要求 7 所述的方法,其中所述第一葡糖脑苷脂酶替代疗法为伊米苷酶或 uplyso。
9. 根据权利要求 7 所述的方法,其中所述样品为血液或血清样品。
10. 根据权利要求 9 所述的方法,其中所述血液或血清样品已经改良。
11. 根据权利要求 10 所述的方法,其中所述样品已与分析试剂或底物接触。
12. 根据权利要求 10 所述的方法,其中所述样品为血液或血清样品的浓缩部分。
13. 根据权利要求 7 所述的方法,其中所述受试者被鉴定为使用维拉苷酶的葡糖脑苷脂酶替代疗法的候选者,且所述方法进一步包括对所述受试者施用维拉苷酶。
14. 一种治疗当前正接受或先前已接受葡糖脑苷脂酶替代疗法的患有 III 型戈谢病的受试者的方法,其包括以下步骤:
根据受试者对于其当前接受或先前接受的戈谢病疗法的抗体的产生测试为阳性来选择受试者,和
对所述受试者施用维拉苷酶。
15. 根据权利要求 14 所述的方法,其中所述受试者对于其当前接受或先前接受的戈谢病疗法的 IgE 抗体的产生测试为阳性。
16. 根据权利要求 14 所述的方法,其中所述受试者对于其当前接受或先前接受的戈谢病疗法的 IgM 抗体的产生测试为阳性。

17. 根据权利要求 14 所述的方法, 其中所述受试者对于其当前接受或先前接受的戈谢病疗法的 IgG 抗体的产生测试为阳性。

18. 根据权利要求 14 所述的方法, 其中所述受试者当前接受或先前接受的戈谢病疗法为伊米苷酶。

19. 根据权利要求 14 所述的方法, 其中所述受试者当前接受或先前接受的戈谢病疗法为 uplyso。

20. 根据权利要求 14 所述的方法, 其中维拉苷酶以 15-60U/kg 的剂量施用。

21. 根据权利要求 14 所述的方法, 其中所述维拉苷酶在 90 分钟或更短时间内通过静脉内输注施用给所述受试者。

用于治疗 III 型戈谢病的组合物和方法

[0001] 相关申请案的交叉引用

[0002] 本申请要求 2012 年 3 月 2 日提交的美国申请系列号 61/606,089 的优先权。先前申请的公开内容被认为是本申请的公开内容的部分（并且通过引用并入本文）。

[0003] 背景

[0004] III 型戈谢病 (Gaucher disease) 为以溶酶体酶、葡糖脑苷脂酶 (GCB) 缺乏为特征的常染色体隐性溶酶体贮积病。GCB 使在白细胞和红细胞的膜中的鞘糖脂降解之后形成的糖脂葡糖脑苷脂水解。该酶的缺乏导致葡糖脑苷脂在位于 III 型戈谢病患者的肝、脾和骨髓中的巨噬细胞的溶酶体中大量积聚。这些分子的积聚引起多种临床表现,包括脾肿大、肝肿大、骨骼病症、血小板减少和贫血。(Beutler 等, Gaucher disease ;在 The Metabolic and Molecular Bases of Inherited Disease (McGraw-Hill, Inc, New York, 1995) 第 2625-2639 页中)。

[0005] 对于患该病的患者的治疗包括施用缓减骨痛的镇痛药、输注血液和血小板和在一些情形下实施脾切除术。对于经受骨侵蚀的患者有时需要关节置换术。

[0006] 已经将用 GCB 的酶替代疗法用作对 III 型戈谢病的治疗。对患有 III 型戈谢病的患者的当前治疗包括施用来源于用 GCB 表达构建体转染的人类胎盘或中国仓鼠卵巢 (CHO) 细胞的碳水化合物重塑 GCB 且所述碳水化合物重塑 GCB 分别称为阿糖脑苷酶或伊米苷酶 (imiglucerase)。

[0007] 概述

[0008] 本发明部分地基于在施用给受试者（例如,患有 III 型戈谢病的受试者）时与伊米苷酶相比维拉苷酶 (velaglucerase) 引起较少免疫响应（例如,产生较少抗体,例如,产生较少中和抗体）的发现。本发明特别涉及用于为患有 III 型戈谢病的受试者选择治疗、为维拉苷酶治疗（例如,单独或与另一疗法组合）选择受试者的组合物和方法、用于减少在经受对 III 型戈谢病的治疗的受试者中的注射部位反应的方法及治疗 III 型戈谢病的方法。本发明也特别涉及在对 III 型戈谢病进行治疗的受试者中测定抗体产生,例如中和抗体产生的方法。本公开内容至少部分地基于以下前提:葡糖脑苷脂酶替代疗法（例如,维拉苷酶、伊米苷酶或 uplyso）能够穿过血脑屏障,从而可用于治疗或预防或阻止神经学参数或神经学症状（例如与 III 型戈谢病相关的神经学参数或神经学症状）的进展。

[0009] 在一些方面,本发明的特点在于一种治疗患有 III 型戈谢病的受试者的方法,所述方法包括以下步骤:

[0010] 通过静脉内输注经小于 2 小时,例如 90 分钟、80 分钟、70 分钟、60 分钟、50 分钟或 45 分钟的时间对所述受试者施用葡糖脑苷脂酶替代疗法（例如,维拉苷酶、伊米苷酶或 uplyso）,由此治疗所述受试者。

[0011] 在一些实施方案中,所述葡糖脑苷脂酶替代疗法（例如,维拉苷酶、伊米苷酶、uplyso）施用不止一次且各额外剂量的葡糖脑苷脂酶替代疗法（例如,维拉苷酶、伊米苷酶或 uplyso）通过经小于 2 小时,例如 90 分钟、80 分钟、70 分钟、60 分钟、50 分钟或 45 分钟的时间静脉内施用给所述受试者来施用。

[0012] 在一些实施方案中,所述葡糖脑苷脂酶替代疗法(例如,维拉苷酶、伊米苷酶、uplyso)以15-60U/kg(例如,30U/kg-60U/kg,例如15U/kg、30U/kg、45U/kg或60U/kg)的剂量、以等于或小于22.5U/kg的剂量、以在22.5U/kg和37.5U/kg之间的剂量、以在37.5U/kg和52.5U/kg之间的剂量或以等于或大于52.5U/kg的剂量施用。在一些实施方案中,葡糖脑苷脂酶替代疗法(例如,维拉苷酶、伊米苷酶、uplyso)以2.5U/kg-60U/kg的剂量施用。在一些实施方案中,所述葡糖脑苷脂酶替代疗法(例如,维拉苷酶、伊米苷酶、uplyso)每隔一周施用。在其他实施方案中,所述葡糖脑苷脂酶替代疗法(例如,维拉苷酶、伊米苷酶、uplyso)每周施用。在一些实施方案中,所述维拉苷酶通过静脉内输注例如以2.5U/kg的剂量每周施用3次。

[0013] 在一些实施方案中,一种或多种剂量(例如,本文所述的剂量)的输注经60分钟发生。

[0014] 在一些实施方案中,所述葡糖脑苷脂酶替代疗法为维拉苷酶且所述方法包括以下步骤:

[0015] 用诸如灭菌注射水的药学上可接受的载体重构冻干的维拉苷酶(例如,用2.2mL灭菌注射水重构200单位小瓶或用4.3mL灭菌注射水重构400单位小瓶),由此形成溶液,例如,其中在加入所述灭菌注射水之后不振荡所述小瓶;任选地检查所述小瓶中的溶液(且,例如任选地测定所述溶液是否变色或是否存在微粒物质,且如果所述溶液变色或存在微粒物质,则任选决定不使用所述溶液);

[0016] 移出一定量的溶液以提供预选剂量(例如,本文所述的剂量,诸如15U/kg、30U/kg、45U/kg或60U/kg);

[0017] 例如在100mL适合于静脉内施用的0.9%氯化钠溶液中稀释所述量的溶液,由此形成稀溶液;任选轻轻摇动所述稀溶液,但不振荡所述稀溶液;和

[0018] 通过静脉内输注向所述受试者施用所述稀溶液,例如,其中经1小时或以1U/kg/min的速率施用所述稀溶液。

[0019] 在一些实施方案中,所述葡糖脑苷脂酶替代疗法以2U/kg/min、1.5U/kg/min、1U/kg/min或0.5U/kg/min的输注速率施用。

[0020] 在一些实施方案中,所述施用包括家庭疗法(例如,在受试者的家、工作场所或其他非临床(例如,非医院)环境中)。在一些实施方案中,所述施用(例如,经输注)由保健专业人员(例如,护士或医师助理)进行。例如,如果受试者例如在施用(例如,经输注)所述葡糖脑苷脂酶替代疗法一次、两次或三次之后,没有经历不良事件(AE)(例如,药物有关的严重AE或输注有关的AE,例如本文所述的事件),则所述受试者适宜接受家庭疗法以便进行后续施用。

[0021] 在另一方面,本发明提供一种鉴定适合于(例如,有希望)用葡糖脑苷脂酶替代疗法(例如,维拉苷酶)治疗的受试者的方法。所述方法包括评估(例如,例如通过本文所述的诸如ELISA或放射免疫沉淀测定(RIP)的方法测量)例如来自所述受试者的样品中是否存在葡糖脑苷脂酶替代疗法(例如,当前正对所述受试者施用的疗法)(例如,伊米苷酶或uplyso)的抗体(例如,中和抗体)(例如,IgE、IgM、IgG和/或IgA抗体)且将所述疗法的抗体的测量值与标准(例如,阴性对照)进行比较。例如,如果所述受试者具有大于阴性对照(例如,在ELISA中的阴性对照)测量值的抗体测量值,则所述受试者被鉴定为具有所

述葡萄糖脑苷脂酶替代疗法的抗体。例如,如果所述测量值比所述标准的值大例如5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%或90%以上,则所述受试者任选被鉴定为适合于用备选的 III 型戈谢病治疗(例如,用维拉苷酶治疗)进行治疗。

[0022] 在一些实施方案中,所述样品为血液或血清样品。在一些实施方案中,所述样品已被改良。例如,抗体测量值通过使样品与分析试剂、例如经标记的试剂(例如,经标记的葡萄糖脑苷脂酶)和/或底物或细胞、例如与葡萄糖脑苷脂酶替代疗法的抗体结合的底物或细胞接触来获得。在一些实施方案中,所述抗体测量值从已经富含抗体的样品、例如血液或血清样品的浓缩部分获得。在一些实施方案中,所述评估通过本文所述的方法获得。

[0023] 在一些实施方案中,所述方法包括例如通过本文所述的方法测量所述抗体测量值。

[0024] 在另一方面,本发明提供一种选择患有 III 型戈谢病的受试者以使用葡萄糖脑苷脂酶替代疗法进行治疗的方法。任选地,所述方法包括根据受试者是否产生其当前接受的 III 型戈谢病治疗的抗体(例如,中和抗体)经测试为阳性来选择所述治疗的受试者。所述方法包括评估(例如,例如通过本文所述的诸如 ELISA 或放射免疫沉淀测定(RIP)的方法测量)在例如来自所述受试者的样品中是否存在葡萄糖脑苷脂酶替代疗法(例如,当前正对受试者施用的疗法)(例如,伊米苷酶或 uplyso)的抗体或获得所述评估且将所述疗法的抗体的测量值与标准(例如,阴性对照)比较。例如,如果所述受试者具有大于阴性对照(例如,在 ELISA 中的阴性对照)测量值的抗体测量值,则所述受试者被鉴定为具有所述葡萄糖脑苷脂酶替代疗法的抗体。所述方法任选地包括:例如,如果所述测量值比所述标准(例如,阴性对照)的值大例如5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%或90%以上,则为所述受试者选择备选的 III 型戈谢病治疗(例如,用维拉苷酶治疗)。

[0025] 在一些实施方案中,所述样品为血液或血清样品。在一些实施方案中,所述样品已被改良。例如,抗体测量值通过使样品与分析试剂、例如经标记的试剂(例如,经标记的葡萄糖脑苷脂酶)和/或底物或细胞、例如与葡萄糖脑苷脂酶替代疗法的抗体结合的底物或细胞接触来获得。在一些实施方案中,所述抗体测量值从已经富含抗体的样品、例如血液或血清样品的浓缩部分获得。在一些实施方案中,所述评估通过本文所述的方法获得。

[0026] 在一些实施方案中,所述方法包括例如通过本文所述的方法测量所述抗体值。

[0027] 在一些实施方案中,受试者关于是否产生其当前接受的 III 型戈谢病治疗(例如,伊米苷酶或 uplyso)的 IgE 抗体经测试为阳性。

[0028] 在一些实施方案中,受试者关于是否产生其当前接受的 III 型戈谢病治疗(例如,伊米苷酶或 uplyso)的 IgM 抗体经测试为阳性。

[0029] 在一些实施方案中,受试者关于是否产生其当前接受的 III 型戈谢病治疗(例如,伊米苷酶或 uplyso)的 IgG 和/或 IgA 抗体经测试为阳性。

[0030] 在另一方面,本发明提供一种治疗患有 III 型戈谢病的受试者的方法。所述方法包括例如通过本文所述的方法、根据受试者是否产生其当前接受的 III 型戈谢病治疗的 IgG 和/或 IgA 抗体经测试为阳性或根据受试者处于产生 III 型戈谢病治疗(例如,伊米苷酶或 uplyso)的抗体(例如,中和抗体)的危险中而选择受试者,和对所述受试者施用维拉

苷酶。

[0031] 在一些实施方案中,受试者关于是否产生其当前接受的 III 型戈谢病治疗(例如,伊米苷酶或 uplyso)的 IgE 抗体经测试为阳性。

[0032] 在一些实施方案中,受试者关于是否产生其当前接受的 III 型戈谢病治疗(例如,伊米苷酶或 uplyso)的 IgM 抗体经测试为阳性。

[0033] 在一些实施方案中,受试者关于是否产生其当前接受的 III 型戈谢病治疗(例如,伊米苷酶或 uplyso)的 IgG 和 / 或 IgA 抗体经测试为阳性。

[0034] 在一些实施方案中,维拉苷酶以 15-60U/kg(例如,30U/kg-60U/kg,例如 15U/kg、30U/kg、45U/kg 或 60U/kg)的剂量、以等于或小于 22.5U/kg 的剂量、以在 22.5U/kg 和 37.5U/kg 之间的剂量、以在 37.5U/kg 和 52.5U/kg 之间的剂量或以等于或大于 52.5U/kg 的剂量施用。在一些实施方案中,维拉苷酶以 2.5-60U/kg 的剂量施用。在一些实施方案中,维拉苷酶每隔一周通过静脉内输注施用。在其他实施方案中,维拉苷酶每周通过静脉内输注施用。在一些实施方案中,维拉苷酶通过静脉内输注例如以 2.5U/kg 的剂量每周施用 3 次。

[0035] 在一些实施方案中,所述剂量(例如,本文所述的剂量)的输注经小于 2 小时、例如 90 分钟、80 分钟、70 分钟、60 分钟、50 分钟或 45 分钟发生。在优选的实施方案中,所述剂量的输注经 60 分钟发生。

[0036] 在一些实施方案中,所述方法包括以下步骤:

[0037] 用诸如灭菌注射水的药学上可接受的载体诸如药学上可接受的载体重构冻干的维拉苷酶(例如,用 2.2mL 灭菌注射水重构 200 单位小瓶或用 4.3mL 灭菌注射水重构 400 单位小瓶),由此形成溶液,例如,其中在加入所述灭菌注射水之后不振荡所述小瓶;任选地,检查所述小瓶中的溶液(且,例如任选地测定所述溶液是否变色或是否存在微粒物质,且如果所述溶液变色或存在微粒物质,则任选地决定不使用所述溶液);

[0038] 移出一定量的溶液以提供预选剂量(例如,本文所述的剂量,诸如 15U/kg、30U/kg、45U/kg 或 60U/kg);

[0039] 例如在 100mL 适合静脉内施用的 0.9%氯化钠溶液中稀释所述量的溶液,由此形成稀溶液;任选地轻轻摇动所述稀溶液,但不振荡所述稀溶液;和

[0040] 通过静脉内输注对所述受试者施用所述稀溶液。

[0041] 在一些实施方案中,维拉苷酶以 2U/kg/min、1.5U/kg/min、1U/kg/min 或 0.5U/kg/min 的速率施用。

[0042] 在一些实施方案中,所述施用包括家庭疗法(例如,在受试者的家、工作场所或其他非临床(例如,非医院)环境中)。在一些实施方案中,所述施用(例如,经输注)由保健专业人员(例如,护士或医师助理)进行。例如,如果受试者例如在施用(例如,经输注)维拉苷酶一次、两次或三次之后,没有经历不良事件(AE)(例如,药物有关的严重 AE 或输注有关的 AE,例如本文所述的事件),则所述受试者适宜接受家庭疗法以便进行连续施用。

[0043] 在另一方面,本发明提供一种鉴定适合于(例如,有希望)用葡糖脑苷脂酶替代疗法(例如,维拉苷酶)治疗的受试者的方法。所述方法包括(例如)评估(例如,测量)所述受试者(例如,在输注葡糖脑苷脂酶替代疗法 12 小时期间或 12 小时之内)(例如,在当前正施用给受试者的疗法中,例如伊米苷酶或 uplyso)是否存在输注部位反应(即,输

注有关的不良事件)且将所述部位反应的测量水平与标准(例如,阴性对照)比较。例如,如果所述受试者在受试者当前接受的 III 型戈谢病治疗中的部位反应的测量值大于阴性对照(例如,在受试者体内输注安慰剂之后观察到的部位反应)的测量值,例如,如果在受试者当前接受的 III 型戈谢病治疗中的部位反应的测量值比所述标准值大例如 5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%或 90%以上,则将所述受试者鉴定为适合用备选的 III 型戈谢病治疗(例如,用维拉昔酶的治疗)进行治疗。

[0044] 在一些实施方案中,所述方法包括例如通过本文所述的方法测定输注部位值。

[0045] 在另一方面,本发明提供一种选择患有 III 型戈谢病的受试者以使用葡萄糖脑苷脂酶替代疗法进行治疗的方法。任选地,所述方法包括根据受试者需要降低输注部位反应(例如,与受试者当前接受的 III 型戈谢病治疗(例如,伊米昔酶或 uplyso)相关或由其引起的反应相比降低)来选择所述治疗的受试者。所述方法包括(例如)评估(例如,测量)所述受试者(例如,在输注葡萄糖脑苷脂酶替代疗法 12 小时期间或 12 小时之内)(例如,在当前施用给受试者的疗法中,例如伊米昔酶或 uplyso))是否存在输注部位反应(即,输注有关的不良事件)或获得所述评估且将所述部位反应的测量水平与标准(例如,阴性对照)比较。例如,如果所述受试者在受试者当前接受的 III 型戈谢病治疗中的部位反应的测量值大于阴性对照(例如,在受试者体内输注安慰剂之后观察到的部位反应)的测量值(例如,大 5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%或 90%以上),例如,如果在受试者当前接受的 III 型戈谢病治疗中的部位反应的测量值比所述标准(例如,阴性对照)值大例如 5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%或 90%以上,则选择所述受试者进行备选的 III 型戈谢病治疗(例如,用维拉昔酶治疗)。

[0046] 在一些实施方案中,所述方法包括例如通过本文所述的方法测量抗体值。

[0047] 在另一方面,本发明提供一种治疗患有 III 型戈谢病的受试者的方法。所述方法包括例如通过本文所述的方法、根据受试者需要降低输注部位反应(例如,与受试者当前接受的 III 型戈谢病治疗(例如,伊米昔酶或 uplyso)相关或由其引起的反应相比降低)来选择所述治疗的受试者,和对所述受试者施用维拉昔酶。

[0048] 在一些实施方案中,维拉昔酶以 15-60U/kg(例如,30U/kg-60U/kg,例如 15U/kg、30U/kg、45U/kg 或 60U/kg)的剂量、以等于或小于 22.5U/kg 的剂量、以在 22.5U/kg 和 37.5U/kg 之间的剂量、以在 37.5U/kg 和 52.5U/kg 之间的剂量或以等于或大于 52.5U/kg 的剂量施用。在一些实施方案中,维拉昔酶以 2.5-60U/kg 的剂量施用。在一些实施方案中,维拉昔酶每隔一周通过静脉内输注施用。在其他实施方案中,维拉昔酶每周通过静脉内输注施用。在一些实施方案中,维拉昔酶通过静脉内输注例如以 2.5U/kg 的剂量每周施用 3 次。

[0049] 在一些实施方案中,所述剂量(例如,本文所述的剂量)的输注经小于 2 小时、例如 90 分钟、80 分钟、70 分钟、60 分钟、50 分钟或 45 分钟发生。

[0050] 在一些实施方案中,所述方法包括以下步骤:

[0051] 用例如灭菌注射水的药学上可接受的载体重构冻干的维拉昔酶(例如,用 2.2mL 灭菌注射水重构 200 单位小瓶或用 4.3mL 灭菌注射水重构 400 单位小瓶),由此形成溶

液,例如,其中在加入所述灭菌注射水之后不振荡所述小瓶;任选地检查所述小瓶中的溶液(且,例如任选地测定所述溶液是否变色或是否存在微粒物质,且如果所述溶液变色或存在微粒物质,则任选地决定不使用所述溶液);

[0052] 移出一定量的溶液以提供预选剂量(例如,本文所述的剂量,诸如 15U/kg、30U/kg、45U/kg 或 60U/kg);

[0053] 例如在 100mL 适合静脉内施用的 0.9%氯化钠溶液中稀释所述量的溶液,由此形成稀溶液;任选地轻轻摇动所述稀溶液,但不振荡所述稀溶液;和

[0054] 通过静脉内输注对所述受试者施用所述稀溶液。

[0055] 在一些实施方案中,维拉昔酶以 2.0U/kg/min、1.5U/kg/min、1.0U/kg/min 或 0.5U/kg/min 的速率施用。

[0056] 在一些实施方案中,所述施用包括家庭疗法(例如,在受试者的家、工作场所或其他非临床(例如,非医院)环境中)。在一些实施方案中,所述施用(例如,经输注)由保健专业人员(例如,护士或医师助理)进行。例如,如果受试者例如在施用(例如,经输注)维拉昔酶一次、两次或三次之后,没有经历不良事件(AE)(例如,药物有关的严重 AE 或输注有关的 AE,例如本文所述的事件),则所述受试者适宜接受家庭疗法以便进行后续施用。

[0057] 在一些方面,本发明的特点在于一种选择患有 III 型戈谢病的受试者以便使用维拉昔酶治疗的方法,所述方法包括以下步骤:

[0058] 鉴定已接受葡糖脑苷脂酶替代疗法(例如,伊米昔酶或 uplyso)且具有小于标准的小血小板计数的患有 III 型戈谢病的受试者;和

[0059] 根据受试者具有小于标准的小血小板计数来选择受试者以便使用维拉昔酶治疗。

[0060] 在一些实施方案中,所述方法包括评估(例如,测量)来自所述受试者的样品的小血小板计数或获得所述血小板计数的估值并将所述血小板计数的测量值与标准(例如,阴性对照)比较。

[0061] 在一些实施方案中,所述样品为血液或血清样品。在一些实施方案中,所述样品已被改良。在一些实施方案中,所述抗体测量值从已经富含血小板的样品、例如血样的浓缩部分获得。在一些实施方案中,所述评估通过本文所述的方法获得。

[0062] 在一些实施方案中,所述方法包括例如通过本文所述的方法测量血小板计数。

[0063] 在一些实施方案中,所述方法进一步包括例如以本文所述的剂量和/或给药方案对所述受试者施用维拉昔酶。

[0064] 在一些实施方案中,维拉昔酶通过静脉内输注经小于 2 小时、例如 90 分钟、80 分钟、70 分钟、60 分钟、50 分钟或 45 分钟的时程施用。在一些实施方案中,所述输注以 2U/kg/min、1.5U/kg/min、1U/kg/min 或 0.5U/kg/min 的速率发生。

[0065] 在一些实施方案中,所述标准为低于或等于 80×10^3 个血小板/立方毫米的血小板计数。在一些实施方案中,所述标准根据用葡糖脑苷脂酶替代疗法治疗 6、9 或 12 个月之后平均血小板计数的增加百分数。例如,与开始葡糖脑苷脂酶替代疗法(例如,伊米昔酶治疗,例如以 60U/kg 的剂量的伊米昔酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后平均血小板计数增加小于 80%、75%、70%、65%、60%或 55%的受试者被鉴定为适合用维拉昔酶治疗。作为另一实例,与开始葡糖脑苷脂酶替代疗法(例如,伊米昔酶治疗,例如以 60U/kg 的剂量的伊米昔酶,例如每隔一周施用历

时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后平均血小板计数增加小于 40%、35%、30%或 25%的受试者被鉴定为适合用维拉昔酶治疗。

[0066] 在一些方面,本发明的特点在于一种治疗患有 III 型戈谢病的受试者的方法,所述方法包括以下步骤:

[0067] 选择已经接受葡糖脑苷脂酶替代疗法(例如,伊米昔酶或 uplyso)且具有小于标准的血小板计数的患有 III 型戈谢病的受试者,例如通过本文所述的方法鉴定出的受试者;和

[0068] 对所述受试者施用维拉昔酶。

[0069] 在一些实施方案中,维拉昔酶以 15-60U/kg(例如,30U/kg-60U/kg,例如,15U/kg、30U/kg、45U/kg 或 60U/kg)的剂量、以等于或小于 22.5U/kg 的剂量、以在 22.5U/kg 和 37.5U/kg 之间的剂量、以在 37.5U/kg 和 52.5U/kg 之间的剂量或以等于或大于 52.5U/kg 的剂量施用。在一些实施方案中,维拉昔酶以 2.5-60U/kg 的剂量施用。在一些实施方案中,维拉昔酶每隔一周通过静脉内输注施用。在其他实施方案中,维拉昔酶每周通过静脉内输注施用。在一些实施方案中,维拉昔酶通过静脉内输注例如以 2.5U/kg 的剂量每周施用 3 次。

[0070] 在一些实施方案中,所述剂量(例如,本文所述的剂量)的输注经小于 2 小时、例如 90 分钟、80 分钟、70 分钟、60 分钟、50 分钟或 45 分钟发生。

[0071] 在一些实施方案中,所述方法包括以下步骤:

[0072] 用诸如灭菌注射水的药学上可接受的载体重构冻干的维拉昔酶(例如,用 2.2mL 灭菌注射水重构 200 单位小瓶或用 4.3mL 灭菌注射水重构 400 单位小瓶),由此形成溶液,例如,其中在加入所述灭菌注射水之后不振荡所述小瓶;任选地检查所述小瓶中的溶液(且,例如任选地测定所述溶液是否变色或是否存在微粒物质,且如果所述溶液变色或存在微粒物质,则任选地决定不使用所述溶液);

[0073] 移出一定量的溶液以提供预选剂量(例如,本文所述的剂量,诸如 15U/kg、30U/kg、45U/kg 或 60U/kg);

[0074] 例如在 100mL 适合静脉内施用的 0.9%氯化钠溶液中稀释所述量的溶液,由此形成稀溶液;任选地轻轻摇动所述稀溶液,但不振荡所述稀溶液;和

[0075] 通过静脉内输注对所述受试者施用所述稀溶液。

[0076] 在一些实施方案中,维拉昔酶以 2U/kg/min、1.5U/kg/min、1U/kg/min 或 0.5U/kg/min 的速率施用。

[0077] 在一些实施方案中,所述施用包括家庭疗法(例如,在受试者的家、工作场所或其他非临床(例如,非医院)环境中)。在一些实施方案中,所述施用(例如,经输注)由保健专业人员(例如,护士或医师助理)进行。例如,如果受试者例如在施用(例如,经输注)维拉昔酶一次、两次或三次之后,没有经历不良事件(AE)(例如,药物有关的严重 AE 或输注有关的 AE,例如本文所述的事件),则所述受试者适宜接受家庭疗法以便进行连续施用。

[0078] 在另一方面,本发明提供一种评估受试者、例如当前正被施用葡糖脑苷脂酶替代疗法(例如,伊米昔酶、维拉昔酶或 uplyso)的受试者的方法。所述方法包括评估(例如,测量)所述受试者的以下参数中的一项或多项(例如,1、2、3、4、5、6 或 7 项)或获得其估值;

[0079] 血红蛋白浓度、血小板计数、肝体积（例如，以占总体重的百分数形式）、脾体积（例如，以占总体重的百分数形式）、输注部位反应、骨骼参数、神经学参数或是否存在葡萄糖脑苷脂酶替代疗法的抗体（例如，中和抗体）（例如，IgE、IgM、IgG 和 / 或 IgA 抗体）（例如，这些参数中一项或多项（例如，1、2、3、4、5、6、7 或 8 项）的平均值）。

[0080] 骨骼参数可以为例如骨矿密度 (BMD)（例如，如通过 Z- 分值的改变（例如，改进）所测量）。BMD 可以例如通过双能量 X 线吸收测定法 (DEXA) 评估。可以评估的其他骨骼参数包括例如 T- 分值（例如，测定正常骨骼、骨量减少和骨质疏松的 WHO 分类）、受试者（例如，儿科受试者）的生长；受试者（例如，儿科受试者）的骨骼年龄；和骨髓负担 (BMB)（例如，在成年受试者中）。

[0081] 神经学参数可以是例如眼运动参数（例如，异常眼运动、异常垂直扫视、异常水平扫视、缓慢水平扫视和核上性凝高麻痹）。评价眼运动参数的方法可包括但不限于例如一般眼科评估和扫视眼球运行评估（例如，巩膜搜索线圈技术、视频技术和红外技术）。神经学参数可以是听力参数（例如，听力丧失）。评估听力参数的方法可包括但不限于一般听觉评估。神经学参数可以是异常脑干听觉诱发电位、颅神经麻痹、咬粘、进行性肌阵挛性癫痫、颤抖、EEG 异常、痴呆、认知缺损、共济失调、肌协调的丧失和较差的移动性 (poor mobility)。评估神经学参数的方法可包括但不限于例如 EEG、脑干听觉诱发响应 (BAER)、听觉诱发电位的测量（例如，电极帽）、体感觉诱发电位的测量（例如，正中神经刺激和拉伸诱发法）、精神状态评估（例如，神经心理学精神状态检查）、脑神经检查、运动技能评估（例如，机动能力的 Bruininks-Oseretsky 测试、学校功能评估测试、视觉 - 动作整合作用的 Beery-Buktenica 发展测试、运动和姿势技巧的临床观察 (COMPS)）、咬粘评估（例如，神经影像（例如，CT、MRI、弥散加权成像）、EEG、患者病史、体格检查）、手灵巧度和手眼协调测试（例如，普渡钉板测试）、标准化 IQ 测试（例如，韦氏智力量表）、口头表演全量表智商测试、雷伊听觉言语学习测试、注意力的测量（例如，注意力的 D2 测试）、连续性能测试、接龙测试、本顿视觉体质测验、记忆力的测量（例如，修订的韦氏记忆量表、刺激呈现后被召回 (presentation of stimuli to later be recalled)、加利福尼亚言语学习测试、雷伊听觉言语学习测试、选择性提醒测试、修订的本顿视觉保持测试、记忆作图测试、Rey-O 复杂图形）、认知功能的评估（例如，韦氏相似性分测试、威斯康辛卡片分类测验、范畴测试、斯特鲁测试、神经系统试验电池 (neurological test batteries)（例如，柔性电池、霍耳斯特德 - 赖坦神经心理学成套测验 (Halstead-Reitan neuropsychological battery)、鲁利亚 - 内布拉斯加神经心理学成套测验 (Luria-Nebraska Neuropsychological Battery)）和心理计量测试。所述评估可以在治疗过程中例如约每周、约每两周、约每三周、约每四周、约每两个月、约每三个月、约每四个月、约每五个月、约每六个月、约每七个月、约每八个月、约每九个月、约每十个月、约每十一个月或约每十二个月进行一次。所述评估也可以在开始治疗之前进行（例如，用以确立基准值）。所述评估可以包括将来自受试者的参数的值与标准（例如，本文所述的标准，例如，阴性对照）比较和任选地测定来自受试者的参数的值与来自标准的值之间是否存在差异。所述标准可以为例如在针对 III 型戈谢病用不同疗法（例如，本文所述的治疗）治疗的患有 III 型戈谢病的受试者中测量的参数的值，或一群这样的受试者（例如，在相同时间长度的治疗之后）的平均值，或在开始疗法之前（例如，在开始葡萄糖脑苷脂酶替代疗法（例如，伊米苷酶或维拉苷酶）之前）受试者的基准值（或一群

患有 III 型戈谢病的受试者的平均基准值)。例如,可以评估骨骼参数(例如,骨矿密度)在例如葡萄糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)1、2、3、4 或更多年之后的长期改变。

[0082] 所述评估可以包括测定是否存在以下一项或多项:

[0083] 在血红蛋白浓度、血小板计数、肝体积、脾体积、神经学参数或骨骼参数中一项或多项之间的差异,与标准(对于给定参数)之间的差异大于 5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85% 或 90%。或者或另外,所述评估可以包括测定是否存在输注部位反应(例如,在输注后 12 小时期间或 12 小时内)和/或是否存在葡萄糖脑苷脂酶替代疗法(例如,伊米苷酶或 uplyso)的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)。

[0084] 在一些实施方案中,所述评估可以为以下一项或多项:血红蛋白浓度、血小板计数和测定葡萄糖脑苷脂酶替代疗法(例如,伊米苷酶或 uplyso)的抗体(例如,中和抗体)是否存在。在一些实施方案中,这些参数中一项或多项的评估是对于来自受试者的样品,例如血液或血清样品进行的。在一些实施方案中,所述样品已被改良。例如,所述值通过使样品与分析试剂和/或底物或细胞,例如与葡萄糖脑苷脂酶替代疗法的抗体结合的底物或细胞接触来获得。在一些实施方案中,所述值从已经富含例如血红蛋白、血小板和/或抗体的样品,例如血液或血清样品的浓缩部分获得。在一些实施方案中,所述评估通过本文所述的方法获得。

[0085] 根据所述测定,可以对受试者作出治疗决策。例如,如果接 III 型戈谢病治疗如葡萄糖脑苷脂酶替代疗法(例如,伊米苷酶或 uplyso)的受试者的一项或多项参数的值与标准(例如,接受不同疗法(维拉苷酶)的患有 III 型戈谢病的受试者)的值不同,则可以作出决策将当前接受葡萄糖脑苷脂酶替代治疗(例如,伊米苷酶治疗)的受试者转变为不同的葡萄糖脑苷脂酶替代治疗(例如,维拉苷酶治疗)。例如,如果在接受伊米苷酶治疗的受试者中检测到伊米苷酶的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体),则可以将受试者转变为用维拉苷酶治疗。作为另一实例,如果在对接受伊米苷酶治疗的受试者施用伊米苷酶的 12 小时期间或 12 小时内检测到输注部位反应,则可以将受试者转变为用维拉苷酶治疗。作为另一实例,如果与以相同剂量和相同治疗施用频率用维拉苷酶治疗相同持续时间的一群患有 III 型戈谢病的受试者获得的平均血小板计数值相比,在接受伊米苷酶治疗的受试者中的平均血小板计数较低(例如,低 5%、10%、%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85% 或 90%),则可以将受试者转变为维拉苷酶治疗。

[0086] 在另一方面,本发明提供一种选择疗法以施用给患有 III 型戈谢病的受试者的方法,所述方法包括以下步骤:

[0087] 根据以下标准来选择治疗:治疗可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶或 uplyso)的一群受试者的可能性)、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和/或减小产生所述治疗的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例

如,伊米苷酶或 uplyso) 的一群受试者的可能性)。任选地,所述方法可以包括对受试者提供治疗,例如,其中提供包括施用或转变受试者所接受的治疗。在一些实施方案中,所述治疗可以本文所述的剂量和 / 或给药方案施用。

[0088] 所述方法可以包括例如通过本文所述的方法评估(例如,测量)这些参数中的一项或多项或获得其估值。

[0089] 一方面,本发明提供一种选择疗法以施用给需要增加血红蛋白浓度、增加血小板水平、减小肝体积、减小脾体积、减小注射部位反应的可能性、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和 / 或减小产生治疗的抗体的可能性的患者,所述方法包括以下步骤:

[0090] 根据以下标准来选择治疗:治疗可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗的一群受试者的可能性)、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和 / 或减小产生所述治疗的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和 / 或 IgA 抗体)的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗的一群受试者的可能性)。任选地,所述方法可以包括对受试者提供治疗,例如,其中提供包括施用或转变受试者所接受的治疗。在一些实施方案中,所述治疗可以本文所述的剂量和 / 或给药方案施用。

[0091] 所述方法可以包括例如通过本文所述的方法评估(例如,测量)这些参数中的一项或多项或获得其估值。

[0092] 在另一方面,本发明提供一种评估受试者、例如被选定以接受用葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso) 治疗的受试者的方法。所述方法包括评估(例如,测量)所述受试者的以下参数中的一项或多项(例如,1、2、3、4、5、6、7 或 8 项):

[0093] 血红蛋白浓度、血小板计数、肝体积(例如,以占总体重的百分数形式)、脾体积(例如,以占总体重的百分数形式)、输注部位反应、骨骼参数、神经学参数或葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和 / 或 IgA 抗体)的存在(例如,这些参数中一项或多项(例如,1、2、3、4、5、6、7 或 8 项)的平均值)。

[0094] 骨骼参数可以为例如骨矿密度(BMD)(例如,如通过 Z- 分值的改变(例如,改进)测量)。BMD 可以例如通过双能量 X 线吸收测定法(DEXA) 评估。可以评估的其他骨骼参数包括例如 T- 分值(例如,测定正常骨骼、骨量减少和骨质疏松的 WHO 分类)、受试者(例如,儿科受试者)的生长;受试者(例如,儿科受试者)的骨骼年龄;和骨髓负担(BMB)(例如,在成年受试者中)。

[0095] 神经学参数可以是例如眼运动参数(例如,异常眼运动、异常垂直扫视、异常水平扫视、缓慢水平扫视和核上性凝高麻痹)。评价眼运动参数的方法可包括但不限于例如一般眼科评估和扫视眼球运行评估(例如,巩膜搜索线圈技术、视频技术和红外技术)。神经学参数可以是听力参数(例如,听力丧失)。评估听力参数的方法可包括但不限于一般听觉评估。神经学参数可以是异常脑干听觉诱发电位、颅神经麻痹、咬粘、进行性肌阵挛性癫痫、颤抖、EEG 异常、痴呆、认知缺损、共济失调、肌协调的丧失和较差的移动性。评估神经学参数的方法可包括但不限于例如 EEG、脑干听觉诱发响应(BAER)、听觉诱发电位的测量(例如,电极帽)、体感觉诱发电位的测量(例如,正中神经刺激和拉伸诱发法)、精神状

态评估（例如，神经心理学精神状态检查）、脑神经检查、运动技能评估（例如，机动能力的 Bruininks-Oseretsky 测试、学校功能评估测试、视觉-动作整合作用的 Beery-Buktenica 发展测试、运动和姿势的技巧的临床观察（COMPS）、咬粘评估（例如，神经影像（例如，CT、MRI、弥散加权成像）、EEG、患者病史、体格检查）、手灵巧度和手眼协调测试（例如，普渡钉板测试）、标准化 IQ 测试（例如，韦氏智力量表）、口头表演全量表智商测试、雷伊听觉言语学习测试、注意力的测量（例如，注意力的 D2 测试）、连续性能测试、接龙测试、本顿视觉体质测验、记忆力的测量（例如，修订的韦氏记忆量表、刺激呈现后被召回、加利福尼亚言语学习测试、雷伊听觉言语学习测试、选择性提醒测试、修订的本顿视觉保持测试、记忆作图测试、Rey-O 复杂图形），认知功能的评估（例如，韦氏相似性分测试、威斯康辛卡片分类测验、范畴测试、斯特鲁测试、神经系统试验电池（例如，柔性电池、霍耳斯特德-赖坦神经心理学成套测验、鲁利亚-内布拉斯加神经心理学成套测验）和心理计量测试。所述评估可以包括将来自受试者的参数的值与标准（例如，本文所述的标准，例如，阴性对照）进行比较和任选地测定在来自所述受试者的参数的值与来自标准的值之间是否存在差异。所述标准可以为例如在用 III 型戈谢病的不同疗法（例如，本文所述的治疗）治疗的患有 III 型戈谢病的受试者中测量的参数的值，或一群这样的受试者（例如，在相同时间长度的治疗之后）的平均值，或在开始疗法之前（例如，在开始疗法之前受试者的基准值（或一群患有 III 型戈谢病的受试者的平均基准值））。

[0096] 所述评估可以包括测定：

[0097] 在血红蛋白浓度、血小板计数、肝体积、脾体积、神经学参数或骨骼参数（例如，测量为骨矿密度（BMD））中的一项或多项与标准（对于给定参数）之间的差异是否大于 5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85% 或 90%。

[0098] 在一些实施方案中，所述评估可以为以下一项或多项：血红蛋白浓度、血小板计数和测定葡糖脑苷脂酶替代疗法（例如，伊米苷酶或 uplyso）的抗体（例如，中和抗体）是否存在。在一些实施方案中，这些参数中一项或多项的评估是对于来自受试者的样品，例如血液或血清样品进行的。在一些实施方案中，所述样品已被改良。例如，所述值通过使样品与分析试剂和 / 或底物或细胞，例如与葡糖脑苷脂酶替代疗法的抗体结合的底物或细胞接触来获得。在一些实施方案中，所述值从已经富含例如血红蛋白、血小板和 / 或抗体的样品、例如血液或血清样品的浓缩部分获得。在一些实施方案中，所述评估通过本文所述的方法获得。

[0099] 在另一方面，本发明提供一种医瞩葡糖脑苷脂酶替代疗法（例如，伊米苷酶、维拉苷酶或 uplyso）的方法，所述方法包括以下步骤：

[0100] 接受所述葡糖脑苷脂酶替代疗法的名标，例如所述葡糖脑苷脂酶替代疗法的化学结构、化学名称、商品名称或通用名称；

[0101] 接受所述葡糖脑苷脂酶替代疗法具有以下性质中的一种或多种的信息：可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性（例如，相对于标准、例如本文所述的标准，例如接受 III 型戈谢病的不同治疗（例如，伊米苷酶或 uplyso）的一群受试者的可能性）、改变骨骼参数（例如，增加骨矿密度）、神经学参数的变化（例如，神经学参数的改善）和 / 或减小产生所述治疗的抗体（例如，中和抗体）

(例如, IgE、IgM、IgG 和 / 或 IgA 抗体) 的可能性 (例如, 相对于标准、例如本文所述的标准, 例如接受 III 型戈谢病的不同治疗 (例如, 伊米苷酶或 uplyso) 的一群受试者的可能性);

[0102] 例如根据受试者需要以下一项或多项来选择需要所述葡糖脑苷脂酶替代疗法的受试者: 增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性 (例如, 相对于标准、例如本文所述的标准, 例如接受 III 型戈谢病的不同治疗 (例如, 伊米苷酶或 uplyso) 的一群受试者的可能性)、改变骨骼参数 (例如, 增加骨矿密度)、神经学参数的变化 (例如, 神经学参数的改善) 和 / 或减小产生所述治疗的抗体 (例如, 中和抗体) (例如, IgE、IgM、IgG 和 / 或 IgA 抗体) 的可能性 (例如, 相对于标准、例如本文所述的标准, 例如接受 III 型戈谢病的不同治疗 (例如, 伊米苷酶或 uplyso) 的一群受试者的可能性); 和

[0103] 将所述葡糖脑苷脂酶替代疗法医嘱、分配或施用给受试者。

[0104] 在一些实施方案中, 所述受试者需要以下一项或多项: 增加血红蛋白浓度、增加血小板计数和减小产生葡糖脑苷脂酶替代疗法 (例如, 伊米苷酶或 uplyso) 的抗体 (例如, 中和抗体) 或神经学参数的变化 (例如, 神经学参数的改善) 的可能性。在一些实施方案中, 受试者需要这些改变中的一项或多项的测定是根据对来自受试者的样品, 例如血液或血清样品进行的这些参数中一项或多项的评估。在一些实施方案中, 所述样品已被改良。例如, 所述值通过使样品与分析试剂和 / 或底物或细胞, 例如与葡糖脑苷脂酶替代疗法的抗体结合的底物或细胞接触来获得。在一些实施方案中, 所述值从已经富含例如血红蛋白、血小板和 / 或抗体的样品, 例如血样的浓缩部分获得。在一些实施方案中, 所述评估通过本文所述的方法获得。

[0105] 在另一方面, 本发明提供一种向接受者提供关于使用葡糖脑苷脂酶替代疗法 (例如, 伊米苷酶或维拉苷酶) 的信息或使用葡糖脑苷脂酶替代疗法 (例如, 伊米苷酶或维拉苷酶) 的准则的方法, 所述方法包括以下步骤:

[0106] 向所述接受者传递所述葡糖脑苷脂酶替代疗法的名标, 例如所述葡糖脑苷脂酶替代疗法的化学结构、化学名称、商品名称或通用名称;

[0107] 向所述接受者传递所述葡糖脑苷脂酶替代疗法具有以下性质中的一种或多种的信息: 可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性 (例如, 相对于标准、例如本文所述的标准, 例如接受 III 型戈谢病的不同治疗 (例如, 伊米苷酶或 uplyso) 的一群受试者的可能性)、改变骨骼参数 (例如, 增加骨矿密度)、神经学参数的变化 (例如, 神经学参数的改善) 和 / 或减小产生所述治疗的抗体 (例如, 中和抗体) (例如, IgE、IgM、IgG 和 / 或 IgA 抗体) 的可能性 (例如, 相对于标准、例如本文所述的标准, 例如接受 III 型戈谢病的不同治疗 (例如, 伊米苷酶或 uplyso) 的一群受试者的可能性);

[0108] 接收来自所述接受者的购买所述葡糖脑苷脂酶替代疗法的请求; 和

[0109] 将所述葡糖脑苷脂酶替代疗法出售、运送或传输到所述接受者。

[0110] 在另一方面, 本发明提供一种向接受者提供关于使用葡糖脑苷脂酶替代疗法 (例如, 伊米苷酶或维拉苷酶) 的信息或使用葡糖脑苷脂酶替代疗法 (例如, 伊米苷酶或维拉苷酶) 的准则的方法, 所述方法包括以下步骤:

[0111] 提供所述葡糖脑苷脂酶替代疗法的名标,例如所述葡糖脑苷脂酶替代疗法的化学结构、化学名称、商品名称或通用名称;

[0112] 提供所述葡糖脑苷脂酶替代疗法具有以下性质中的一种或多种的信息:可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶)的一群受试者的可能性)、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和/或降低产生所述治疗的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶)的一群受试者的可能性);

[0113] 例如在数据库中记录所述名标和所述信息;和

[0114] 将所述记录(例如,记录的名标和信息)传输给所述接受者。

[0115] 在另一方面,本发明提供一种向接受者提供关于葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)的信息或使用葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)的准则的方法,所述方法包括以下步骤:

[0116] 提供所述葡糖脑苷脂酶替代疗法的名标,例如所述葡糖脑苷脂酶替代疗法的化学结构、化学名称、商品名称或通用名称;

[0117] 提供所述葡糖脑苷脂酶替代疗法具有以下性质中的一种或多种的信息:可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶或 uplyso)的一群受试者的可能性)、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和/或减小产生所述治疗的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病(例如,伊米苷酶或 uplyso)的一群受试者的可能性);

[0118] 例如在数据库中或通过物理关联使所述名标与所述信息关联;和

[0119] 将关联的名标和信息传输给所述接受者。

[0120] 在另一方面,本发明提供一种包含或经程序设计以包含以下项目的数据库、媒体或计算机:

[0121] 葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)的名标,例如所述葡糖脑苷脂酶替代疗法的化学结构、化学名称、商品名称或通用名称;

[0122] 所述葡糖脑苷脂酶替代疗法具有以下性质中的一种或多种的信息:可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病(例如,伊米苷酶或 uplyso)的一群受试者的可能性)、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和/或减小产生所述治疗的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病(例如,伊米苷酶或 uplyso)的一群受试者的可能性);和

[0123] 例如在数据库中或通过物理关联使所述名标与所述信息关联的关联功能。

[0124] 在另一方面,本发明提供一种使得葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)可由受试者获得的方法,所述方法包括以下步骤:

[0125] 向所述受试者提供所述葡糖脑苷脂酶替代疗法的名标,例如所述葡糖脑苷脂酶替代疗法的化学结构、化学名称、商品名称或通用名称;

[0126] 向所述受试者提供所述葡糖脑苷脂酶替代疗法具有以下性质中的一种或多种的信息:可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶或 uplyso)的一群受试者的可能性)、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和/或减小产生所述治疗的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶或 uplyso)的一群受试者的可能性);和

[0127] 将可以施用给受试者、提供给受试者或由受试者购买的一定剂量的葡糖脑苷脂酶替代疗法投入市场。

[0128] 在另一方面,本发明提供一种促使受试者要求葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)的方法,所述方法包括以下步骤:

[0129] 向所述受试者提供所述葡糖脑苷脂酶替代疗法的名标,例如所述葡糖脑苷脂酶替代疗法的化学结构、化学名称、商品名称或通用名称;

[0130] 向所述受试者提供所述葡糖脑苷脂酶替代疗法具有以下性质中的一种或多种的信息:可以增加血红蛋白浓度、增加血小板计数、减小肝体积、减小脾体积、减小输注部位反应的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶或 uplyso)的一群受试者的可能性)、改变骨骼参数(例如,增加骨矿密度)、神经学参数的变化(例如,神经学参数的改善)和/或减小产生所述治疗的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)的可能性(例如,相对于标准、例如本文所述的标准,例如接受 III 型戈谢病的不同治疗(例如,伊米苷酶或 uplyso)的一群受试者的可能性);和

[0131] 将可以施用给受试者、提供给受试者或由受试者购买的一定剂量的葡糖脑苷脂酶替代疗法投入市场。

[0132] 在另一方面,本发明的特点在于一种为患有 III 型戈谢病的患者的葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)的治疗过程选择支付种类的方法。所述方法包括提供(例如,接受)受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体)的评估;和进行以下至少一项(1)如果受试者经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体),则选择第一支付种类,和(2)如果受试者没有经历葡糖脑苷脂酶替代疗法的输注部位反应或没有产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和/或 IgA 抗体),则选择第二支付种类。

[0133] 在一些实施方案中,指派所述受试者为第一种类且所述指派使治疗过程(例如,维拉苷酶)的支付得到授权。

[0134] 在一些实施方案中,指派所述受试者为第二种类且所述指派使治疗过程(例如,伊米苷酶、维拉苷酶或 uplyso)的支付得到授权。

[0135] 在一些实施方案中,所述评估为受试者是否产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG和/或IgA抗体)且所述评估通过本文所述的方法获得。

[0136] 在另一方面,本发明的特点在于一种为患有III型戈谢病的患者的葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或uplyso)的治疗过程选择支付种类的方法。所述方法包括提供(例如,接受)以下评估:与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以60U/kg剂量的伊米苷酶,例如每隔一周施用历时9个月)之前的基准平均血小板计数相比,在治疗9或12个月之后受试者的神经学参数是否已改变(例如,神经学参数已改善)和/或受试者的平均血小板计数增加是否小于80%、75%、70%、65%、60%或55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以60U/kg剂量的伊米苷酶,例如每隔一周施用历时6个月)之前的基准平均血小板计数相比,在治疗6个月之后受试者的神经学参数是否已改变(例如,神经学参数已改善)和/或受试者的平均血小板计数增加是否小于40%、35%、30%或25%;和进行以下至少一者:(1)如果与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以60U/kg剂量的伊米苷酶,例如每隔一周施用历时9个月)之前的基准平均血小板计数相比,在治疗9或12个月之后受试者的神经学参数已改变(例如,神经学参数已改善)和/或如果受试者的平均血小板计数增加小于80%、75%、70%、65%、60%或55%或者如果与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以60U/kg剂量的伊米苷酶,例如每隔一周施用历时6个月)之前的基准平均血小板计数相比,在治疗6个月之后如果受试者的神经学参数已改变(例如,神经学参数已改善)和/或如果受试者的平均血小板计数增加小于40%、35%、30%或25%,则选择第一支付种类,和(2)如果与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以60U/kg剂量的伊米苷酶,例如每隔一周施用历时9个月)之前的基准平均血小板计数相比,在治疗9或12个月之后受试者的神经学参数已改变(例如,神经学参数已改善)和/或如果受试者的平均血小板计数增加80%、75%、70%、65%、60%或55%或者如果与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以60U/kg剂量的伊米苷酶,例如每隔一周施用历时6个月)之前的基准平均血小板计数相比,在治疗6个月之后受试者的神经学参数已改变(例如,神经学参数已改善)和/或如果受试者的平均血小板计数增加40%、35%、30%或25%,则选择第二支付种类。

[0137] 在一些实施方案中,指派所述受试者为第一种类且所述指派使治疗过程(例如,维拉苷酶)的支付得到授权。

[0138] 在一些实施方案中,指派所述受试者为第二种类且所述指派使治疗过程(例如,伊米苷酶、维拉苷酶或uplyso)的支付得到授权。

[0139] 在一些实施方案中,所述评估通过本文所述的方法获得。

[0140] 一方面,本发明的特点在于一种提供关于对患有III型戈谢病的受试者作出决策的信息或作出这一决策的方法。所述方法包括提供(例如,通过接受)受试者的评估,其中所述评估通过本文所述的方法、例如通过任选地对所述受试者施用葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或uplyso)来进行;提供受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG和/或IgA抗体)的施用后测定,由此提供施用后测定;提供所述施用后测定与标

准（例如，本文所述的标准）的比较，由此提供对受试者作出决策的信息或作出这一决策。

[0141] 在一些实施方案中，所述方法包括作出所述决策。

[0142] 在一些实施方案中，所述方法也包括将所述信息传递给另一方（例如，通过计算机、光盘、电话、传真、电子邮件或信件）。

[0143] 在一些实施方案中，所述决策包括：选择进行支付的受试者，或如果受试者经历葡萄糖脑苷脂酶替代疗法的输注部位反应或产生葡萄糖脑苷脂酶替代疗法的抗体（例如，中和抗体）（例如，IgE、IgM、IgG 和 / 或 IgA 抗体），则为第一行动步骤（例如，用维拉昔酶治疗））进行支付或授权支付，并且如果受试者没有经历葡萄糖脑苷脂酶替代疗法的输注部位反应或没有产生葡萄糖脑苷脂酶替代疗法的抗体（例如，中和抗体）（例如，IgE、IgM、IgG 和 / 或 IgA 抗体），则为第二行动步骤（例如，用伊米昔酶、维拉昔酶或 uplyso 治疗））进行支付或授权支付。

[0144] 在一些实施方案中，所述受试者经历葡萄糖脑苷脂酶替代疗法的输注部位反应或产生葡萄糖脑苷脂酶替代疗法的抗体（例如，中和抗体）（例如，IgE、IgM、IgG 和 / 或 IgA 抗体）且行动步骤为对疗法过程（例如，用维拉昔酶治疗）进行授权。

[0145] 在一些实施方案中，所述受试者经历葡萄糖脑苷脂酶替代疗法的输注部位反应或产生葡萄糖脑苷脂酶替代疗法的抗体（例如，中和抗体）（例如，IgE、IgM、IgG 和 / 或 IgA 抗体）且行动步骤为将受试者指派为第一种类。在一些实施方案中，指派为第一种类将使得能够为提供给受试者的治疗（例如，维拉昔酶）进行支付。在一些实施方案中，支付由第一方给第二方。在一些实施方案中，第一方不是受试者。在一些实施方案中，第一方选自第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构。在一些实施方案中，第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中，第一方为保险公司且第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中，第一方为政府机构且第二方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应治疗的机构。

[0146] 在一些实施方案中，所述受试者没有经历葡萄糖脑苷脂酶替代疗法的输注部位反应或没有产生葡萄糖脑苷脂酶替代疗法的抗体（例如，中和抗体）（例如，IgE、IgM、IgG 和 / 或 IgA 抗体）且行动步骤为对疗法过程（例如，伊米昔酶、维拉昔酶或 uplyso）进行授权。

[0147] 在一些实施方案中，所述受试者没有经历葡萄糖脑苷脂酶替代疗法的输注部位反应或没有产生葡萄糖脑苷脂酶替代疗法的抗体（例如，中和抗体）（例如，IgE、IgM、IgG 和 / 或 IgA 抗体）且行动步骤为将受试者指派为第二种类。在一些实施方案中，指派为第二种类将使得能够为提供给受试者的治疗（例如，伊米昔酶、维拉昔酶或 uplyso）进行支付。在一些实施方案中，支付由第一方给第二方。在一些实施方案中，第一方不是受试者。在一些实施方案中，第一方选自第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构。在一些实施方案中，第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中，第一方为保险公司且第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中，第一方为政府机构且第二方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应治疗的机构。

[0148] 一方面，本发明的特点在于一种提供关于对患有 III 型戈谢病的受试者作出决策

的信息或作出这一决策的方法。所述方法包括提供（例如，通过接受）受试者的评估，其中所述评估通过本文所述的方法、例如通过任选对所述受试者施用葡糖脑苷脂酶替代疗法（例如，伊米苷酶、维拉苷酶或 uplyso）来进行；提供施用后测定，与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 9 个月）之前的基准平均血小板计数相比，在治疗 9 或 12 个月之后受试者的神经学参数是否已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加是否小于 80%、75%、70%、65%、60% 或 55% 或者与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 6 个月）之前的基准平均血小板计数相比，在治疗 6 个月之后受试者的神经学参数是否已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加是否小于 40%、35%、30% 或 25%，由此提供施用后测定；提供所述施用后测定与标准（例如，本文所述的标准）的比较，由此提供对受试者作出决策的信息或作出这一决策。

[0149] 在一些实施方案中，所述方法包括作出所述决策。

[0150] 在一些实施方案中，所述方法也包括将所述信息传递给另一方（例如，通过计算机、光盘、电话、传真、电子邮件或信件）。

[0151] 在一些实施方案中，所述决策包括选择进行支付的受试者，如果与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 9 个月）之前的基准平均血小板计数相比，在治疗 9 或 12 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或如果受试者的平均血小板计数增加小于 80%、75%、70%、65%、60% 或 55% 或者如果与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 6 个月）之前的基准平均血小板计数相比，在治疗 6 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或如果受试者的平均血小板计数增加小于 40%、35%、30% 或 25%，则对第一行动步骤（例如，用维拉苷酶治疗）作出支付或授权支付，或者与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 9 个月）之前的基准平均血小板计数相比，在治疗 9 或 12 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或如果受试者的平均血小板计数增加 80%、75%、70%、65%、60% 或 55% 或者如果与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 6 个月）之前的基准平均血小板计数相比，在治疗 6 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或如果受试者的平均血小板计数增加 40%、35%、30% 或 25%，则对第二行动步骤（例如，用伊米苷酶、维拉苷酶或 uplyso 治疗）作出支付或授权支付。

[0152] 在一些实施方案中，与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 9 个月）之前的基准平均血小板计数相比，在治疗 9 或 12 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加小于 80%、75%、70%、65%、60% 或 55%，或者与在开始葡糖脑苷脂酶替代疗法（例如，伊米苷酶治疗，例如以 60U/kg 剂量的伊米苷酶，例如每隔一周施用历时 6 个月）之前的基准平均血小板计数相比，在治疗 6 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加小于 40%、

35%、30%或25%，且行动步骤为对疗法过程（例如，用维拉昔酶治疗）进行授权。

[0153] 在一些实施方案中，指派所述受试者为第一种类且所述指派使治疗过程（例如，维拉昔酶）的支付得到授权。

[0154] 在一些实施方案中，指派所述受试者为第二种类且所述指派使治疗过程（例如，伊米昔酶、维拉昔酶或 uplyso）的支付得到授权。

[0155] 在一些实施方案中，与在开始葡糖脑苷脂酶替代疗法（例如，伊米昔酶治疗，例如以 60U/kg 剂量的伊米昔酶，例如每隔一周施用历时 9 个月）之前的基准平均血小板计数相比，在治疗 9 或 12 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加小于 80%、75%、70%、65%、60%或 55%，或者与在开始葡糖脑苷脂酶替代疗法（例如，伊米昔酶治疗，例如以 60U/kg 剂量的伊米昔酶，例如每隔一周施用历时 6 个月）之前的基准平均血小板计数相比，在治疗 6 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加小于 40%、35%、30%或 25%，且行动步骤为指派所述受试者为第一种类。在一些实施方案中，指派为第一种类将使得能够为提供给受试者的治疗（例如，维拉昔酶）进行支付。在一些实施方案中，支付由第一方给第二方。在一些实施方案中，第一方不是受试者。在一些实施方案中，第一方选自第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构。在一些实施方案中，第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中，第一方为保险公司且第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中，第一方为政府机构且第二方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应治疗的机构。

[0156] 在一些实施方案中，与在开始葡糖脑苷脂酶替代疗法（例如，伊米昔酶治疗，例如以 60U/kg 剂量的伊米昔酶，例如每隔一周施用历时 9 个月）之前的基准平均血小板计数相比，在治疗 9 或 12 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加 80%、75%、70%、65%、60%或 55%，或者与在开始葡糖脑苷脂酶替代疗法（例如，伊米昔酶治疗，例如以 60U/kg 剂量的伊米昔酶，例如每隔一周施用历时 6 个月）之前的基准平均血小板计数相比，如果在治疗 6 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或如果受试者的平均血小板计数增加 40%、35%、30%或 25%，且行动步骤为对疗法过程（例如，伊米昔酶、维拉昔酶或 uplyso）进行授权。

[0157] 在一些实施方案中，与在开始葡糖脑苷脂酶替代疗法（例如，伊米昔酶治疗，例如以 60U/kg 剂量的伊米昔酶，例如每隔一周施用历时 9 个月）之前的基准平均血小板计数相比，在治疗 9 或 12 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加 80%、75%、70%、65%、60%或 55%，或者与在开始葡糖脑苷脂酶替代疗法（例如，伊米昔酶治疗，例如以 60U/kg 剂量的伊米昔酶，例如每隔一周施用历时 6 个月）之前的基准平均血小板计数相比，如果在治疗 6 个月之后受试者的神经学参数已改变（例如，神经学参数已改善）和 / 或受试者的平均血小板计数增加 40%、35%、30%或 25%，且行动步骤为指派所述受试者为第二种类。在一些实施方案中，指派为第二种类将使得能够为提供给受试者的治疗（例如，伊米昔酶、维拉昔酶或 uplyso）进行支付。在

一些实施方案中,支付由第一方给第二方。在一些实施方案中,第一方不是受试者。在一些实施方案中,第一方选自第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构。在一些实施方案中,第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中,第一方为保险公司且第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中,第一方为政府机构且第二方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应治疗的机构。

[0158] 在另一方面,本发明的特点在于一种为患有 III 型戈谢病的患者的葡糖脑苷脂酶替代疗法(例如,维拉苷酶)治疗过程选择支付种类的方法。所述方法包括例如通过本文所述的方法测定在输注疗法之后的 12 小时期间或 12 小时之内在受试者中存在输注部位反应或在受试者中存在所述疗法的抗体(例如,中和抗体)(例如, IgE、IgM、IgG 和 / 或 IgA 抗体),和批准、作出、授权、接受、传送或以其他方式允许对选定的治疗过程(例如,维拉苷酶)进行支付。

[0159] 在另一方面,本发明的特点在于一种为患有 III 型戈谢病的患者的葡糖脑苷脂酶替代疗法(例如,伊米苷酶、维拉苷酶或 uplyso)治疗过程选择支付种类的方法。所述方法包括例如通过本文所述的方法测定在输注疗法之后的 12 小时期间或 12 小时之内在受试者中不存在输注部位反应或在受试者中不存在所述疗法的抗体(例如,中和抗体)(例如, IgE、IgM、IgG 和 / 或 IgA 抗体),和批准、作出、授权、接受、传送或以其它方式允许对选定的治疗过程(例如,伊米苷酶、维拉苷酶或 uplyso)进行支付。

[0160] 一方面,本发明的特点在于一种作出数据记录的方法。所述方法包括将本文所述的方法的结果输入记录,例如计算机可读记录中。在一些实施方案中,所述记录可在全球信息网上得到。在一些实施方案中,所述记录由第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构或者保健提供者、治疗医师、HMO、医院、政府机构或销售或供应药物的机构评估,或在其他方面以本文所述的方法来利用。

[0161] 另一方面,本发明的特点在于一种数据记录(例如,计算机可读记录),其中所述记录包括来自本文所述的方法的结果。在一些实施方案中,所述记录可在全球信息网上得到。在一些实施方案中,所述记录由第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构或者保健提供者、治疗医师、HMO、医院、政府机构或销售或供应药物的机构评估和 / 或传送给第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构或者保健提供者、治疗医师、HMO、医院、政府机构或销售或供应药物的机构。

[0162] 一方面,本发明的特点在于一种提供数据的方法。所述方法包括提供本文所述的数据(例如,通过本文所述的方法产生的数据)以提供记录(例如,本文所述的记录),从而测定是否将提供支付。在一些实施方案中,所述数据由计算机、光盘、电话、传真、电子邮件或信件提供。在一些实施方案中,所述数据由第一方提供给第二方。在一些实施方案中,第一方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中,第二方选自第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构。在一些实施方案中,第一方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应治疗的机构且第二方为政府机构。在一些实施方案中,第一方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应治疗的机构且第二方为保险

公司。

[0163] 一方面,本发明的特点在于一种传送本文所述的记录的方法。所述方法包括第一方例如通过计算机、光盘、电话、传真、电子邮件或信件传送所述记录给第二方。在一些实施方案中,第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中,第一方为保险公司或政府机构且第二方选自受试者、保健提供者、治疗医师、HMO、医院、政府机构或销售或供应治疗的机构。在一些实施方案中,第一方为政府机构或保险公司且第二方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应治疗的机构。

[0164] 在一种方法中,将例如关于患有 III 型戈谢病的受试者是否经历神经学参数的变化、关于患有 III 型戈谢病的受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和 / 或 IgA 抗体)的信息(例如,其中所述信息如本文所述获得)提供(例如,传递,例如通过电子手段传递)给第三方,例如医院、诊所、政府机构、偿付方或保险公司(例如人寿保险公司)。例如,医学程序的选择、医学程序的支付、偿付方的支付或服务或保险的成本可以根据所述信息。例如,第三方接受信息,至少部分根据所述信息进行测定和任选地传递所述信息或根据所述信息进行程序、支付、支付水平、承保范围等的选择。

[0165] 在一种方法中,将例如与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和 / 或患有 III 型戈谢病的受试者的平均血小板计数增加是否小于 80%、75%、70%、65%、60% 或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后所述受试者的平均血小板计数增加是否小于 40%、35%、30% 或 25% 的信息(例如,其中所述信息如本文所述获得)提供(例如,传递,例如通过电子手段传递)给第三方,例如医院、诊所、政府机构、偿付方或保险公司(例如,人寿保险公司)。例如,医学程序的选择、医学程序的支付、偿付方的支付或服务或保险的成本可以根据所述信息。例如,第三方接受信息,至少部分根据所述信息作出测定和任选地传递所述信息或根据所述信息进行程序、支付、支付水平、承保范围等的选择。

[0166] 在一个实施方案中,保险费(例如,人寿或医疗)根据关于患有 III 型戈谢病的受试者是否经历神经学参数的变化和 / 或患有 III 型的受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和 / 或 IgA 抗体)的信息而评估。例如,如果受试者经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如,IgE、IgM、IgG 和 / 或 IgA 抗体),则可以增加保险费(例如,增加某一百分数)。

[0167] 在一个实施方案中,保险费(例如,人寿或医疗)根据关于与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和 / 或患有 III 型戈谢病的受试者的平均血小板计数增加是否小于 80%、75%、70%、65%、60% 或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例

如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后所述受试者的平均血小板计数增加是否小于 40%、35%、30%或 25%的信息(例如,其中所述信息如本文所述获得)而评估。例如,如果与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后受试者的平均血小板计数增加小于 80%、75%、70%、65%、60%或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后所述受试者的平均血小板计数增加小于 40%、35%、30%或 25%,则可以增加保险费(例如,增加某一百分数)。

[0168] 例如,在人寿保险的核保过程中,可以使用关于患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或患有 III 型戈谢病的受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如, IgE、IgM、IgG 和/或 IgA 抗体)的信息。所述信息可以并入关于受试者的概况中。在所述概况中的其他信息可以包括例如出生日期、性别、婚姻状况、银行信息、信用信息、子女等。保险单可以根据关于患 III 型戈谢病的受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如, IgE、IgM、IgG 和/或 IgA 抗体)的信息而推荐。保险费和危险评价也可以根据患 III 型戈谢病的受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如, IgE、IgM、IgG 和/或 IgA 抗体)而评估。

[0169] 例如,在人寿保险的核保过程中,可以使用关于与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或受试者的平均血小板计数增加是否小于 80%、75%、70%、65%、60%或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后所述受试者的平均血小板计数增加是否小于 40%、35%、30%或 25%的信息。所述信息可以并入关于受试者的概况中。在所述概况中的其他信息可以包括例如出生日期、性别、婚姻状况、银行信息、信用信息、子女等。保险单可以根据关于与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或受试者的平均血小板计数增加是否小于 80%、75%、70%、65%、60%或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后所述受试者的平均血小板计数增加是否小于 40%、35%、30%或 25%的信息而推荐。保险费和危险评价也可以根据与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或受试者的平均血小板计数增加是否是否小于 80%、75%、70%、65%、60%或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治

疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或所述受试者的平均血小板计数增加是否小于 40%、35%、30%或 25%而评估。

[0170] 在一个实施方案中,分析关于患有患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或患有 III 型戈谢病的受试者是否经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如, IgE、IgM、IgG 和/或 IgA 抗体)的信息用以测定是否授权资金转拨以支付提供给受试者的服务或治疗(或作出本文中提到的另一决策)。例如,结果(例如,受试者经历葡糖脑苷脂酶替代疗法的输注部位反应或产生葡糖脑苷脂酶替代疗法的抗体(例如,中和抗体)(例如, IgE、IgM、IgG 和/或 IgA 抗体)可以指示受试者适合于治疗(例如,维拉苷酶),提示需要治疗过程(例如,用维拉苷酶),由此触发指示或引起对提供给受试者的服务或治疗(例如,维拉苷酶)的支付进行授权的结果。例如,机构(例如,医院、护理给予者、政府机构或保险公司或支付或偿付医药费的其他机构)可以使用本文所述的方法的结果以测定一方(例如,除了受试患者以外的一方)是否将对提供给患者的服务(例如,特定疗法)或治疗进行支付。例如,第一机构(例如,保险公司)可以使用本文所述的方法的结果来测定是否提供财政支付给患者或为了患者的利益例如是否偿付第三方(例如,商品或服务的供应商、医院、医师或其他护理给予者)提供给患者的服务或治疗(例如,维拉苷酶)。例如,第一机构(例如,保险公司)可以使用本文所述方法的结果以测定在保险计划或程序(例如,健康保险或人寿保险计划或程序)中是否继续、中断、登记个体。

[0171] 在一个实施方案中,分析关于与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或受试者的平均血小板计数增加是否小于 80%、75%、70%、65%、60%或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后患有 III 型戈谢病的受试者是否经历神经学参数的变化和/或所述受试者的平均血小板计数增加是否小于 40%、35%、30%或 25%的信息用以测定是否授权资金转拨以支付提供给受试者的服务或治疗(或作出本文中提到的另一决策)。例如,结果(例如,与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 9 个月)之前的基准平均血小板计数相比,在治疗 9 或 12 个月之后受试者的平均血小板计数增加小于 80%、75%、70%、65%、60%或 55%,或者与在开始葡糖脑苷脂酶替代疗法(例如,伊米苷酶治疗,例如以 60U/kg 剂量的伊米苷酶,例如每隔一周施用历时 6 个月)之前的基准平均血小板计数相比,在治疗 6 个月之后所述受试者的平均血小板计数增加小于 40%、35%、30%或 25%)可以指示受试者适合于治疗(例如,维拉苷酶),提示需要治疗过程(例如,用维拉苷酶),由此触发指示或引起对提供给受试者的服务或治疗(例如,维拉苷酶)的支付进行授权的结果。例如,机构(例如,医院、护理给予者、政府机构或保险公司或支付或偿付医药费的其他机构)可以使用本文所述方法的结果以测定一方(例如,除了受试患者以外的一方)是否将对提供给患者的服务(例如,特定疗法)或治疗进行支付。例如,第一机构(例如,保险公司)可以使用本文所述方法的结果来测定是否提供财政支付给

患者或为了患者的利益例如是否偿付第三方（例如，商品或服务的供应商、医院、医师或其他护理给予者）提供给患者的服务或治疗（例如，维拉苷酶）。例如，第一机构（例如，保险公司）可以使用本文所述方法的结果以测定是否在保险计划或程序（例如，健康保险或人寿保险计划或程序）中继续、中断、登记个体。

[0172] 一方面，本发明的特点在于一种提供数据的方法。所述方法包括提供本文所述的数据（例如，通过本文所述的方法产生的数据）以提供记录（例如，本文所述的记录），从而测定是否将提供支付。在一些实施方案中，所述数据由计算机、光盘、电话、传真、电子邮件或信件提供。在一些实施方案中，所述数据由第一方提供给第二方。在一些实施方案中，第一方选自受试者、保健提供者、治疗医师、健康维护组织（HMO）、医院、政府机构或销售或供应药物的机构。在一些实施方案中，第二方选自第三方付款人、保险公司、雇主、雇主发起的健康计划、HMO 或政府机构。在一些实施方案中，第一方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应药物的机构且第二方为政府机构。在一些实施方案中，第一方选自受试者、保健提供者、治疗医师、HMO、医院、保险公司或销售或供应药物的机构且第二方为保险公司。

[0173] 在一些方面，本发明提供葡糖脑苷脂酶替代疗法（例如，维拉苷酶、伊米苷酶或 uplyso）单独或与本文所述的另一药剂（例如，异法戈明酒石酸盐（isofagomine tartrate）、美格鲁特（miglustat）或 Genz112638）组合用于治疗的用途。

[0174] 在一些方面，本发明提供葡糖脑苷脂酶替代疗法（例如，维拉苷酶或伊米苷酶）单独或与本文所述的另一药剂（例如，异法戈明酒石酸盐、美格鲁特或 Genz112638）组合用于制备例如用于治疗 III 型戈谢病的药物的用途。

[0175] 在另一方面，本发明提供一种维拉苷酶的药物组合物。所述组合物包含：维拉苷酶、冻干保护剂（例如，碳水化合物（例如，蔗糖）、缓冲盐（例如，柠檬酸盐和 / 或柠檬酸，例如柠檬酸钠和柠檬酸）和稳定剂（例如，聚山梨醇酯，例如聚山梨醇酯 20）。

[0176] 在一些实施方案中，可以冻干所述组合物。在一些实施方案中，所述冻干组合物的湿含量为 1% -6%，例如 1.3% -6.2%。在一些实施方案中，所述冻干组合物的湿含量为 1% -5%。在一些实施方案中，所述冻干组合物的湿含量为 3% -5%。在一些实施方案中，所述湿含量大于或等于 3%。在一些实施方案中，所述湿含量为 3%。

[0177] 在一些实施方案中，可以评估所述冻干组合物。例如，可以例如通过 FT-IR 评估所述冻干组合物的二级结构。

[0178] 在其他实施方案中，所述组合物可以为重构的溶液。例如，所述组合物为在诸如灭菌注射水的药学上可接受的载体中的重构溶液（例如，用 2.2mL 灭菌注射水重构 200 单位小瓶或用 4.3mL 灭菌注射水重构 400 单位小瓶）。在一些实施方案中，所述组合物可以进一步包含适于静脉内施用的氯化钠溶液（例如，适于静脉内施用的 0.9% 氯化钠溶液）或由所述氯化钠溶液组成。

[0179] 在一些实施方案中，可以评估所述重构溶液，例如，降解。例如，可以通过 SE-HPLC 和 / 或 RP-HPLC 评估所述重构溶液，例如，降解产物的存在。

[0180] 在一些实施方案中，可以评估所述重构溶液的氧化。例如，可以通过肽作图评估所述重构溶液。

[0181] 在一些方面，本发明的特点在于一种检测在样品（例如，患者样品，例如血液或血

清)中的抗葡糖脑苷脂酶抗体的测定(例如,方法)。所述方法包括以下步骤:

[0182] 提供固定在表面(例如,微孔)上的葡糖脑苷脂酶(例如,维拉苷酶、伊米苷酶或 uplyso)(例如,所述表面可以用诸如链霉亲和素(strepavidin)的偶合剂涂覆且所述葡糖脑苷脂酶(例如,维拉苷酶、伊米苷酶或 uplyso)可以与药剂(例如,生物素)结合,所述药剂与所述偶合剂缔合,例如结合,例如所述葡糖脑苷脂酶(例如,维拉苷酶、伊米苷酶或 uplyso)经与链霉亲和素结合的生物素固定到表面上);

[0183] 使所述样品与固定的葡糖脑苷脂酶(例如,维拉苷酶、伊米苷酶或 uplyso)在允许在所述样品中的抗葡糖脑苷脂酶抗体(如果存在的话)与所述固定的葡糖脑苷脂酶结合的条件下接触,由此形成混合物;

[0184] 任选地进行洗涤步骤以从所述混合物中除去在所述样品中的不与所述固定的葡糖脑苷脂酶结合的任何材料;

[0185] 在允许经标记的葡糖脑苷脂酶与所述抗葡糖脑苷脂酶抗体(例如,与固定的葡糖脑苷脂酶结合)(如果存在的话)(优选,所述标记物与所述偶合剂和/或与所述偶合剂结合的药剂不同,例如,如果使用生物素来将葡糖脑苷脂酶固定到表面上,则所述标记物不是生物素)的条件下向所述混合物中加入经标记的葡糖脑苷脂酶(例如,维拉苷酶或伊米苷酶),其中所述经标记的葡糖脑苷脂酶用可检测的标记物标记(例如,钆标记的葡糖脑苷脂酶);

[0186] 任选地进行洗涤步骤以从所述混合物中除去不与所述抗葡糖脑苷脂酶抗体结合的经标记的葡糖脑苷脂酶;和

[0187] 检测(和任选地量化)在所述混合物中的标记物,例如,其中所述标记物的检测指示在所述样品中存在抗葡糖脑苷脂酶抗体。

[0188] 在一些实施方案中,将检测的标记物量化为值且与对照(例如,阴性对照)比较,其中如果检测的标记物的值大于阴性对照,则所述样品含有抗葡糖脑苷脂酶抗体。在一些实施方案中,所述阴性对照为多个阴性对照的平均阴性对照值(例如,背景)。例如,所述阴性对照可以为正常人类血清(NHS),且所述平均阴性对照值可以为多批NHS的平均值或从多种测定中获得的阴性对照值的平均值。例如,所述阴性对照值可以为1、2、3、4、5或6ng/ml抗体的值,例如,大于所述数值的样品值指示样品中存在抗葡糖脑苷脂酶抗体。作为另一实例,所述阴性对照值可以为200、250、300(例如,306)、350或400,例如,大于所述数值的样品值指示样品中存在抗葡糖脑苷脂酶抗体。

[0189] 在一些实施方案中,所述测定用于检测抗维拉苷酶抗体。在另一实施方案中,所述测定用于检测抗伊米苷酶抗体。在一些实施方案中,所述测定用于检测抗 uplyso 抗体。

[0190] 在一些方面,本发明的特点在于一种检测样品(例如,患者样品,例如血液或血清)中的抗葡糖脑苷脂酶抗体(例如,IgG抗葡糖脑苷脂酶抗体)的测定。所述方法包括以下步骤:

[0191] 使所述样品与经标记的葡糖脑苷脂酶(例如,维拉苷酶或伊米苷酶)(其中所述葡糖脑苷脂酶用可检测的标记物标记(例如,葡糖脑苷脂酶为¹²⁵I标记的))在允许在所述样品中的抗葡糖脑苷脂酶抗体(如果存在的话)与经标记的葡糖脑苷脂酶结合的条件下接触,由此形成混合物;

[0192] 在允许所述抗葡糖脑苷脂酶抗体(如果存在的话)与树脂(例如,蛋白质G、蛋白

质 A、蛋白质 A/G 或蛋白质 L 树脂) (例如蛋白质 G 纯化柱) 结合的条件下将所述混合物施加到所述树脂;

[0193] 任选地进行洗涤步骤以从所述混合物中除去不与所述抗葡萄糖脑苷脂酶抗体结合的经标记的葡萄糖脑苷脂酶;和

[0194] 检测(和任选地量化)所述混合物中(例如,在所述树脂上)的标记物,例如,其中所述标记物的检测指示在所述样品中存在抗葡萄糖脑苷脂酶抗体。

[0195] 在一些实施方案中,所述测定用于检测抗维拉昔酶抗体。在另一实施方案中,所述测定用于检测抗伊米昔酶抗体。在一些实施方案中,所述测定用于检测抗 uplyso 抗体。

[0196] 在一些实施方案中,将检测的标记物量化为值且与对照(例如,阴性对照)比较,其中如果检测的标记物的值大于阴性对照,则所述样品含有抗葡萄糖脑苷脂酶抗体。在一些实施方案中,所述阴性对照为多种阴性对照的平均阴性对照值(例如,背景)。例如,所述阴性对照可以为正常人类血清(NHS),且所述平均阴性对照值可以为多批 NHS 的平均值或从多种测定中获得的阴性对照值的平均值。

[0197] 在一些方面,本发明的特点在于一种检测样品(例如,患者样品,例如血液或血清)中的人类抗葡萄糖脑苷脂酶抗体的测定。所述方法包括以下步骤:

[0198] 提供固定在表面(例如,微孔)上的葡萄糖脑苷脂酶(例如,维拉昔酶、伊米昔酶或 uplyso)(例如,所述表面可以用诸如链霉亲和素的偶合剂涂覆且所述葡萄糖脑苷脂酶可以与药剂(例如,生物素)结合,所述药剂与所述偶合剂缔合,例如结合,例如所述葡萄糖脑苷脂酶经与链霉亲和素结合的生物素固定到表面上);

[0199] 使所述样品与固定的葡萄糖脑苷脂酶在允许在所述样品中的人类抗葡萄糖脑苷脂酶抗体(如果存在的话)与固定的葡萄糖脑苷脂酶结合的条件下接触,由此形成混合物;

[0200] 任选地进行洗涤步骤以从所述混合物中除去在所述样品中的不与固定的葡萄糖脑苷脂酶结合的任何材料;

[0201] 在允许可结合人类抗葡萄糖脑苷脂酶抗体的经标记的抗体与所述人类抗葡萄糖脑苷脂酶抗体(例如,与经固定的葡萄糖脑苷脂酶结合的所述抗体)(如果存在的话)结合的条件下向所述混合物中加入与所述人类抗葡萄糖脑苷脂酶抗体结合的抗体,其中与人类抗葡萄糖脑苷脂酶抗体结合的抗体用可检测的标记物(例如,钯或生物素)标记(优选地,所述标记物与所述偶合剂和/或与所述偶合剂结合的药剂不同,例如,如果使用生物素来将葡萄糖脑苷脂酶固定到表面上,则所述标记物不是生物素);

[0202] 任选地进行洗涤步骤以从所述混合物中除去不与所述人类抗葡萄糖脑苷脂酶抗体结合的可结合所述人类抗葡萄糖脑苷脂酶抗体的经标记的抗体;和

[0203] 检测(和任选地量化)所述混合物中的标记物,例如,其中所述标记物的检测指示在所述样品中存在所述人类抗葡萄糖脑苷脂酶抗体。

[0204] 在一些实施方案中,所述测定检测抗维拉昔酶抗体。在一些实施方案中,所述测定检测抗伊米昔酶抗体。在一些实施方案中,所述测定检测抗 uplyso 抗体。

[0205] 在一些实施方案中,将检测的标记物量化为值且与对照(例如,阴性对照)比较,其中如果检测的标记物的值大于阴性对照,则所述样品含有抗维拉昔酶抗体。在一些实施方案中,所述阴性对照为多种阴性对照的平均阴性对照值(例如,背景)。例如,所述阴性对照可以为正常人类血清(NHS),且所述平均阴性对照值可以为多批 NHS 的平均值或从多种

测定中获得的阴性对照值的平均值。

[0206] 在一些实施方案中,与所述人类抗葡糖脑苷脂酶抗体结合的抗体为同种型特异性的,其中与所述人类抗葡糖脑苷脂酶抗体结合的所述同种型特异性抗体与对其具有特异性的同种型的人类抗体特异性结合。

[0207] 在一些实施方案中,与所述人类抗葡糖脑苷脂酶抗体结合的抗体为 IgA 特异性抗体且与在样品中的 IgA 人类抗葡糖脑苷脂酶抗体结合。

[0208] 在一些实施方案中,与所述人类抗葡糖脑苷脂酶抗体结合的抗体为 IgE 特异性抗体且与在样品中的 IgE 人类抗葡糖脑苷脂酶抗体结合。

[0209] 在一些实施方案中,与所述人类抗葡糖脑苷脂酶抗体结合的抗体为 IgM 特异性抗体且与在样品中的 IgM 人类抗葡糖脑苷脂酶抗体结合。

[0210] 在一些实施方案中,与所述人类抗葡糖脑苷脂酶抗体结合的抗体为 IgG 特异性抗体且与在样品中的 IgG 人类抗葡糖脑苷脂酶抗体结合。

[0211] 在一些方面,本发明的特点在于一种测定抗葡糖脑苷脂酶抗体(例如,在样品中)是否中和(例如,抑制)葡糖脑苷脂酶(例如,维拉苷酶或伊米苷酶)活性的方法。所述方法包括以下步骤:

[0212] 提供表达人类巨噬细胞甘露糖受体(MMR)的细胞(例如,人类细胞,例如人类成纤维细胞);

[0213] 使所述抗葡糖脑苷脂酶抗体与所述细胞接触,由此形成混合物;

[0214] 使经标记的葡糖脑苷脂酶(例如,维拉苷酶、伊米苷酶或 uplyso)与所述混合物在允许经标记的葡糖脑苷脂酶与 MMR 在缺乏抗葡糖脑苷脂酶抗体的情况下结合(例如,其中葡糖脑苷脂酶与 MMR 的结合允许葡糖脑苷脂酶的细胞吸收)的条件下接触,其中葡糖脑苷脂酶用可检测的标记物标记(例如,所述葡糖脑苷脂酶用荧光标记物,例如绿色荧光染料,诸如 Alexa **FLUOR**® 488 或异硫氰酸荧光素(fluorescein isothiocyanate, FITC)标记);

[0215] 除去未结合的经标记的葡糖脑苷脂酶和与细胞表面结合的经标记的葡糖脑苷脂酶(例如,经胰蛋白酶消化);和

[0216] 测量在细胞中的经标记的葡糖脑苷脂酶的量。

[0217] 在一些实施方案中,将经标记的葡糖脑苷脂酶的水平与对照,例如在缺乏抗葡糖脑苷脂酶抗体的情况下在相同条件下检测的经标记的葡糖脑苷脂酶的水平比较。

[0218] 在一些实施方案中,所述细胞不表达 Fc 受体(例如,人类 Fc 受体)。

[0219] 在一些实施方案中,所述方法检测是否存在中和抗维拉苷酶抗体。在一些实施方案中,所述方法检测是否存在中和抗伊米苷酶抗体。在一些实施方案中,所述方法检测是否存在中和抗 uplyso 抗体。

[0220] 在一些实施方案中,所述方法检测抗伊米苷酶抗体是否中和伊米苷酶活性。在一些实施方案中,所述方法检测抗维拉苷酶抗体是否中和维拉苷酶活性。在一些实施方案中,所述方法检测抗 uplyso 抗体是否中和 uplyso 活性。

[0221] 在一些实施方案中,所述方法检测抗伊米苷酶抗体是否中和维拉苷酶和/或 uplyso 活性。在一些实施方案中,所述方法检测抗伊米苷酶抗体是否中和伊米苷酶和/或 uplyso 活性。在一些实施方案中,所述方法检测抗 uplyso 抗体是否中和伊米苷酶和/或维

拉昔酶活性。

[0222] 在一些方面,本发明的特点在于一种测定抗维拉昔酶抗体(例如,在样品中)是否中和(例如,抑制)伊米昔酶活性的方法。所述方法包括以下步骤:

[0223] 提供表达人类巨噬细胞甘露糖受体(MMR)的细胞(例如,人类细胞,例如人类成纤维细胞);

[0224] 使所述抗维拉昔酶抗体与所述细胞接触,由此形成混合物;

[0225] 使经标记的伊米昔酶与所述混合物在允许经标记的伊米昔酶与 MMR 在缺乏抗维拉昔酶抗体的情况下结合(例如,其中伊米昔酶与 MMR 的结合允许伊米昔酶的细胞吸收)的条件下接触,其中所述伊米昔酶用可检测的标记物标记(例如,所述伊米昔酶用荧光标记物,例如绿色荧光染料,诸如 Alexa **FLUOR®** 488 或异硫氰酸荧光素(FITC)标记);

[0226] 除去未结合的经标记的伊米昔酶和与细胞表面结合的经标记的伊米昔酶(例如,经胰蛋白酶消化);和

[0227] 测量在所述细胞中的经标记的伊米昔酶的量。

[0228] 在一些实施方案中,将经标记的伊米昔酶的水平与对照,例如在缺乏抗维拉昔酶抗体的情况下在相同条件下检测的经标记的伊米昔酶的水平比较。

[0229] 在一些实施方案中,所述细胞没有表达 Fc 受体(例如,人类 Fc 受体)。

[0230] 在一些方面,本发明的特点在于一种测定抗维拉昔酶抗体(例如,在样品中)是否中和(例如,抑制)维拉昔酶活性的方法。所述方法包括以下步骤:

[0231] 提供表达人类巨噬细胞甘露糖受体(MMR)的细胞(例如,人类细胞,例如人类成纤维细胞);

[0232] 使所述抗伊米昔酶抗体与所述细胞接触,由此形成混合物;

[0233] 使标记的维拉昔酶与所述混合物在允许标记的维拉昔酶与 MMR 在缺乏抗伊米昔酶抗体的情况下结合(例如,其中维拉昔酶与 MMR 的结合允许维拉昔酶的细胞吸收)的条件下接触,其中所述维拉昔酶用可检测的标记物标记(例如,所述维拉昔酶用荧光标记物,例如绿色荧光染料,诸如 Alexa **FLUOR®** 488 或异硫氰酸荧光素(FITC)标记);除去未结合的标记的维拉昔酶和与细胞表面结合的标记的维拉昔酶(例如,经胰蛋白酶消化);和

[0234] 测量在所述细胞中标记的维拉昔酶的量。

[0235] 在一些实施方案中,将标记的维拉昔酶的水平与对照,例如在缺乏抗伊米昔酶抗体的情况下在相同条件下检测的标记的维拉昔酶的水平比较。

[0236] 在一些实施方案中,所述细胞没有表达 Fc 受体(例如,人类 Fc 受体)。

[0237] 在一些方面,本发明的特点在于一种混合抗体,其中所述混合抗体包含非人类抗药物抗体和人免疫球蛋白(Ig)。

[0238] 在一些实施方案中,所述非人类抗药物 IgG 抗体为绵羊抗药物 IgG 抗体。

[0239] 在一些实施方案中,所述抗药物抗体与维拉昔酶结合。

[0240] 在一些实施方案中,所述抗药物抗体与伊米昔酶结合。

[0241] 在一些实施方案中,所述抗药物抗体与 uplyso 结合。

[0242] 在一些实施方案中,所述人类 Ig 为 IgA。

[0243] 在一些实施方案中,所述人类 Ig 为 IgE。

- [0244] 在一些实施方案中,所述人类 Ig 为 IgM。
- [0245] 在一些实施方案中,所述人类 Ig 为 IgG。
- [0246] 在一些实施方案中,所述非人类抗药物抗体为 IgG 抗体。
- [0247] 在一些实施方案中,所述非人类抗药物抗体和所述人类 Ig 通过化学交联剂、例如长间隔臂交联剂、例如 6-[3'-2-吡啶二硫代-丙酰胺基]己酸琥珀酰亚胺酯 (LC-SPDP) 结合在一起。
- [0248] 在一些实施方案中,所述混合抗体在检测和 / 或测量样品中的抗药物抗体的水平和 / 或同种型的测定中、例如在本文所述的方法中用作阳性对照。
- [0249] 在一些实施方案中,所述混合抗体在用于测定葡糖脑苷脂酶抗体 (例如,在样品中) 是否中和 (例如,抑制) 葡糖脑苷脂酶 (例如,维拉苷酶或伊米苷酶) 活性的测定中、例如在本文所述的方法中用作阳性对照。例如,所述混合抗体在测定中用作抗葡糖脑苷脂酶抗体 (例如,在样品中)。
- [0250] 在一些方面,本发明的特点在于一种测量细胞中葡糖脑苷脂酶 (例如,维拉苷酶或伊米苷酶) 的细胞吸收 (例如,内化) 的方法。所述方法包括以下步骤:
- [0251] 使葡糖脑苷脂酶 (例如,维拉苷酶或伊米苷酶) 与细胞 (例如,人类白血病单核淋巴瘤细胞系 (例如, U937) 或鼠巨噬细胞细胞系 (例如, J774)) 接触,由此形成混合物;
- [0252] 孵育所述混合物 (例如,1、2、3、4、5、6 或 7 小时或过夜),例如以允许葡糖脑苷脂酶 (例如,维拉苷酶或伊米苷酶) 细胞吸收到所述细胞中;和
- [0253] 测量所述细胞中的葡糖脑苷脂酶 (例如,维拉苷酶或伊米苷酶) 的吸收量。
- [0254] 在一些实施方案中,所述吸收量通过测量所述细胞中葡糖脑苷脂酶的酶活性来测量。在一些实施方案中,使用在裂解后发荧光的合成底物 (例如,4-MU-gluc)。
- [0255] 在一些实施方案中,所述吸收量通过测量细胞内葡糖脑苷脂酶蛋白质水平来测量。在一些实施方案中,使用蛋白免疫印迹分析。在一些实施方案中,使用免疫组织化学分析 (例如,透性化细胞的免疫组织化学)。
- [0256] 在一些实施方案中,在测量步骤之前将细胞洗涤一次或多次。
- [0257] 在一些实施方案中,所述混合物的 pH 值为 7.5。
- [0258] 在一些实施方案中,甘露糖-6-磷酸 (M6P) 存在于所述混合物中。
- [0259] 在一些实施方案中,聚甘露糖存在于所述混合物中。
- [0260] 在一些实施方案中,钙存在于所述混合物中。
- [0261] 在一些实施方案中,将所述吸收量与标准、例如在不使葡糖脑苷脂酶 (例如,维拉苷酶或伊米苷酶) 与细胞接触的情况下测量的吸收量比较,或比较在存在和缺乏甘露糖-6-磷酸的情况下测量的吸收量,或比较在存在和缺乏聚甘露糖的情况下测量的吸收量,或比较在存在和缺乏钙的情况下测量的吸收量。
- [0262] 在一些实施方案中,所述葡糖脑苷脂酶为维拉苷酶。
- [0263] 在一些实施方案中,所述葡糖脑苷脂酶为伊米苷酶。
- [0264] 在一些实施方案中,将维拉苷酶的吸收量与伊米苷酶的吸收量 (例如,在相同条件下) 相比较。
- [0265] 术语“受试者”是指任何哺乳动物,包括但不限于按此分类的任何动物,包括人类、非人类灵长类动物、灵长类动物、狒狒、黑猩猩、猴子、啮齿类动物 (例如,小鼠、大鼠)、兔、

猫、狗、马、奶牛、绵羊、山羊、猪等。术语“受试者”可以与术语“患者”互换使用。

[0266] 术语“分离”是指基本脱离其自然环境的分子。例如，分离蛋白基本脱离产生其的细胞或组织源的细胞物质或其他蛋白质。该术语是指其中分离蛋白的纯度足以作为治疗组合物施用的制剂，或其纯度为至少 70% -80% (w/w)，更优选至少 80% -90% (w/w)，甚至更优选 90-95% 且最优选至少 95%、96%、97%、98%、99%、99.5%、99.8% 或 100% (w/w)。

[0267] 如本文所使用的术语“约”是指高达 $\pm 10\%$ 的由该术语限定的值。例如，约 50mM 是指 $50\text{mM} \pm 5\text{mM}$ ；约 4% 是指 $4\% \pm 0.4\%$ 。

[0268] 术语“治疗有效剂量”和“治疗有效量”是指引起症状预防（例如，预防 80%、85%、90%、95%、96%、97%、98% 或 99% 的症状，例如在诊断为患有 III 型戈谢病的受试者中的 III 型戈谢病的症状）、延缓症状起始或改善 III 型戈谢病的症状的化合物的量。治疗有效剂量将例如足以治疗、预防、降低与 III 型戈谢病相关的病症的一种或多种症状的严重性、延缓所述症状的起始和 / 或降低所述症状出现的危险性。所述有效量可以通过本领域熟知的方法且如本描述的后续部分中所述来测定。

[0269] 术语“治疗”和“疗法”是指现有病症的治疗和 / 或预防 (prophylactic) / 预防 (preventative) 措施。需要治疗者可以包括已经患有特定医学病症的个体以及处于患上所述病症的危险中或可能最终患上所述病症的个体。对治疗的需要例如通过与病症的发展相关的一种或多种危险因子的存在、病症的存在或进展或患有病症的受试者的治疗的可能接受性来评价。治疗可以包括减慢或逆转病症的进展。

[0270] 术语“治疗”是指以在统计上显著的程度或本领域技术人员可检测的程度有效改善或预防与病症（例如，本文所述的病症）相关的病状、症状或参数或预防所述病症的起始、进展或恶化的量、方式和 / 或模式施以疗法。因此，治疗可以获得治疗和 / 或预防益处。有效量、方式或模式可以根据受试者而变且可对于受试者定制。

[0271] 如本文所使用的“输注部位反应”是指在输注葡糖脑苷脂酶替代疗法期间或之后不久（例如，在对受试者输注葡糖脑苷脂酶替代治疗的 12 小时内）发展的一种或多种过敏症状。症状包括例如输注部位的瘙痒、刺痛、肿胀或脓肿、变红、荨麻疹 / 血管性水肿、胸部不适、心动过速、紫绀、呼吸症状和感觉异常。

[0272] 术语“组合”是指使用两种或更多种药剂或疗法来治疗同一患者，其中所述药剂或疗法的使用和作用在时间上重叠。所述药剂或疗法可以同时（例如，作为施用给患者的单一制剂或作为同时施用的两种单独制剂）或以任何顺序相继施用。

[0273] 本文提到的所有出版物、专利申请、专利及其他参考文献都通过引用全部并入本文中。在存在冲突的情形下，则以本发明说明书（包括定义）为准。另外，材料、方法和实施例仅是说明性的，而并非用来限制。

[0274] 在附图和以下描述中阐述本发明的一个或多个实施方案的细节。

[0275] 附图简述

[0276] 图 1(a)-(f) 描绘在 I/II 期试验中血液学值、器官值和生物标志物的平均变化%。

[0277] 图 2 描绘在 I/II 期和扩展试验中关键临床参数的平均变化%。

[0278] 图 3 描绘在 TKT025 中相对于基准的 TKT025EXT (N = 10) 平均血液学参数变化。

[0279] 图 4 描绘在 TKT025 中相对于基准的 TKT025EXT (N = 10) 平均正规化器官体积变化。

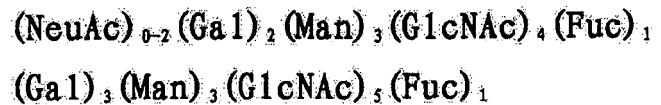
- [0280] 图 5 描绘腰脊柱的平均 Z- 分值相对于基准的 TKT025 和 TKT025EXT 时间变化。
- [0281] 图 6 描绘股骨颈的平均 Z- 分值相对于基准的 TKT025 和 TKT025EXT 时间变化。
- [0282] 图 7 描绘相对于基准的 TKT032 (N = 25) 平均血红蛋白浓度变化。
- [0283] 图 8 描绘相对于基准的 TKT032 (N = 25) 平均血小板计数变化。
- [0284] 图 9 描绘相对于基准的 TKT032 (N = 25) 平均正规化脾体积变化。
- [0285] 图 10 描绘相对于基准的 TKT032 (N = 25) 平均正规化肝体积变化。
- [0286] 图 11 描绘相对于基准的 HGT-GCB-039 (N = 34) 平均血红蛋白浓度变化。
- [0287] 图 12 描绘相对于基准的 HGT-GCB-039 (N = 34) 平均血小板计数变化。
- [0288] 图 13 描绘在无脾的患者中血小板计数相对于基准的 HGT-GCB-039 (N = 34) 平均变化。
- [0289] 图 14 描绘相对于基准的 HGT-GCB-039 (N = 34) 平均正规化肝体积变化。
- [0290] 图 15 描绘血红蛋白浓度相对于基准的 TKT034 平均变化。
- [0291] 图 16 描绘血小板计数相对于基准的 TKT034 平均 n 变化%。
- [0292] 图 17 描绘正规化肝体积相对于基准的 TKT034 平均变化%。
- [0293] 图 18 描绘正规化脾体积相对于基准的 TKT034 平均变化%。
- [0294] 图 19 描绘血浆壳三糖苷酶相对于基准的 TKT034 平均变化%。
- [0295] 图 20 描绘血浆 CCL18 相对于基准的 TKT034 平均变化%。
- [0296] 图 21 描绘在维拉苷酶 α 临床研究中患者的免疫原性评估。
- [0297] 图 22 描绘通过电致化学发光 (ECL) 免疫测定的抗药物抗体筛选。
- [0298] 图 23 描绘筛选测定剂量响应曲线。
- [0299] 图 24 描绘 IgG ADA 验证性测定。
- [0300] 图 25 描绘 RIP 测定剂量响应曲线。
- [0301] 图 26 描绘 IgA、IgM 和 IgE ADA 验证性测定。
- [0302] 发明详述
- [0303] 本发明部分地根据在施用给受试者 (例如, 患有戈谢病的受试者) 时与伊米苷酶相比维拉苷酶引起较少免疫响应 (例如, 产生较少抗体, 例如, 产生较少中和抗体) 的发现。人们发现, 在施用给受试者 (例如, 患有戈谢病的受试者) 后与伊米苷酶相比维拉苷酶引起较少输注部位反应, 且在施用给受试者 (例如, 患有戈谢病的受试者) 时与伊米苷酶相比维拉苷酶可引起血小板计数增加。本发明特别涉及用于为患有戈谢病的受试者选择治疗、选择受试者以使用维拉苷酶治疗 (例如, 单独或与另一疗法组合) 的组合物和方法、用于减少经受戈谢病治疗的受试者的注射部位反应的方法。
- [0304] 维拉苷酶
- [0305] 维拉苷酶为在人类细胞系中通过基因活化产生的人类 β - 葡糖脑苷脂酶。基因活化是指在所选择的人类细胞系中用活化内源性 β - 葡糖脑苷脂酶基因的启动子的目标重组。维拉苷酶作为约 63kDa 的单体糖蛋白分泌且由具有与天然人类蛋白质相同的序列的 497 个氨基酸组成。维拉苷酶的氨基酸序列描述于 Zimran 等 (2007) Blood Cells Mol Dis, 39:115-118 中。
- [0306] 维拉苷酶 α 的糖基化通过在细胞培养期间使用 kifunensine 甘露糖苷酶 I 抑制剂来改变, 这导致分泌主要含有具有 6-9 个甘露糖单位 / 聚糖的高甘露糖型聚糖的蛋白质。

下文提供维拉昔酶的聚糖结构的概述。

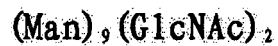
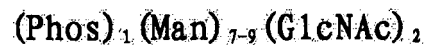
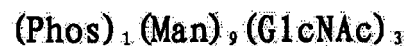
[0307]

糖基化部位	主要聚糖	其他聚糖
Asn19	<u>高甘露糖</u> (Man) ₉ (GlcNAc) ₂	<u>高甘露糖</u> (Man) ₆₋₈ (GlcNAc) ₂ <u>磷酸化高甘露糖</u> (Phos) ₁ (Man) ₈₋₉ (GlcNAc) ₂ <u>GlcNAc 盖帽磷酸酯</u> (Phos) ₁ (Man) ₈₋₉ (GlcNAc) ₃ <u>混合体</u> (Hex) ₂ (Man) ₃ (GlcNAc) ₃ (Fuc) ₁
Asn59	<u>高甘露糖</u> (Man) ₉ (GlcNAc) ₂	<u>高甘露糖</u> (Man) ₅₋₈ (GlcNAc) ₂ <u>磷酸化高甘露糖</u> (Phos) ₁ (Man) ₇₋₉ (GlcNAc) ₂ <u>GlcNAc 盖帽磷酸酯</u> (Phos) ₁ (Man) ₈₋₉ (GlcNAc) ₃ <u>混合体</u> (NeuAc) ₁ (Gal) ₁ (Man) ₅ (GlcNAc) ₃ (Fuc) ₁

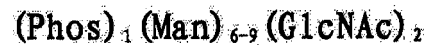
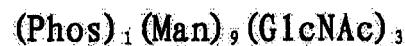
[0308]

复合体

Asn146

高甘露糖高甘露糖磷酸化高甘露糖GlcNAc 盖帽磷酸酯混合体

Asn270

高甘露糖高甘露糖磷酸化高甘露糖GlcNAc 盖帽磷酸酯混合体复合体

Asn462

未检出

未检出

[0309] 维拉苷酶具有三个非邻近的结构域,其中催化部位位于 III 结构域(残基 76-381 和 416-430)(β/α)8(TIM)桶中。

[0310] 维拉苷酶(VPRIVTM)自 Shire Human Genetics Therapies, Inc. 购得。制备维拉苷酶的方法描述于例如美国专利第 7,138,262 号中。

[0311] 药物形式。维拉苷酶(在本文中也称作维拉苷酶 α)是一次性使用小瓶中的供溶

解使用的无菌、白色至灰白色的无防腐剂的冻干粉末,其在用灭菌注射水重构之后静脉内(IV)输注。

[0312] 定性和定量组合物。在用灭菌注射水重构后,各小瓶含有约 2.5mg/mL(40U/mL) 维拉苷酶 α 、50mg/mL 蔗糖、12.9mg/mL 柠檬酸钠二水合物、1.3mg/mL 柠檬酸单水化物和 0.11mg/mL 聚山梨醇酯 20。各小瓶含有对于 200U 小瓶为 2.0mL 的可提取体积和对于 400U 小瓶为 4.0mL 的可提取体积。维拉苷酶以 200U/ 小瓶 (5mg) 或 400U/ 小瓶 (10mg) 的维拉苷酶 α 供应,一单位 (U) 的酶活性定义为在 37°C 下每分钟将 1 微摩尔对硝基苯基 β -D-吡喃葡萄糖苷转化为对硝基苯酚需要的酶的量。

[0313] 容器和含量。维拉苷酶为在 5mL(200U/ 小瓶形式) 或 20mL(400U/ 小瓶形式) I 型玻璃小瓶中供应的供溶解使用的无菌冻干粉末。各小瓶含有 200U(5mg) 或 400U(10mg) 的维拉苷酶 α 。小瓶用具有氟树脂涂层的丁基橡胶塞封闭且用具有弹开塑料盖的铝外封件密封。

[0314] 使用说明书。维拉苷酶为欲用于静脉内输注的供溶解使用的冻干粉末。小瓶为一次性使用小瓶。在同一输注中维拉苷酶不与其他产品一起输注。总输注体积经 60 分钟的时间递送。维拉苷酶应该如下处理:

[0315] 1. 根据个体患者的体重和医嘱剂量测定待重构的小瓶的数量。

[0316] 2. 从冰箱中移出所需数量的小瓶。用 2.2mL 的灭菌注射水重构 200 单位小瓶且用 4.3mL 灭菌注射水重构 400 单位小瓶。不振荡。

[0317] 3. 在稀释之前,目视检查小瓶中的溶液。如果溶液变色或如果存在微粒物质,则不使用。

[0318] 4. 从适当数量的小瓶中取出计算体积的药物。

[0319] 5. 在 100mL 适于 IV 施用的 0.9%氯化钠溶液中稀释所需要的总体积,轻轻摇动,但不振荡。

[0320] 剂量。维拉苷酶以在(且包括)2.5U/kg 和 60U/kg 受试者体重之间,例如 15U/kg 至 60U/kg(例如,15U/kg、30U/kg、45U/kg 或 60U/kg) 的剂量施用。维拉苷酶可以 2U/kg/min、1.5U/kg/min、1U/kg/min 或 0.5U/kg/min 的速率施用。维拉苷酶的剂量每隔一周施用给受试者。

[0321] 维拉苷酶可以 15-60U/kg(例如,30U/kg-60U/kg,例如,15U/kg、30U/kg、45U/kg 或 60U/kg) 的剂量、以等于或小于 22.5U/kg 的剂量、以在 22.5U/kg 和 37.5U/kg 之间的剂量、以在 37.5U/kg 和 52.5U/kg 之间的剂量或以等于或大于 52.5U/kg 的剂量施用。在一些实施方案中,维拉苷酶可以 2.5-60U/kg 的剂量施用。在一些实施方案中,维拉苷酶可每隔一周通过静脉内输注施用。在其他实施方案中,维拉苷酶可以每周通过静脉内输注施用。在一些实施方案中,维拉苷酶可以通过静脉内输注例如以 2.5U/kg 的剂量每周施用 3 次。

[0322] 在一些实施方案中,所述剂量(例如,本文所述的剂量)的输注经小于 2 小时、例如小于 90 分钟、80 分钟、70 分钟、60 分钟、50 分钟或 45 分钟发生。

[0323] 重组产生的人类葡萄糖脑苷脂酶。其他形式的重组产生的人类葡萄糖脑苷脂酶可以用于本文所述的组合物、测定和方法中。例如,伊米苷酶(**Cerazyme®**)在中国仓鼠卵巢(CHO)细胞中重组产生且为市售的。另外,可使用 uplyso,其为在植物细胞中表达的重组葡萄糖脑苷脂酶(prGCD)。植物重组葡萄糖脑苷脂酶可以通过例如在美国公开案第

US 20090208477 号和第 US 20080038232 号和 PCT 公开案第 WO 2004/096978 号和第 WO 2008/132743 号中描述的方法获得。

[0324] 维持剂量

[0325] 在改善受试者的病状后,如果需要,则可以施用治疗的维持剂量。随后,可以根据症状将施用的剂量或频率或两者降低到保持改善的病状的水平。然而,在疾病症状任何反复后受试者可能需要以长期方式的间歇治疗。

[0326] 用 60U/kg 维拉苷酶的剂量每隔一周治疗且在用维拉苷酶完全治疗约 15-18 个月之后在四种推荐治疗标准(血红蛋白浓度、血小板计数、肝体积、脾体积)中的两种显示出改善后,将受试者转变到 30U/kg 的降低剂量每隔一周治疗。尽管有该剂量降低,但这些受试者显示这四种参数(血红蛋白浓度、血小板计数、脾体积和肝体积)和生物标志物(壳三糖苷酶和 CCL18)经 48 个月的时程临床上有意义的变化。

[0327] 在维拉苷酶和伊米苷酶之间的定性比较指示戈谢受试者的潜在额外益处在于,尽管剂量降低到伊米苷酶剂量的几乎一半,患者在该四种参数方面继续临床上改善,证明血红蛋白浓度的显著增加、在 36 个月之后血小板计数继续增加和肝/脾体积下降率。

[0328] 备选疗法

[0329] 维拉苷酶(有或没有额外药剂)的施用可用作例如先前用另一疗法(即,除了维拉苷酶以外的疗法,例如伊米苷酶、阿糖脑苷酶、uplyso、异法戈明酒石酸盐、美格鲁特或 Genz112638)治疗的受试者的备选治疗。例如,例如如果受试者经历来自另一疗法的副作用或不良作用,则接受另一疗法的戈谢病治疗的受试者可转变为用维拉苷酶治疗。例如,接受伊米苷酶的戈谢病治疗的受试者可转变为用维拉苷酶治疗,例如维拉苷酶可以与施用伊米苷酶时相同的剂量和相同的频率施用。例如,受试者在施用伊米苷酶时或之后可能经历输注部位反应和/或发展出抗伊米苷酶抗体(例如,伊米苷酶的中和抗体)。

[0330] 组合疗法

[0331] 可对患有戈谢病的受试者施用包括适量的维拉苷酶且持续适当时间以提供总治疗作用的疗法。维拉苷酶可单独或与额外药剂组合施用。在组合疗法的情况下,施用的量和时间可为提供例如增效治疗作用或相加治疗作用的施用量和时间。

[0332] 在一些实施方案中,维拉苷酶可与对戈谢病的另一疗法、例如除了酶替代疗法以外的疗法、例如异法戈明酒石酸盐、美格鲁特或 Genz112638 组合使用。

[0333] 异法戈明酒石酸盐。异法戈明酒石酸盐(AT-2101, HGT-34100, **PLICERA®**)((3R, 4R, 5R)-3, 4-二羟基-5-(羟基甲基)哌啶 L-(+)-酒石酸盐;CAS No. 957230-65-8)与葡萄糖脑苷脂酶选择性结合且使葡萄糖脑苷脂酶稳定并促进所述酶恰当运输到溶酶体,即在需要破坏葡萄糖脑苷脂的细胞中的隔室。也参见美国专利第 7,501,439 号。

[0334] 美格鲁特。美格鲁特(**ZAVESCA®**)((2R, 3R, 4R, 5S)-1-丁基-2-(羟基甲基)哌啶-3, 4, 5-三醇;CAS No. 72599-27-0)为 N-烷基化亚氨基糖,即右旋葡萄糖的合成类似物且具有苦味的白色至灰白色的结晶固体。美格鲁特显示出大量分布且具有进入诸如脑、骨骼和肺的深部器官的能力。

[0335] 美格鲁特抑制葡萄糖神经酰胺合酶,即用于合成大多数鞘糖脂的主要酶。美格鲁特为葡萄糖神经酰胺合酶抑制剂。其通过阻断降低在体内形成某些化学物质(基于葡萄糖

神经酰胺的鞘糖脂)的酶而起作用。美格鲁特用以治疗患有轻度至中度 1 型戈谢病的成人。

[0336] Genz112638。Genz112638 为经口给予的葡萄糖神经酰胺类似物且设计用来部分地抑制葡萄糖神经酰胺合酶,其导致葡萄糖神经酰胺的产量降低。

[0337] 在一些实施方案中,当维拉苷酶与额外药剂组合施用,该组合可导致所需要的额外药剂或维拉苷酶的剂量降低,使得副作用减少。所述组合可引起一种或两种药剂的递送和功效增强。

[0338] 所述药剂或疗法可同时(例如,作为施用给患者的单一制剂或同时施用的两种单独制剂)或以任何顺序相继施用。相继施用为在不同时间给予的施用。在一种药剂的施用与另一药剂的施用之间的时间可为数分钟、数小时、数天或数周。维拉苷酶的使用也可用以降低另一疗法的剂量,例如降低与正施用的另一药剂相关的副作用,例如降低除了酶替代疗法之外的疗法的副作用。因此,组合可包括以比在缺乏维拉苷酶的情况下使用的剂量低至少 10%、20%、30% 或 50% 的剂量施用第二药剂。

[0339] 组合疗法可包括施用降低其他疗法的副作用的药剂。例如,在施用对戈谢病的治疗之前可对受试者施用皮质类固醇以降低输注部位反应。作为另一实例,在维拉苷酶疗法的过程中可给予铁补充疗法。

[0340] 戈谢病

[0341] 戈谢病为最常见的溶酶体贮积病。其由酶葡萄糖脑苷脂酶(也称作酸 β -葡萄糖苷酶)的遗传性缺乏引起。所述酶作用于脂质葡萄糖脑苷脂(也称作葡萄糖神经酰胺)。当所述酶缺乏时,所述物质尤其积聚在单核细胞系的细胞中。脂肪物质可集中在脾、肝、肾、肺、脑和骨髓中。症状可包括脾肿大和肝肿大、肝功能障碍和可能为痛苦的重度神经功能并发症的骨骼病症和骨病变、淋巴结和(偶尔)邻近关节肿胀、腹部膨胀、皮肤褐色着色、贫血、低血小板和眼白(巩膜)上的黄色脂肪沉积。受影响最严重的人也可能更易于感染。该疾病由染色体 1 上的隐性基因引起且影响男性和女性。

[0342] 戈谢病具有三种常见的临床亚型:

[0343] I 型(或非神经病型)为该疾病的最常见形式,在 50,000 个活产婴儿中出现约 1 个。其在德系犹太人(Ashkenazi Jewish)后裔的人中最常发生。症状可在年轻时或成年开始且包括肝肿大和脾肿大(通称肝脾肿大);脾可能破裂且引起额外的并发症。骨骼衰弱和骨病可为广泛的。脾肿大和骨髓置换引起贫血、血小板减少症和白细胞减少症。脑未受影响,但可能有肺损伤和罕见的肾损伤。该组中的患者通常易于瘀伤(由于血小板水平低)且经历由红细胞数量较低引起的疲劳。根据疾病起始和严重性,1 型患者可很好地活到成年。许多患者具有该疾病的轻微形式且可能不显示任何症状。在一些实施方案中,本文所述的方法和组合物用以治疗 I 型戈谢病。

[0344] II 型(或急性婴儿神经戈谢病)通常在初生 6 个月内开始且发生率为在 100,000 个活产婴儿中有约 1 个。症状包括肝脾肿大、广泛且渐进性脑损伤、眼球运动病症、痉挛、发作、四肢强直和吮吸吞咽能力弱。感染的儿童通常在 2 岁前死去。

[0345] III 型(慢性神经病形式)可在儿童或甚至成年的任何时候开始且在 100,000 个活产婴儿中出现约 1 个。其特征在于,与急性或 2 型型式相比,其为缓进型但较轻微的症状。主要症状包括脾和/或肝肿大、发作、机能协调弱、骨不规则、眼球运动病症、包括贫血的血液病症和呼吸问题。患者常活到其青少年和成年。

[0346] 骨矿密度

[0347] 如本文所使用的骨骼密度（或骨矿密度）是指每平方厘米骨骼中物质的量。骨骼密度可在临床医学中用作骨质疏松和/或骨折危险的间接指示。BMD 可通过许多程序测量：例如双能量 X 线吸收测定法（DXA 或 DEXA）、定量计算机断层摄影（QCT）、定性超声波（QUS）、单光子吸收测定法（SPA）、双光子吸收测定法（DPA）、数字 X 射线放射照片测量（DXR）和单能量 X 射线吸收测定法（SEXA）。可对例如腰脊柱、臀上部或前臂进行测量。

[0348] 平均骨矿密度可定义为 $BMC/W[g/cm]$ ，其中 $BMC = \text{骨矿含量} = g/cm$ ，且 $W = \text{扫描线处的宽度}$

[0349] 密度测定法结果可以例如测量密度 ($g\ cm^{-3}$)、z-分值和 t-分值报道。负分指示骨密度较低，且正分指示骨密度较高。

[0350] Z-分值是指高于或低于患者年龄、性别和种族的平均值的标准偏差的数目。

[0351] T-分值是指高于或低于与患者相同性别和种族的健康 30 岁成人的平均值的标准偏差的数目。世界卫生组织的标准为：

[0352] • 正常为 -1.0 或更高的 T-分值。

[0353] • 骨量减少定义为小于 -1.0 且大于 -2.5。

[0354] • 骨质疏松定义为 -2.5 或更低，意思是骨密度比 30 岁妇女的平均值低 2.5 倍标准偏差。

[0355] 施用

[0356] 本文所述的葡糖脑苷脂酶替代疗法可例如通过静脉内、动脉内、皮下 (subdermally)、腹腔内、肌肉内或皮下 (subcutaneously) 注射来施用。优选所述葡糖脑苷脂酶替代疗法以约 10、15、20、25、30、35、40、45、50、55、60、65、70、75 或 80U/kg 的范围内的剂量静脉内每隔一周施用或根据特定化合物的要求来施用。本文的方法预期施用有效量的化合物或化合物组合物以实现期望的或规定的作用。通常，葡糖脑苷脂酶替代疗法可以连续输注、例如经 60 分钟、90 分钟、120 分钟或 150 分钟连续输注来施用。可以与载体材料组合以产生单一剂型的活性成分的量将取决于所治疗的宿主和特定施用模式而改变。典型制剂将含有约 5% - 约 95% 的活性化合物 (w/w)。或者，这类制剂含有约 20% - 约 80% 的活性化合物。

[0357] 可能需要比上述剂量更高或更低的剂量。任何特定受试者的具体剂量和治疗方案将取决于多种因素，包括所用具体化合物的活性、年龄、体重、一般健康状况、性别、饮食、施用时间、排泄率、药物组合、疾病、病状或症状的严重性和过程、受试者受疾病、病状或症状影响的倾向和治疗医师的判断。

[0358] 在改善受试者的病状时，如果需要，可以施用维持剂量的本发明的化合物、组合物或组合。随后，可以根据症状将施用的剂量或频率或二者降低到保持改善的病状的水平。然而，在疾病症状任何复发时受试者可能需要长期的间歇治疗。

[0359] 本发明的化合物、组合物或组合可作为家庭疗法（例如，在受试者的家、工作场所或其他非临床（例如，非医院）环境中）施用。其可由保健专业人员（例如，护士或医师助理）施用（例如，经输注）。例如，如果受试者例如在施用（例如，经输注）所述化合物、组合物或组合一次、两次或三次之后，没有经历不良事件 (AE)（例如，药物有关的严重 AE 或输注有关的 AE，例如本文所述的事件），则所述受试者适接受家庭疗法以便进行后续施用。

[0360] 药物组合物

[0361] 葡糖脑苷脂酶替代疗法（例如，维拉苷酶）可并入药物组合物中以便施用给受试者。这类组合物通常包含所述葡糖脑苷脂酶替代疗法（例如，维拉苷酶）和药学上可接受的载体。

[0362] 如本文所使用的语言“药学上可接受的载体或佐剂”包括与药物施用相容的溶剂、分散介质、覆料、抗菌剂和抗真菌剂、等渗且吸收延迟剂等。补充活性化合物也可并入所述组合物中。术语“药学上可接受的载体或佐剂”是指可以与葡糖脑苷脂酶一起施用给受试者并且在足以递送治疗量的葡糖脑苷脂酶的剂量施用不会破坏其药理学活性且无毒的载体或佐剂。

[0363] 配制药组合物以与其预定施用路径相容。施用路径的实例包括肠胃外路径，例如静脉内、皮内和皮下路径。用于肠胃外、皮内或皮下应用的溶液或悬浮液可包括以下组分：无菌稀释剂，诸如注射用水、盐水溶液、固定油、聚乙二醇、甘油、丙二醇或其他合成溶剂；抗菌剂，诸如苄醇或对羟基苯甲酸甲酯；抗氧化剂，诸如抗坏血酸或亚硫酸氢钠；螯合剂，诸如乙二胺四乙酸；缓冲剂，诸如乙酸盐、柠檬酸盐或磷酸盐；和用于调节张力的试剂，诸如氯化钠或右旋糖。pH值可用诸如盐酸或氢氧化钠的酸或碱调节。肠胃外制剂可装入安瓿、一次性注射器或由玻璃或塑料制成的多剂量小瓶中。

[0364] 对于静脉内施用，合适的载体包括灭菌注射水、生理盐水、抑菌水、CREMOPHOR EL (BASF, Parsippany, NJ) 或磷酸盐缓冲盐水 (PBS)。在所有情况下，所述组合物必须为无菌的且应为流体从而存在容易的可注射性。所述组合物应该在制造和储存条件下稳定且必须被保藏以防诸如细菌和真菌的微生物的污染作用。所述载体可为溶剂或分散介质，其包含例如水、乙醇、多元醇（例如，甘油、丙二醇和液体聚乙二醇等）及其合适的混合物。恰当的流动性可例如通过使用诸如卵磷脂的覆料、在分散液的情况下通过保持所需粒度或通过使用表面活性剂来保持。防止微生物作用可通过例如对羟基苯甲酸、氯丁醇、苯酚、抗坏血酸、硫柳汞等的各种抗菌剂和抗真菌剂来获得。在很多情况下，将优选在组合物中包含等渗剂，例如糖，诸如蔗糖；多元醇，诸如甘露醇、山梨糖醇；氯化钠。可注射组合物的延长吸收可通过在所述组合物中包含例如单硬脂酸铝和明胶的延迟吸收的试剂而达到。

[0365] 所述药物组合物可包含例如灭菌注射水、蔗糖、柠檬酸钠、柠檬酸和聚山梨酯。

[0366] 无菌注射溶液可通过在适当溶剂中并入所需量的葡糖脑苷脂酶以及根据需要上文列举的成分中的一种或组合，接着过滤灭菌来制备。通常，分散液通过将活性化合物并入含有基础分散介质和来自以上列举成分的所需其他成分的无菌赋形剂中来制备。在供制备无菌注射溶液的无菌粉末的情况下，优选的制备方法为真空干燥和冷冻干燥，其产生活性成分外加来自其先前无菌过滤的溶液的任何额外期望的成分的粉末。

[0367] 有利的是以便于施用和可实现剂量均匀性的剂量单位形式配制肠胃外组合物。如本文所使用的剂量单位形式是指适合作为待治疗的受试者的单一剂量的实体离散单位；各单位含有在与所需药物载体缔合的情况下可产生期望的治疗作用的预定量的活性化合物。

[0368] 所述药物组合物可与施用说明书一起包括在容器、包装或分配器中。

[0369] 试剂盒

[0370] 葡糖脑苷脂酶替代疗法（例如，维拉苷酶）可提供在试剂盒中。所述试剂盒包含 (a) 葡糖脑苷脂酶替代疗法，例如包含所述葡糖脑苷脂酶替代疗法的组合物；和 (b) 信息材

料。所述信息材料可以是涉及本文所述的方法和 / 或在本文所述的方法中使用的葡糖脑苷脂酶替代疗法的描述性、指导性、销售性或其他材料。例如,所述信息材料描述施用葡糖脑苷脂酶替代疗法来治疗戈谢病的方法。

[0371] 在一个实施方案中,所述信息材料可以包括以合适的方式、例如以合适的施用剂量、剂型或模式(例如,本文所述的施用剂量、剂型或模式)施用葡糖脑苷脂酶替代疗法的说明。在另一实施方案中,所述信息材料可以包括用于鉴定例如人类的合适受试者的说明。所述试剂盒的信息材料在其形式方面不受限制。在很多情况下,所述信息材料(例如说明)以印刷品,例如印刷文本、图画和 / 或照片,例如标签或印刷纸张提供。然而,所述信息材料也可以以其他格式提供,诸如盲文、计算机可读材料、视频记录或录音。在另一实施方案中,所述试剂盒的信息材料为链接或联系信息,例如实际地址、电子邮件地址、超链接、网址或电话号码,其中所述试剂盒的使用者可以获得关于效应物和 / 或其在本文所述方法中的用途的实质性信息。当然,所述信息材料也可以以格式的任何组合提供。

[0372] 除了所述葡糖脑苷脂酶替代疗法外,所述试剂盒的组合物可以包含其他成分,诸如溶剂或缓冲剂、稳定剂或防腐剂和 / 或用于治疗戈谢病的第二试剂。或者,其他成分可以包含在试剂盒中,但在与所述葡糖脑苷脂酶替代疗法不同的组合物或容器中。在这类实施方案中,所述试剂盒可以包含用于混合所述葡糖脑苷脂酶替代疗法与所述其他成分(例如,在施用之前重构冻干的疗法和 / 或稀释重构的疗法)或用于与其他成分一起使用所述葡糖脑苷脂酶替代疗法的说明。

[0373] 所述葡糖脑苷脂酶替代疗法可以以任何形式(例如液态、干燥或冻干形式)提供。优选所述葡糖脑苷脂酶替代疗法为实质上纯和 / 或无菌的。当所述葡糖脑苷脂酶替代疗法以液体溶液提供时,所述液体溶液优选为水溶液,优选无菌水溶液。当所述葡糖脑苷脂酶替代疗法以干燥形式提供时,通常通过加入合适的溶剂来进行重构。所述溶剂(例如,无菌水或缓冲剂)可以任选地提供于试剂盒中。

[0374] 所述试剂盒可以包括含有所述葡糖脑苷脂酶替代疗法的组合物的一个或多个容器。在一些实施方案中,所述试剂盒含有用于所述葡糖脑苷脂酶替代疗法(例如,在组合物中)和信息材料的单独容器、分配器或隔室。例如,所述葡糖脑苷脂酶替代疗法(例如,在组合物中)可以容纳于瓶子、小瓶或注射器中,且所述信息材料可以容纳于塑料套或小袋中。在其他实施方案中,所述试剂盒的单独元件容纳于单个未分开的容器内。例如,所述葡糖脑苷脂酶替代疗法(例如,在组合物中)容纳于具有以标签形式附着到其上的信息材料的瓶子、小瓶或注射器中。在一些实施方案中,所述试剂盒包括多个(例如,一包)单独容器,其各自包含所述葡糖脑苷脂酶替代疗法(例如,在组合物中)的一个或多个单位剂型(例如,本文所述的剂型)。例如,所述试剂盒包括多个注射器、安瓿、铝箔包装或泡罩包装,其各自含有所述葡糖脑苷脂酶替代疗法的单个单位剂量。所述试剂盒的容器可以是气密的和 / 或防水的。

[0375] 所述葡糖脑苷脂酶替代疗法(例如,在组合物中)可以施用给患有戈谢病的受试者。所述方法可以包括例如如本文所述评估受试者且由此鉴定需要用所述葡糖脑苷脂酶替代疗法治疗的受试者。

[0376] 以下实施例提供本发明的说明性实施方案。本领域的普通技术人员应了解可以在不改变本发明的精神或范围的情况下进行的许多改进和变化。这类改进和变化涵盖在本发

明的范围内。所述实施例不以任何形式限制本发明。

实施例

[0377] 酶疗法的引进显著影响 1 型戈谢病的自然史。遗憾的是,单一治疗选择的存在代表在患有 1 型戈谢病的患者的治疗中的内在脆弱性。据报道约 15% 的伊米苷酶治疗的患者发展出 IgG 抗体且据报道这些患者中大致一半具有过敏症状 (Starzyk K 等, Molec Genet Metab. 2007 ;90:157-163)。在全球,近期伊米苷酶的短缺加剧了在戈谢病的治疗中对于单一产品的依赖性 (Steinbrook R 等, N Engl J Med. 2009 ;361:1525-1527)。在那些接受伊米苷酶输注的患者中,未知数量的患者需要预投药来减轻潜在的免疫介导的响应。在一些事例中,患者需要氢化可的松 (hydrocortisone),其本身与包括 AVN 的医疗危险相关。此外,近来公布的数据提示 59% 的用伊米苷酶治疗的 GD1 患者在治疗最少 4 年之后未能实现至少一个治疗目标,而这与治疗剂量和持续时间无关 (Weinreb N 等, Am J Hematol. 2008 ;83:890-895)。

[0378] 维拉苷酶 α 为区别于伊米苷酶的具有独有的特征 (野生型氨基酸序列和高 α -甘露糖基含量) 的新型酶替代疗法 (ERT)。以下提供的一些实施例描述评估维拉苷酶 α 的安全性和功效的临床试验和扩展研究。这些是涉及 ERT 以实现剂量降低和家庭疗法的首次临床试验。

[0379] 实施例 1:TKT025 和 TKT025EXT 研究

[0380] 概述

[0381] 该实施例描述所进行的用于评估维拉苷酶 α 的安全性和功效的维拉苷酶 α 的 9 个月 I/II 期开放标签单一中心试验 (TKT025) 和进行以评估维拉苷酶 α 的安全性和功效的进行中的扩展 (TKT025EXT) 研究。

[0382] I/II 期试验的第一目的在于评价以 60U/kg 的剂量每隔一周静脉内施用给患有有症状的 1 型 (非神经病变型) 戈谢病 (GD1) 的成年患者 9 个月的维拉苷酶 α 的安全性。该试验的第二目的在于评价维拉苷酶 α 对关键疾病特点的临床活动 (Barton NW 等, N Engl J Med. 1991 ;324:1464-1470)。类似地设计扩展研究以评估长期安全性和评价维拉苷酶 α 对四种疾病量度:血红蛋白浓度、血小板计数、肝体积和脾体积的影响 (Barton NW 等, N Engl J Med. 1991 ;324:1464-1470)。

[0383] 在 I/II 期研究中 12 个具有 1 型戈谢病和完整脾的有症状的成年患者接受维拉苷酶 α (60U/kg/ 输注)。最初筛选出 13 个患者参与研究,而一个患者由于存在抗伊米苷酶抗体而被排除。对完成试验的患者提供扩展研究且实行逐步剂量降低 (至 30U/kg/ 输注)。11 个患者完成 I/II 期,10 个患者进入扩展研究;9 个患者在扩展研究中达到 39 个月。没有观察到药物有关的严重不良事件或退出,也没有观察到抗体。在扩展研究中成功实施了家庭疗法。记录以下项目的从基准到 9 个月和从基准到 48 个月的平均变化%的统计上显著的改善 ($p < 0.004$):血红蛋白 (分别为 +19.2%、+21.7%)、血小板计数 (分别为 +67.6%、+157.8%)、正规化肝体积 (分别为 -18.2%、-42.8%) 和正规化脾体积 (分别为 -49.5%、-79.3%)。这些显著的临床变化和安全性概况导致 III 期试验且突出了维拉苷酶 α 作为 1 型戈谢病的备选疗法的潜力。

[0384] 方法

[0385] I/II 期研究和扩展研究在单一中心 (Gaucher Clinic, Shaare Zedek Medical Center ;Jerusalem, Israel) 中进行。

[0386] 患者 :筛选成年有症状的酶证实的患有 GD1 的患者。合格标准包括年龄 ≥ 18 岁、脾完整、疾病有关的贫血 (血红蛋白值比对于性别的正常值下限 (LLN) 低至少 1g/dL)、血小板减少 (血小板计数低于 LLN) 且 B 和 C 肝炎抗原和艾滋病毒的结果为阴性。如果患者对 ERT 为雏期且在登记之前在 12 个月内没有接受伊米苷酶且伊米苷酶抗体为阴性,则这些患者符合条件。如果患者在登记之前 30 天以内接受过任何其他适应症的研究性疗法或如果他们出于医疗或非医疗原因不能遵循实验方案,则排除这些患者。

[0387] 制备和给药 :维拉苷酶 α 由 Shire HGT 以冻干产品形式来供应且在 2°C 至 8°C 下运输。所述产品用无防腐剂的灭菌注射水重构。将适当量的维拉苷酶 α (基于体重计) 与生理盐水缓慢混合,达到 100mL 的最终体积。稀释的维拉苷酶 α 穿过 $0.2\mu\text{m}$ 过滤器静脉内施用 60 分钟的时间 (最大速率 :1.5mg/kg/hr ;1U/kg/min)。

[0388] 在 I/II 期研究期间,前三个患者在试验地点按每隔一周的方案接受维拉苷酶 α 。对于该前三个患者进行剂量增加,其中给药从 15U/kg 的初始剂量加倍,直至达到 60U/kg 的最终剂量。刚好在第一个患者和第二个患者完成 7 天观察期之后使第二个患者和第三个患者分别接受他们的初始 15U/kg 输注。第三个患者一旦接受 60U/kg 的单一剂量且观察 7 天时间,登记 9 个额外患者且每隔一周接受 60U/kg 的输注,总共 20 剂。经受剂量增加的患者继续每隔一周的方案,另外以 60U/kg 输注 17 次,总共 20 次输注。

[0389] 在扩展研究期间,所有患者每隔一周继续以 60U/kg/ 输注。在约 6-9 个月的扩展研究之后,将对于贫血、血小板减少、肝肿大和 / 或脾肿大改善的 4 个治疗目标中实现至少两个的患者 (Pastores GM 等,Semin Hematol. 2004 ;41:4-14) 转换为每隔一周 45U/kg/ 输注历时 3 个月,且随后转换为每隔一周 30U/kg/ 输注。根据治疗目标的实现来减少剂量的惯例是与涉及伊米苷酶的差别对待患者中的 ERT 的建议相一致的 (Andersson HC 等,Genet Med. 2005 ;7:105-110)。

[0390] 另外,居住在以色列的 7 个患者在扩展期转换为家庭疗法。

[0391] 安全性评价 :贯穿研究通过在输注之前、输注期间和输注之后进行不良事件 (包括输注有关的反应)、伴随的投药和生命体征的每隔一周评价来评估安全性。大致每 12 周进行额外安全性评价,且包括在试验地点的身体检查、临床实验室检验 (血液学、血清化学、尿检和妊娠试验)、12- 导联心电图和超声心电图。在 Shire HGT 以 3 个月间隔进行抗维拉苷酶 α 抗体的存在的测定。

[0392] 抗体测定 :使用经批准的间接 ELISA 对于循环抗维拉苷酶 α 抗体筛选所有参与者。微孔板用维拉苷酶 α 涂覆,洗涤且用牛血清蛋白阻断以限制非特异性抗体结合。在 37°C 下将它们与在含有 0.05% 吐温 20 的磷酸盐缓冲盐水中稀释 100 倍的患者血清样品孵育 60 分钟。洗涤微孔且随后用适当辣根过氧化物酶 (HRP)- 结合的二级抗体孵育。将它们用以下 HRP- 抗体、同种型特异性结合物单独探试 :1) 山羊抗人类 IgG Fc、2) 山羊抗人类 IgA α - 链、3) 山羊抗人类 IgM μ - 链或 4) 山羊抗人类 IgE ϵ - 链二级抗体。将微孔洗涤最后一次且用 HRP 产色性底物 3,3',5,5'- 四甲基联苯胺孵育。反应通过加入 2N 硫酸而停止,且使用 Molecular Devices SPECTRAmax Plus384 板阅读器和 SOFTMax PRO 软件量化各孔在 450nm 下的吸光度 (A_{450})。从接受伊米苷酶的患者获得抗体阳性血清样品。这些患者

的样品与维拉昔酶 α 交叉反应且在抗维拉昔酶抗体的筛选测定中用作人类阳性对照。因此,这些血清为抗伊米昔酶和抗维拉昔酶 α 阳性。在每一测定板内包括阴性人类血清对照以及阳性人类血清对照。

[0393] 由 ERT 雏期戈谢病患者血清样品 ($N = 108$) 的平均吸光度确立抗维拉昔酶 α 抗体的实用 ELISA 抗体阳性切割点吸光度。使用 ELISA 吸光度数据的参数分析来计算各抗体同种型的抗体阳性下限 (平均值加 $1.645 \times$ 标准偏差;其中 1.645 为正态分布的第 95 百分位数,因此潜在地接受 5% 假阳性率) (Mire-Sluis AR 等, J Immunol Methods. 2004; 289:1-16)。对于所有抗维拉昔酶 α 抗体同种型测定,计算 ERT 雏期戈谢血清样品的 ELISA A_{450} 背景为 0.040。ELISA 抗体阳性截止点确立为比率 ≥ 2.0 且 $A_{450} \geq 0.040$, 其中该比率为在任何时间点取得的患者样品的 A_{450} 除以在第一 ERT 治疗之前在基准处取得的患者样品的 A_{450} 。超过 ELISA 阳性截止点的任何样品通过定量放射免疫沉淀测定证实且测试中和抗体;然而,没有样品达到确立的截止标准 (Mire-Sluis AR 等, J Immunol Methods. 2004; 289:1-16)。

[0394] 临床活动:以预定的每三个月间隔评估血红蛋白浓度和血小板计数的主要功效评价。在扩展研究期间使用在基准、6 个月和 9 个月 (在 Hadassah-Hebrew University Medical Center) 和在 24 个月、33 个月和 45 个月 (在 MOR-MAR Imaging 装置) 进行的定量腹部 MRI (在相同型号的设备上) 测量肝体积和脾体积。在试验的结束时由不知晓患者身份和进行定量腹部 MRI 的顺序的放射科医师评价肝体积和脾体积。在荷兰阿姆斯特丹的 Academic Medical Center;Amsterdam 测量壳三糖苷酶和 CCL18。

[0395] 统计分析:将安全性群 (其也为意向治疗群) 定义为接受维拉昔酶 α 的至少一次输注 (部分或全部) 的所有登记的患者,且其用于所有临床活性。不使用归集。

[0396] 对于血红蛋白浓度、血小板计数、肝体积和脾体积的主要临床活动参数,零假设在于在基准值与研究结束 (9 个月) 值之间和在基准值与 48 个月结束值之间没有差异。(即,在成员之间的各对观察结果的差异具有中值零。) 在 5% 显著性水平下使用双尾假设检验进行比较。在基准与期末值之间的差异使用 Wilcoxon 符号秩次检验分析。计算相对于基准的变化且使用描述性统计概述相对于基准的变化百分数。

[0397] 对于次要临床活动参数,通过访问概述观察数据、相对于基准的变化和相对于基准的变化%,包括平均值、标准误差 (SE) 和中值。对这些数据集不采用形式统计检验。

[0398] 来自 TKT025 研究 (9 个月, 60U/kg 维拉昔酶 α) 的结果

[0399] 维拉昔酶 α 耐受性良好且患者没有发展出抗体。早在 3 至 6 个月就发现了在血液学参数和器官体积方面的临床和统计上显著的改善 (表 1)。

[0400] 表 1 TKT025 临床结果 ($N = 12$)

[0401]

临床活动参数	中值 基准值	相对于基准的平均变化±标准偏差		
		3个月	6个月	9个月
血红蛋白浓度(g/dL)	10.95	1.24 ± 0.90	1.92 ± 0.82	2.24 ± 0.89
血小板计数($\times 10^9/L$)	57.5	8.9 ± 11.20	23.4 ± 24.63	40.6 ± 30.68
肝(体重%)	4.43	NA	-0.67 ± 0.64	-0.79 ± 0.48
脾(体重%)	3.63	NA	-1.62 ± 0.67	-1.89 ± 0.75

[0402] 人口资料和处置:筛选出总共13个患者且全部同意参与该研究:一个患者(0004)由于伊米苷酶抗体而被排除。尽管在更远的过去,2个患者(0008和0009)各自接受3次伊米苷酶输注,1个患者(0003)暴露于美格鲁特且2个患者(0005和0007)暴露于美格鲁特和伊米苷酶两者,但是因为在登记之前12个月内,所有患者未暴露于任何戈谢特异性疗法,所以根据实验方案他们在到来时都处于治疗初期。

[0403] 意向治疗群(表2)包括12个接受至少一剂量维拉苷酶 α 的患者;其中,11个患者(92%)完成I/II期研究(1个患者0006在接受三次输注之后因家人突然死亡而同意退出)。

[0404] 在登记I/II期试验时,7个患者(58%)为女性;平均年龄为41.7(SD±17.3;范围19至70)岁;平均体重为59.6(SD±9.1;范围50至73)kg且平均身高为169(SD±8.0;范围160至184)cm。两个患者(16.7%)在登记时具有髌关节无血管形成性坏死(AVN)且另一患者的各踝具有破坏性病变。表2提供在I/II期研究内在基准的人口统计、遗传性和临床特征以及各意向治疗患者在关键数据收集点的临床发现。

[0405]

表 2 患者的人口资料 and 特征

患者	年龄	性别	基因型	血红蛋白 g/dL					血小板 10 ⁹ /L					正常化脾脏, 正常脾脏					正常化脾脏, 正常脾脏				
				BL	6 Mo	9 Mo	24 Mo	48 Mo	BL	6 Mo	9 Mo	24 Mo	48 Mo	BL	6 Mo	9 Mo	24 Mo	45 Mo	BL	6 Mo	9 Mo	24 Mo	45 Mo
1	24	F	N370S/N370S	10.8	12.2	12.5	12.4	12.7	80	123	149	155	130	37.5	11.0	10.0	8.0	6.5	2.3	1.8	1.9	1.5	1.2
2	62	M	N370S/L44P	12.7	14.1	14.8	14.3	14.2	69	74	90	127	142	11.0	7.5	6.0	4.0	3.0	1.0	1.0	0.9	1.0	0.8
3	35	M	N370S/其他	13.5	15.7	16.5	15.7	16.0	48	95	128	140	151	17.5	8.0	6.5	□	□	2.2	1.6	1.7	1.3	1.2
5	35	F	N370S/N370S	10.6	11.7	12.0			52	68	72			32.5	23.0	24.5			1.7	1.8	1.6		
6	44	F	N370S/T182-I	10.9					56					16.0					2.1				
7	42	F	N370S/N370S	10.5	12.5	12.5	13.8	13.0	37	32	39	42	95	21.0	13.5	13.0	7.0	4.0	1.8	1.7	1.6	1.4	1.0
8	23	F	N370S/N370S	10.0	12.1	11.8	12.7	14.2	65	137	150	154	203	39.0	9.0	7.0	3.5	3.5	2.2	1.6	1.6	1.2	1.2
9	25	M	N370S/N370S	12.6	15.8	15.9	15.8	15.1	69	97	120	178	183	28.0	12.5	10.0	4.0	2.5	2.0	1.4	1.4	1.1	0.9
10	60	F	N370S/N370S	10.1	11.1	12.5	12.4	12.4	48	60	86	121	139	39.0	11.0	8.5	3.5	3.0	1.4	1.2	1.0	0.8	0.8
11	18	F	N370S/其他	11.0	13.8	13.6	13.6	12.6	59	98	110	122	130	35.0	9.0	8.0	5.0	3.5	1.4	1.1	1.0	1.1	0.8
12	69	M	N370S/N370S	13.5	14.4	14.1			59	65	99			13.0	9.0	7.5			1.0	1.0	1.0		
13	56	M	N370S/Y394L	12.9	15.9	16.6	15.4	16.0	46	40	36	111	135	39.5	10.0	8.0	4.5	2.5	1.0	0.8	0.8	0.8	0.7

在 I/II 期试验和扩展试验期间, 在基准和选择的评估点的患者人口资料 and 特征包括对于群体的各临床参数所收集的最后的值。正常化肝体积和正常化脾脏体积分别定义为总体重 (以 kg 计) 的 2.5% 和 0.2% (Pastores GM 等, *Semin Hematol.* 2004; 41: 4-14)。正常的倍数为观察到器官体积除以正常化器官体积。在 24 个月内, 所有患者证明了血红蛋白浓度的正常化且除了 1 个患者外都证明血小板计数大于 $100 \times 10^9/L$ 。BL: 基准; Mo: 月; M: 男性; F: 女性; *脾脏由于技术误差而不能解释。

[0406] 肝和脾磁共振成像 (MRI) 扫描的结果经隐瞒且由单个审阅者解释以使偏差最小化。在研究中的各个时间点也进行包括 PFT (肺功能检验)、股骨和腰脊柱的 MRI 和骨密度

的其他临床参数的基准数据的收集。

[0407] 研究由两期：剂量增加期和继续给药期组成。在剂量增加期，登记第一个患者并静脉内（IV）施用维拉苷酶 α 15U/kg，随后跟踪 7 天以便评价安全性。在对第一个患者证实了安全之后，登记 2 个额外患者并 IV 施用维拉苷酶 α 15U/kg，且跟踪 7 天以便评价安全性。在对接受 15U/kg 剂量的所有 3 个患者证实了安全之后，以相同方式施用接下来的两种较高剂量（30U/kg 和 60U/kg）。一旦对所有 3 个患者都证实了安全，则登记额外 9 个患者并施用 60U/kg 剂量。所有患者随后每隔一周接受 60U/kg 剂量，总共 20 剂维拉苷酶 α 。

[0408] 在 TKT025 中在 39 周中的第 1 周、第 3 周、第 5 周和第 37 周评估人类的药物动力学（PK）。在研究 TKT025EXT 的第 65 周进行额外 PK 研究。在各评估中维拉苷酶 α 以一阶消除动力学自血液迅速清除。在初始暴露于维拉苷酶 α 之后和在重复施用维拉苷酶 α 之后患者的消除半衰期值是类似的。相比之下，在重复施用维拉苷酶 α 之后的清除率和表观分布容积值降低。

[0409] 在研究 TKT025 中在开始每两周给药 60U/kg 维拉苷酶 α 之后 3 个月观察到血红蛋白浓度和血小板计数相对于基准的统计上显著的平均增加。在 TKT025 中的第 25 周（第 6 月），平均血红蛋白浓度为 13.57g/dL（在正常范围内）且在该研究的剩余期内保持在正常范围内。在该研究结束时，11 个患者中的 10 个经历血红蛋白浓度的正常化。在第 13 周（在第 3 月）观察到平均血小板计数相对于基准（ $57.3 \times 10^3/\text{mm}^3$ ）的统计上显著的增加并且在整个研究中持续。在第 37 周，平均血小板计数增加到 $98.1 \times 10^3/\text{mm}^3$ 。

[0410] 另外，在开始维拉苷酶 α 治疗之后 6 个月（第 25 周）和 9 个月（第 37 周）观察到平均和正规化（通过体重百分数校正）肝体积和脾体积相对于基准的统计上显著的减少。第 3 个月观察到生物标志物值（血清壳三糖苷酶和趋化因子（C-C 基元）配体 18[CCL18]）的显著减小。总体上，在维拉苷酶 α 施用过程期间所有这些参数的平均改善连续不断。此外，各患者在对戈谢病具有特异性的四种治疗参数（例如，血红蛋白浓度、血小板计数、脾体积和肝体积）中的至少两种经历改善。

[0411] 总之，图 1A-1F 提供 I/II 期研究中血液学参数、器官体积和生物标志物的平均改变%。在 25 周和 37 周期间观察到 (a) 血红蛋白浓度和 (b) 血小板计数的显著增加以及 (c) 肝体积和 (d) 脾体积的显著减小。虽然 (e) 壳三糖苷酶和 (f) CCL18 的生物标志物取样不完全，但每个患者随时间而观察到两种生物标志物相对于基准的一般减少。记录到以下项目从基准到第 9 个月的平均变化%的统计上显著的改善 ($p < 0.003$)：血红蛋白浓度 (+21.7%)、血小板计数 (+67.6%)、正规化肝体积 (-18.2%) 和正规化脾体积 (-49.5%)，其中血红蛋白浓度和血小板计数两者相对于基准的统计上显著的改善在最初的 3 个月内达到（图 2）。在图 2 中，作图将血红蛋白浓度、血小板计数、肝体积和脾体积的平均变化%相对于时间作图且对于 I/II 期和扩展研究进行区分。观察到各参数从基准到第 9 个月的相对于基准的统计上显著的变化 ($p < 0.003$)。对于血小板计数和脾体积观察到最显著的变化。

[0412] 在 TKT025 中，所有 12 个患者报道经历 1 件或多件 AE（不良事件），总共 103 件 AE。在 15U/kg 剂量期间由 1 个患者报道两件 AE（眩晕和多汗），由接受 60U/kg 剂量的患者报道剩余 101 件 AE。最常报道的 AE 为眩晕、骨痛和头痛（各有 5 个患者；41.7%）。关节痛、背痛、手足痛、流行性感、上腹痛和乏力各自出现在 3 个患者（25%）中。除了 1 件骨痛

之外,所有 AE 都是轻微的 (1 级),所述骨痛为中度 (2 级) 严重性且与研究药物无关。103 件 AE 中无一被视为重度或危及生命的 (3 级或 4 级)。没有患者由于不良事件而被从研究中退出。

[0413] 在 TKT025 期间观察到的大多数 AE 由研究者测定为与药物施用无关 (表 3A)。十个 (10 个) 患者经历被视为可能或者或许与研究药物有关的 22 件 AE;最常为眩晕 (3 个患者);背痛、骨痛、头痛、体温增加和恶心 (各 2 个患者)。九 (9) 个患者经历总共 17 件输注有关的不良事件,其在实验方案中定义为在输注当天发生、在输注期间或之后开始的 AE,且判断为可能或者或许与研究药物有关。这些 AE 包括眩晕、头痛、背痛、骨痛、体温增加,各自在 2 个患者中出现。对于接受 60U/kg 剂量的患者,报道了所有输注有关的不良事件。一个患者在剂量减少之后由于骨痛恶化而增加到每隔一周剂量 60U/kg/ 输注历时 24 个月,但在剂量增加之后没有经历缓减。因此,不存在药物有关的严重不良事件,不管输注环境为何。不施用预投药且没有患者由于不良事件而退出。患者没有发展出维拉昔酶 α 的抗体。

[0414] 表 3A 在 I/II 期试验 (TKT025) 期间观察到的治疗突现的不良事件被测定为可能或者或许与维拉昔酶 α (GA-GCB) 施用有关

[0415]

系统器官种类优先项	维拉昔酶 α 60 U/kg EOW n = 12 个患者	
	患者, n (%)	事件, n (%)
任何不良事件	10 (83.3)	22 (21.8)
神经系统病症		
眩晕	3 (25.0)	4 (4.0)
头痛	2 (16.7)	2 (2.0)
刺痛感	1 (8.3)	1 (1.0)
偏头痛	1 (8.3)	3 (3.0)
颤振	1 (8.3)	1 (1.0)
肠胃病症		
恶心	2 (16.7)	2 (2.0)
上腹痛	1 (8.3)	1 (1.0)
肌骨骼和结缔组织病症		
背痛	2 (16.7)	2 (2.0)
骨痛	2 (16.7)	2 (2.0)
手足痛	1 (8.3)	1 (1.0)
一般病症和施用部位病状		
乏力	1 (8.3)	1 (1.0)
调查		
体温增加	2 (16.7)	2 (2.0)

[0416] 患者百分数基于治疗组中患者的总数计。不良事件使用 MedDRA 7.0 版词典编码。EOW:每隔一周。

[0417] TKT025EXT (长期, 30-60U/Kg 维拉苷酶 α) 的结果

[0418] 概述: TKT025EXT 为在完成研究 TKT025 的患有 1 型戈谢病的患者中的维拉苷酶 α 疗法的开放标签扩展研究。TKT025EXT 的第一目的在于当以 30 或 60U/kg 的剂量每隔一周 IV 施用历时总共 4 年维拉苷酶 α 的长期安全性。第二目的在于继续评价在这些患者中如以血液学参数和肝体积和脾体积减小所测量的维拉苷酶 α 对临床活动的影响。也评估血浆壳三糖苷酶和 CCL18 以及 PFT、股骨颈和腰脊柱的 MRI、骨骼调查和骨密度测定法。

[0419] 选择十个患者参与该临床研究。在 60 个月的累积治疗 (TKT025+TKT025EXT) 之后, 维拉苷酶 α 耐受性良好且患者没有发展出抗体。继续观察到血液学参数和器官体积的继续且维持的临床和统计上显著的改善 (表 4)。图 3 显示在用维拉苷酶 α 治疗 60 个月的患者中平均血红蛋白浓度和血小板计数相对于基准的增加。图 4 显示在用维拉苷酶 α 治疗 60 个月的患者中平均肝体积和平均脾体积相对于基准的减小。

[0420] 表 4 TKT025EXT 临床结果 (N = 10)

[0421]

临床活动 参数	中值 基准值	相对于基准的平均变化			
		[95%置信区间]			
		24 个月	36 个月*	48 个月*	60 个月*
血红蛋白浓度(g/dL)	10.90	2.30 [1.78, 2.82]	2.13 [1.59, 2.67]	2.31 [1.66, 2.96]	2.38 [1.60, 3.16]
血小板计数($\times 10^3/L$)	55.5	66.5 [45.3, 87.7]	71.1 [53.6, 88.6]	82.3 [60.1, 104.5]	85.1 [59.8, 110.4]
肝(体重%)	4.40	-1.29 [-1.91, -0.67]	-1.37 [-1.90, -0.84]	-1.74 [-2.40, -1.08]	-1.70 [-2.33, -1.07]
脾(体重%)	3.80	-2.63 [-3.44, -1.83]	-2.69 [-3.51, -1.87]	-2.88 [-3.72, -2.03]	-2.89 [-3.72, -2.06]

[0422] a- 器官体积在从 TKT025 开始的 33 个月、45 个月和 57 个月评价。

[0423] 人口资料和处置: 在 TKT025 中在完成第 41 周评估后, 患者符合条件参与 TKT025EXT。在完成研究 TKT025 的 11 个患者中, 10 个患者进入研究 TKT025EXT。1 个患者 (0012) 由于不方便每隔一周医院出席而没有同意进入扩展研究, 且 1 个患者 (0005) 由于妊娠而从扩展研究中退出。表 2 提供在扩展研究内在基准的人口统计、遗传性和临床特征以及各意向治疗患者在关键数据收集点的临床发现。

[0424] 最初登记的所有患者每隔一周接受维拉苷酶 α 60U/kg。如果在 1 年累积治疗之后患者血液学参数 (血红蛋白浓度或血小板水平) 改善和 / 或器官体积 (肝或脾) 改善, 剂量滴定降至 30U/kg。在 TKT025EXT 中最初登记的所有 10 个患者符合所需要的剂量减少标准且转换到 30U/kg 剂量。

[0425] 在 TKT025EXT 中的所有 9 个患者直到第 42 个月具有可用的血红蛋白浓度和血小板计数数据, 而 3 个患者直至第 45 个月具有可用的数据。在第 42 个月血红蛋白相对于预处理基准的平均 (标准误差 [SE]) 变化为 2.18 (0.25) g/dL ($p = 0.004$), 相当于相对于预处理基准的平均变化 % 为 19.0 %。关于血小板计数, 在第 42 个月相对于预处理基准的平均 (SE) 变化为 82.1 (8.4) $\times 10^3/mm^3$ ($p = 0.004$), 相当于相对于预处理基准的平均变化 %

为 149.8%。

[0426] 分析直到第 33 个月的相对于体重%经正规化的肝体积和脾体积；对于该评价所有 9 个患者都具有可用的肝数据且 8 个患者具有可用的脾数据。在第 33 个月正规化肝体积和正规化脾体积相对于预治疗基准的平均 (SE) 变化分别为 -1.5 (0.22) 和 -2.8 (0.37)。结果对应于正规化肝体积和正规化脾体积相对于预治疗基准的平均变化%：-34.0% 和 -73.5% (分别 $p = 0.004$ 和 $p = 0.008$)。对于血浆壳三糖苷酶和 CCL18 参数两者也观察到了进一步改善。

[0427] 总之，图 2 提供相对于时间作图且对于扩展研究标定的血红蛋白浓度、血小板计数、肝体积和脾体积的平均变化%。对于各参数自基准到 48 个月观察到统计上显著的变化 ($p < 0.004$)。对于血小板计数和脾体积观察到最显著的变化。对于所有患者血红蛋白值经 24 个月正常化。肝体积趋近正常。在整个扩展研究中注意到这些临床参数的连续改善 (图 2) 且在所有患者中经 24 个月观察到血红蛋白的正常化 (表 2)。血红蛋白浓度 (+21.7%)、血小板计数 (+157.8%)、肝体积 (-42.8%) 和脾体积 (-79.3%) 的自基准到 48 个月的平均变化%为统计上显著的 ($p < 0.004$)。

[0428] 分析直到某一日期可用的研究 TKT025EXT 的安全性和临床活动数据。在该研究中登记的患者具有 43.2 个月的累积平均暴露时间 (范围：13.2-45.0 个月)。在所登记的所有 10 个患者中报道了总共 248 件不良事件。大多数 AE 在严重性方面为轻微到中度的。对于 2 个患者报道了研究者视为与研究药物无关的三件重度 (3 度) 不良事件 (在 1 个患者中，关节痛和无菌性骨坏死，和在 1 个患者中，头痛)。报道了三 (3) 件 SAE (在 1 个患者中，2 件无菌性骨坏死，且在 1 个患者中，1 件瘢痕)。这些 SAE 中的每一件被研究者视为与研究药物无关，且各自在无后发病的情况下得到解决。最常报道的治疗突现的不良事件为流行性感冒 (8 个患者，24 件)；关节痛 (8 个患者，21 件)；头痛 (6 个患者，13 件)；背痛 (6 个患者，10 件)；咽喉疼痛 (5 个患者，7 件)；上腹痛 (5 个患者，7 件)；和牙龈出血、发热及疲劳 (各 4 个患者，4 件)。

[0429] 在 TKT025EXT 中报道的大多数不良事件被研究者视为与研究药物无关 (表 3B)。在 4 个患者中报道了总共 7 件治疗有关的不良事件，包括鼻出血 (2 个患者，各 1 件)、腹痛 (1 个患者，1 件)、上腹痛 (1 个患者，1 件)、疲劳 (1 个患者，1 件) 和剧烈疼痛 (1 个患者，1 件)。两个患者各自经历 1 件输注有关的 AE：1 个患者有中度 (2 级) 手足痛，1 个患者有轻微 (1 级) 颤振。因此，不存在药物有关的严重不良事件，不管输注环境为何。另外，没有患者发生需要中断维拉昔酶 α 施用的输注有关的不良事件。

[0430] 患者没有发展出维拉昔酶 α 的抗体。在扩展研究的第一年，所有 7 个以色列居民成功转换到家庭疗法。

[0431] 表 3B 在扩展研究 (TKT025 EXT) 期间观察到的治疗突现的不良事件被测定为可能或者或许与维拉昔酶 α (GA-GCB) 施用有关

[0432]

系统器官种类优先项	维拉昔酶 α 30 U/kg-60 U/kg EOW	
	患者, n (%) N = 10	事件, n (%) N = 311
任何有关的不良事件	4 (40.0)	7 (2.8)
神经系统病症		
颤振	1 (10.0)	1 (0.32)
呼吸、胸部或纵隔病症		
鼻出血	2 (20.0)	2 (0.6)
肠胃病症		
腹痛	2 (20.0)	2 (0.6)
肌骨骼和结缔组织病症		
手足痛	1 (10.0)	1 (0.3)
一般病症和施用部位病状		
疲劳	1 (10.0)	1 (0.3)

[0433] 患者百分数基于安全群中患者的总数计。事件百分数基于安全群中的患者经历的事件总数计。不良事件使用 MedDRA 9.0 版词典编码。EOW:每隔一周。

[0434] 在 TKT025 和 TKT025EXT 中在 1 型戈谢病患者中的骨矿密度的结果

[0435] 在用维拉昔酶 α 治疗的 1 型戈谢病患者中评估长期骨矿密度 (BMD) 变化。患者证明了 BMD 的显著且连续的改善。

[0436] 评价:在 TKT025 登记期间,使用骨骼概况和双能量 X 线吸收测定法 (DEXA) 来评估骨骼病理。贯穿该研究在预定时间分析腰脊柱和股骨颈的 Z- 分值。对于一些患者,进行 DEXA 扫描,但不能根据所述扫描来评价 Z- 分值。缺失的 Z- 分值包括:1 个患者 - 在基准和第 9 个月时的股骨颈;1 个患者 - 在基准时的腰脊柱;和 1 个患者 - 在第 9 个月时的股骨颈。在两个患者从研究中退出(在第 24 个月之前 1 个患者和在第 57 个月之前 1 个患者)之后没有评估他们的腰脊柱和股骨颈的 Z- 分值。

[0437] 使用 T- 分值来将骨密度与“年轻正常”健康 30 岁成人的峰值骨密度比较。在基准和 69 个月时的临床骨骼状况根据 WHO 标准来对 T- 分值表征:≥ -1 为正常;>-2.5 且 <-1 为骨量减少;≤ -2.5 为骨质疏松。

[0438] 方法:

[0439] 分析群:在意向治疗 (ITT) 群 (N = 10) 中进行第一分析,所述意向治疗群定义为签字告知同意书参与长期扩展研究且接受 ≥ 1 的全部或部分剂量的维拉昔酶 α 的所有患者。在伴随 ERT 接受或不接受双磷酸盐的亚组中也评价维拉昔酶 α 对 BMD 的影响。

[0440] 统计方法论:缺失的基准 Z- 分值替换为下一个值。对于后续缺失的 Z- 分值使用末次观察结转 (LOCF) 分析。将线性混合模型用作重复测量分析以随时间分析 Z- 分值。显示出 Z- 分值单位的每年增加的 y- 截距和斜率估计值。记录并报道从骨质疏松到骨量减少或正常和从骨量减少到正常的转变。

[0441] 结果:

[0442] 基准特征:在 TKT025EXT 中登记的所有患者具有 GD1 有关的骨病理。腰椎脊椎

(LS) 骨病理的临床状况为:1 个患者 (10%) 在正常范围内;8 个患者 (80%) 具有骨量减少;且 1 个患者 (10%) 具有骨质疏松。股骨颈 (FN) 骨病理的临床状况为:1 个患者 (10%) 处于正常范围;5 个患者 (50%) 具有骨量减少;且 4 个患者 (40%) 患者具有骨质疏松。DXA Z-分值为 (中值 [范围]):LS, -1.8[-2.9 至 -0.4], FN-1.5[-2.9 至 -0.2]。经过 69 个月,平均维拉昔酶 α 剂量为 40U/kg。十个患者中的四个也用双磷酸盐治疗。

[0443] Z-分值:对于 ITT 群 ($n = 10$)、接受伴随的双磷酸盐的患者和没有接受双磷酸盐的患者在基准和各种时间点的 Z-分值分别在表 5-7 中示出。腰脊柱和股骨颈的平均 Z-分值的时间变化也分别在图 5 和图 6 中示出。

[0444] 表 5 Z-分值;N = 10, ITT

[0445]

时间	Z-分值	
	腰脊柱 平均 [95% CI]	股骨 平均 [95% CI]
基准	-1.59 [-2.17, -1.01]	-1.46 [-2.11, -0.81]
第 9 个月	-1.50 [-2.05, -0.95]	-1.39 [-2.06, -0.72]
第 24 个月	-1.20 [-1.80, -0.60]	-1.23 [-2.04, -0.42]
第 33 个月	-1.16 [-1.80, -0.52]	-1.07 [-1.82, -0.32]
第 45 个月	-1.12 [-1.72, -0.52]	-1.14 [-1.86, -0.42]
第 57 个月	-1.07 [-1.68, -0.46]	-1.10 [-1.89, -0.31]
第 69 个月	-0.91 [-1.55, -0.27]	-1.06 [-1.83, -0.29]

[0446] 表 6 Z-分值;n = 4[用双磷酸盐]

[0447]

时间	Z-分值	
	腰脊柱 平均 [95% CI]	股骨 平均 [95% CI]
基准	-1.88 [-3.25, -0.50]	-1.70 [-3.13, -0.27]
第 9 个月	-1.90 [-3.14, -0.66]	-1.68 [-3.15, -0.20]
第 24 个月	-1.78 [-2.89, -0.66]	-1.75 [-3.52, 0.02]
第 33 个月	-1.68 [-3.14, -0.21]	-1.45 [-3.19, 0.29]
第 45 个月	-1.60 [-2.74, -0.46]	-1.48 [-3.23, 0.28]
第 57 个月	-1.50 [-2.96, -0.04]	-1.40 [-3.25, 0.45]
第 69 个月	-1.23 [-2.78, 0.33]	-1.48 [-3.22, 0.27]

[0448] 表 7 Z- 分值 ;n = 6[无双磷酸盐]

[0449]

时间	Z-分值	
	腰脊柱 平均 [95% CI]	股骨 平均 [95% CI]
基准	-1.40 [-2.22, -0.58]	-1.30 [-2.30, -0.30]
第 9 个月	-1.23 [-1.97, -0.50]	-1.20 [-2.22, -0.18]
第 24 个月	-0.82 [-1.57, -0.06]	-0.88 [-2.03, 0.26]
第 33 个月	-0.82 [-1.62, -0.02]	-0.82 [-1.91, 0.28]
第 45 个月	-0.80 [-1.64, 0.04]	-0.92 [-1.95, 0.11]
第 57 个月	-0.78 [-1.55, -0.01]	-0.90 [-2.07, 0.27]
第 69 个月	-0.70 [-1.61, 0.21]	-0.78 [-1.91, 0.34]

[0450] 在 69 个月的过程期间腰脊柱 (LS) 和股骨颈 (FN) 的 Z- 分值相对于基准 Z- 分值的变化示于表 8 中。

[0451] 表 8

[0452]

排定访问	无双磷酸盐 n = 6	双磷酸盐* n = 4	ITT 群 N = 10
相对于基准 Z-分值的腰脊柱变化(95% CI)			
第 9 个月	0.17 (-0.03, 0.36)	-0.03 (-0.18, 0.13)	0.09 (-0.04, 0.22)
第 24 个月	0.58 (0.08, 1.09)	0.10 (-0.27, 0.47)	0.39 (0.06, 0.72)
第 33 个月	0.58 (-0.06, 1.22)	0.20 (-0.14, 0.54)	0.43 (0.06, 0.80)
第 45 个月	0.60 (0.03, 1.17)	0.28 (-0.12, 0.67)	0.47 (0.14, 0.80)
第 57 个月	0.62 (0.05, 1.18)	0.38 (-0.04, 0.79)	0.52 (0.20, 0.84)
第 69 个月	0.70 (0.16, 1.24)	0.65 (-0.04, 1.34)	0.68 (0.35, 1.01)
相对于基准 Z-分值的股骨颈变化(95% CI)			
第 9 个月	0.10 (-0.03, 0.23)	0.03 (-0.18, 0.23)	0.07 (-0.02, 0.16)
第 24 个月	0.42 (-0.04, 0.87)	-0.05 (-0.47, 0.37)	0.23 (-0.08, 0.54)
第 33 个月	0.48 (0.10, 0.87)	0.25 (-0.13, 0.63)	0.39 (0.16, 0.62)
第 45 个月	0.38 (0.18, 0.59)	0.23 (-0.15, 0.60)	0.32 (0.17, 0.47)
第 57 个月	0.40 (0.06, 0.74)	0.30 (-0.20, 0.80)	0.36 (0.14, 0.58)
第 69 个月	0.52 (0.22, 0.81)	0.23 (-0.25, 0.70)	0.40 (0.18, 0.62)

[0453] 相对于基准值, $P \leq 0.05$

[0454] 如表 8 中所示, 意向治疗群的 BMD 经 24 个月显著改善 (LS: 0.39 (0.06, 0.72)) 且经 33 个月显著改善 (FN: 0.39 (0.16, 0.62))。没有接受伴随的双磷酸盐的患者的 BMD 经 24 个月显著改善 (LS: 0.58 (0.08, 1.09)) 且经 33 个月显著改善 (FN: 0.48 (0.10, 0.87))。

[0455] 线性混合模型评估 Z- 分值: Z- 分值 (ITT 群, $n = 10$, 95% CI) 显著低于参考群 (LS y- 截距 = -1.56 [-2.09, -1.03]; $P < 0.0001$) 和 FN (y- 截距 = -1.42 [-2.06, -0.79]; $P = 0.0007$)。两参数随时间而显著改善 (LS 斜率 / 月 = +0.011 [0.005, 0.017], $P = 0.0021$ (斜率 +0.011Z- 分值单位 / 月对应于 +0.132 / 年); FN 斜率 / 月 = +0.007 [0.004, 0.009], $P = 0.0005$ (斜率 +0.007Z- 分值单位 / 月对应于 +0.084 / 年))。

[0456] 在仅接受维拉苷酶 α 的患者 ($n = 6$, 95% CI) 中, Z- 分值 LS y- 截距为 -1.29 [-1.97, -0.62] ($P = 0.0045$), FN y- 截距为 -1.24 [-2.21, -0.27] ($P = 0.0216$) 且随时间出现显著改善 [LS 斜率 / 月 = +0.013 [0.002, 0.024] ($P = 0.028$) (对应于 +0.158 / 年)、FN 斜率 / 月 = +0.009 [0.004, 0.013] ($P = 0.0055$) (对应于 +0.103 / 年)]。

[0457] 在伴随接受双磷酸盐的患者 ($n = 4$, 95% CI) 中, Z- 分值 LS y- 截距为 -1.97 [-3.06, -0.88] ($P = 0.0104$), FN y- 截距为 -1.71 [-3.02, -0.40] ($P = 0.0252$) 且随时间出现改善 [LS 斜率 / 月 = +0.009 [0.001, 0.017] ($P = 0.0351$) (对应于 +0.111 / 年)、FN 斜率 / 月 = +0.004 [-0.001, 0.010] ($P = 0.0867$) (对应于 +0.048 / 年)]。

[0458] 在根据观察数据库 (International Collaborative Gaucher Group Gaucher Registry) 的分析的线性混合模型中, 用 ERT 伊米苷酶治疗的 GD1 患者具有比在开始跟踪时的参考群显著更低的 Z- 分值 ($n = 340$, Y- 截距 -1.17, $P < 0.001$) (Wenstrup 等, J Bone Min Res. 2007; 22:119-26)。然而, 在 Z- 分值斜率 / 年中观察到了剂量响应改善 (15U/kg [$n = 113$]: +0.064; 30U/kg [$n = 116$]: +0.086; 60U/kg [$n = 111$]: +0.132; 所有 $P < 0.001$)

(Wenstrup 等, J Bone Min Res. 2007 ;22:119-26)。

[0459] 在基准和 69 个月时的 BMD 状况:在基准和 69 个月时的临床骨骼状况使用 WHO 标准表征 (正常 = T- 分值 ≥ -1 ; 骨量减少 = T- 分值 >-2.5 且 <-1 ; 骨质疏松 = T- 分值 ≤ -2.5)。在接受维拉苷酶 α 而没有伴随双磷酸盐的患者 ($n = 6$) 中观察到状况变化。经过 69 个月, 两个 LS 和一个 FN 骨质减少患者正常化且一个 FN 骨质疏松患者变成骨质减少。双磷酸盐的所有四个患者在 WHO 分类方面没有变化。

[0460] 重复测量分析 (纵向) 斜率估计值:在重复测量分析中, ITT 群 ($N = 10$) 的 Z- 分值估计值 [95 % CI] 为:LS 截距 = $-1.56[-2.09, -1.03]$, $P < 0.0001$; LS 斜率 (/ 月) = $0.011[0.005, 0.017]$, $P = 0.0021$; FN 截距 = $-1.42[-2.06, -0.79]$, $P = 0.0007$; LS 斜率 (/ 月) = $0.007[0.004, 0.009]$, $P = 0.0005$ 。腰脊柱斜率每月增加 0.011 对应于每年增加 0.132, 且股骨颈斜率每月增加 0.007 对应于每年增加 0.084。

[0461] 没有接受双磷酸盐的患者 ($N = 6$) 的 Z- 分值估计值 [95 % CI] 为:LS 截距 = $-1.29[-1.97, -0.62]$, $P = 0.0045$; LS 斜率 (/ 月) = $0.013[0.002, 0.024]$, $P = 0.0280$; FN 截距 = $-1.24[-2.21, -0.27]$, $P = 0.0216$; LS 斜率 (/ 月) = $0.009[0.004, 0.013]$, $P = 0.0055$ 。腰脊柱斜率每月增加 0.013 对应于每年增加 0.158, 且股骨颈斜率每月增加 0.009 对应于每年增加 0.103。

[0462] 接受双磷酸盐的患者 ($N = 4$) 的 Z- 分值估计值 [95 % CI] 为:LS 截距 = $-1.97[-3.06, -0.88]$, $P = 0.0104$; LS 斜率 (/ 月) = $0.009[0.001, 0.017]$, $P = 0.0351$; FN 截距 = $-1.71[-3.02, -0.40]$, $P = 0.0252$; LS 斜率 (/ 月) = $0.004[-0.001, 0.010]$, $P = 0.0867$ 。腰脊柱斜率每月增加 0.009 对应于每年增加 0.111, 且股骨颈斜率每月增加 0.004 对应于每年增加 0.048。

[0463] 统计模型使用所有可用的数据且没有使用数据归集。

[0464] 结论:在用维拉苷酶 α 治疗的具有戈谢病和基准骨量减少 / 骨质疏松的患者中, 在腰脊柱 (第 24 个月) 和股骨颈 (第 36 个月) 两者中的 BMD 改善。因为维拉苷酶 α 剂量在第 2 年期间从 60 单位 / 千克 / 输注减少到 30 单位 / 千克 / 输注, 所以骨病理的改善不取决于连续高剂量疗法。

[0465] 长期治疗目标的实现

[0466] 已经描述了用于监测接受 ERT 的患有 1 型戈谢病的患者中治疗响应的实现、维持和连续性的治疗目标 (Pastores G 等, (2004) Seminars in Hematology, 41 (增刊 5):4-14)。

[0467] 为了将维拉苷酶 α 治疗的影响相对于 1 型戈谢病的 5 种关键临床参数 (贫血、血小板减少、肝肿大、脾肿大和骨骼病理) 的治疗目标进行比较, 将在基准时处于贫血、血小板减少、肝肿大和脾肿大的目标下的患者的比例与在 4 年时实现这些目标中的每一个的比例相比较。对于 8 个患者 (3 个男性, 5 个女性) 可使用在基准和 4 年时贫血、血小板减少、肝肿大、脾肿大和骨骼病理的完整数据。在 4 年时实现骨骼病理目标的比例基于从基准到 4 年的 Z- 分值改善来测定。另外, 将在 4 年时实现所有 5 个目标的患者的比例与在基准时处于所有 5 种参数的目标下的比例比较。

[0468] 在基准时, 没有患者处于所有临床参数的目标下 (表 9)。在 1 年治疗之后, 所有患者维持基准时存在的目标, 且全都实现了 ≥ 2 个目标。在 15 个月和 18 个月之间, 所有 8 个患者开始逐步剂量降低到维拉苷酶 α 30U/kg EOW。经过 4 年治疗, 所有患者满足了所有

5 种临床参数的目标 ; 因此, 对于 5 个长期治疗目标中的每一个观察到 100% 实现 (表 9)。

[0469] 表 9

[0470]

	基准	4 年
贫血	4/8(50%)	8/8(100%)
血小板减少	0/8(0%)	8/8(100%)
肝肿大	4/8(50%)	8/8(100%)
脾肿大	0/8(0%)	8/8(100%)
骨骼病理	-----	8/8(100%)
所有 5 个目标	0/8(0%)	8/8(100%)

[0471] 以在第 4 年时的骨矿密度 (BMD) 相对于基准的改善来测量骨骼病理。

[0472] 在该维拉苷酶 α I/II 期和扩展研究中, 尽管在 1 年之后剂量减少, 对于各患者也观察到临床上有意义地实现了各长期治疗目标。这是接受 1 型戈谢病的 ERT 的所有患者在开始治疗的 4 年内实现了所有 5 个长期治疗目标的群组的首次报道。

[0473] TKT025 和 TKT025EXT 的结果的概述

[0474] 本文中报道的发现证明了与维拉苷酶 α 相关的不良事件在严重性方面通常为轻微的且大多数与疗法无关。治疗突现不良事件为轻微至中度的且大多数与药物无关。在这些研究中登记的患者没有发展出抗体, 没有观察到药物有关的严重不良事件, 不论输注环境和暴露事件为何, 且没有患者由于不良事件而从研究中退出。在试验地点的初期观察之后, 符合条件的患者成功转换到基于家庭的护士施用的维拉苷酶 α 。

[0475] 维拉苷酶 α 在所研究的四种疾病参数中展示了功效, 在最初的 6 个月治疗内和贯穿试验和扩展研究过程观察到相对于基准的具有统计上显著且临床上有意义的改善。在开始疗法的 24 个月内, 所有患者都实现了血红蛋白水平正常化, 除一个以外的所有患者都实现了大于 100,000/mm³ 的血小板计数, 所有患者都实现了肝体积的接近正常化, 且除一个以外的所有患者都显示出脾体积减小大于 50%。此外, 在包括剂量减少期的整个研究持续期中观察到了这些改善。回到原始剂量 60U/kg/ 每隔一周的唯一患者在 15 个月初始剂量减少之后, 在 39 个月的骨痛后回到所述原始剂量。该患者在登记时在两个脚裸中具有骨破坏性病变 (成像病理无法排除 AVN) 且具有骨髓炎的先前病史。主要研究者 (AZ) 将疼痛恶化归因于先前存在的破坏性病变和先前的病理, 且可能与剂量减少或治疗失败无关。

[0476] 包括转换到家庭治疗和尽管剂量减少但仍然观察到的临床参数的显著变化的所观察到的安全概况已进一步产生了维拉苷酶 α 的三种后续 III 期试验 (其考虑了儿童) 以及全球早期访问项目和 FDA 认可的治疗方案。

[0477] 实施例 2: TKT032 研究 (12M, 45 或 60U/kg 维拉苷酶 α)

[0478] 实施例 2.1: 概述

[0479] 该实施例描述了评估维拉昔酶 α 在 1 型 GD 中的功效和安全性的全局多中心试验。二十五个治疗雏期的贫血 1 型 GD 患者 (4 至 62 岁) 随机分组至每隔一周静脉内施用维拉昔酶 α 60U/kg ($n = 12$) 或 45U/kg ($n = 13$) 历时 12 个月随机分组。

[0480] 患者以 1:1 比率随机分组以接受 45U/kg ($N = 13$) 或 60U/kg ($N = 12$) 维拉昔酶 α 。分层因子包括年龄 (2-17 ; ≥ 18) 和性别 (男性 ; 女性)。患者的基准特征列在表 10 中。在 12 个月观察到了血液学参数和脾体积的临床和统计上显著的改善 (表 11)。图 7 和图 8 示出在用 45U/kg 或 60U/kg 维拉昔酶 α 治疗 12 个月的患者中平均血红蛋白浓度和血小板计数分别相对于基准增加。图 9 和图 10 示出在用 45U/kg 或 60U/kg 维拉昔酶 α 治疗 12 个月的患者中平均正规化脾体积和平均正规化肝体积分别相对于基准减小。

[0481] 表 10 TKT032 基准特征

[0482]

基准因子	维拉昔酶 α 45 U/kg $N = 13$	维拉昔酶 60 U/kg $N = 12$	总计 $N = 25$
2 至 17 岁 n (%)	3 (23.1)	4 (33.3)	7 (28.0)
2 至 4 岁 n (%)	0	1 (8.3)	1 (4.0)
5 至 17 岁 n (%)	3 (23.1)	3 (25.0)	6 (24.0)
> 18 岁 n (%)	10 (76.9)	8 (66.7)	18 (72.0)
男性 n (%)	8 (61.5)	7 (58.3)	15 (60.0)
女性 n (%)	5 (38.5)	5 (41.7)	10 (40.0)
血红蛋白浓度 (g/dL) 中值 [Min, Max]	10.90 [8.45, 12.85]	10.83 [7.05, 12.25]	10.85 [7.05, 12.85]
血小板计数 ($\times 10^3/L$) 中值 [Min, Max]	58.00 [13.0, 264.0]	66.75 [47.0, 438.0]	65.50 [13.0, 438.0]

[0483] 表 11 TKT032 临床结果 ($N = 25$)

[0484]

	n	基准中值	自基准到第 12 个月的 平均变化	95% CI	P-值
第一终点					
血红蛋白 60 U/kg	12	10.83	2.43	[1.72, 3.14]	< 0.0001
第二终点					
血红蛋白 45 U/kg	13	10.90	2.44	[1.49, 3.39]	0.0001
血小板 60 U/kg	12	66.75	50.88	[23.97, 77.78]	0.0016
血小板 45 U/kg	13	58.00	40.92	[11.20, 70.64]	0.0111
脾 60 U/kg	12	2.80	-1.92	[-3.04, -0.79]	0.0032
脾 45 U/kg	13	2.90	-1.87	[-3.17, -0.57]	0.0085
肝 60 U/kg	12	3.65	-0.84	[-1.58, -0.11]	0.0282a
肝 45 U/kg	13	3.50	-0.30	[-0.92, 0.32]	0.3149

[0485] a- 在对于多个试验调节之后统计上不显著。

[0486] 维拉昔酶 α 耐受性良好。一个患者在研究结束时（第 12 个月）发展出抗体。表 12 汇总 TKT032 安全试验的结果。

[0487] 表 12 TKT032 安全性

[0488]

描述	患者 n (%)		
	维拉昔酶 α 45 U/kg N = 13	维拉昔酶 α 60 U/kg N = 12	总计 N = 25
没有经历不良事件	2 (15.4)	0	2 (8.0)
经历至少 1 件不良事件	11 (84.6)	12 (100.0)	23 (92.0)
经历至少 1 件药物有关的不良事件	9 (69.2)	6 (50.0)	15 (60.0)
经历至少 1 件输注有关的不良事件	8 (61.5)	6 (50.0)	14 (56.0)
经历至少 1 件重度或危及生命的不良事件	2 (15.4)	0	2 (8.0)
经历至少 1 件严重的不良事件	0	1 (8.3)	1 (4.0)
由于不良事件而中断	0	0	0
死亡	0	0	0
发展出抗维拉昔酶 α 抗体	1 (7.7)	0	1 (4.0)

[0489] 在第 12 个月, 平均血红蛋白浓度增加 (60U/kg :+23 % ;+2.4g/dL[95 % 置信区间 (CI) :1.5, 3.4 ;P<0.001] ;45U/kg:+24 % ;+2.4g/dL[95 % CI:1.5, 3.4 ;P<0.001]) 平

均血小板计数增加 (60U/kg: +65.9 % ; +51×10⁹/L [95 % CI: 24, 78 ; P = 0.002] ; 45U/kg: +66 % ; +41×10⁹/L [95 % CI: 11, 71 ; P = 0.01])。平均脾体积减小 (60U/kg: -50 % [95 % CI: -62, -39 %] , 从正常的倍数 14.0 减小到 5.8 [MN] [P = 0.003] ; 45U/kg: -40 % [95 % CI: -52, -28 %] , 从 14.5 减小到 9.5 MN [P = 0.009]) ; 平均肝体积减小 (60U/kg: -17 % [95 % CI: -27, -7 %] , 从 1.5 减小到 1.2 MN [P = 0.03] ; 45U/kg: -6 % [95 % CI: -18, 6 %] , 从 1.4 减小到 1.2 MN [P = 0.32]。没有观察到药物有关严重不良事件或退出。一个患者发展出抗体。

[0490] 研究目的

[0491] 该研究的第一目的在于测定如由血红蛋白浓度增加所测量的在患有 I 型戈谢病的患者中以 60U/kg 剂量每隔一周给药维拉苷酶 α 的功效。

[0492] 该研究的第二目的在于评估以 60U/kg 和 45U/kg 剂量每隔一周给药维拉苷酶 α 的安全性 ; 评估如由血红蛋白浓度增加所测量的以 45U/kg 剂量每隔一周给药维拉苷酶 α 的功效 ; 通过评价血小板计数增加、脾体积和肝体积减小和血浆壳三糖苷酶和趋化因子 (C-C 基元) 配体 18 (CCL18) 水平降低来评估以 60U/kg 和 45U/kg 剂量每隔一周给药维拉苷酶 α 的功效 ; 评估以 60U/kg 和 45U/kg 剂量每隔一周给药维拉苷酶 α 对生命综合质量 (QoL) 的影响 ; 和评估当以 60U/kg 和 45U/kg 剂量施用时隔一周给药维拉苷酶 α 的单一剂量和重复剂量的药物动力学。

[0493] 该研究的第三目的在于测定自基准到实现血红蛋白响应的时间, 定义为在以 60U/kg 或 45U/kg 剂量每隔一周给药用维拉苷酶 α 之后血红蛋白浓度增加 ≥ 1g/dL ; 评估以 60U/kg 和 45U/kg 剂量每隔一周给药维拉苷酶 α 对 ≥ 18 岁患者的肺功能检验 (PFT) 的影响 ; 评估在 2 岁至 17 岁之间的患者中的生长速度和 Tanner 分期 ; 通过放射照相左手和腕部评估在 2 岁至 17 岁之间的患者中的骨骼年龄的变化 ; 确立基准, 据以通过腰脊柱和股骨髓的磁共振成像 (MRI) 来评估在 2 岁至 17 岁之间的患者中的骨病 ; 和确立基准, 据以通过以下项目来评估维拉苷酶 α 疗法对在 ≥ 18 岁的患者中戈谢有关的局部和全身性骨病的长期影响 : 腰脊柱和股骨髓的 DXA (包括冠状面成像) ; 和血清碱性磷酸酶 N- 末端肽交联 (NTx) 和 C- 末端肽交联 (CTx)。

[0494] 总体研究设计

[0495] 这是设计用来评估维拉苷酶 α 疗法对患有 I 型戈谢病的患者的功效和安全性的多中心 III 期随机分组双盲平行组 2 剂量研究。

[0496] 该研究由以下 5 期组成 : (1) 筛选 : 第 -21 天至第 -4 天 ; 基准 : 第 -3 天至第 0 天 (在第一剂量之前) ; 治疗期 : 第 1 周 (第 1 天 ; 第一剂量) 至第 51 周 (每个患者施用总共 26 次输注) ; 研究访问结束 : 第 53 周 ; 跟踪 : 在最后输注之后 30 天 (对于在第 53 周评估之前中断 / 退出的患者, 或对于完成该研究但没有选择登记在后续长期临床研究中的患者)。

[0497] 在筛选时, 提供书写的告知同意书以参与该研究的患者相对于研究加入标准经历复查以测定研究合格性并进行筛选评估。具体地说, 患者提供血样以测量血红蛋白浓度。只有具有对于年龄和性别来说低于正常下限至少 1g/dL 的血红蛋白浓度的那些患者适宜继续进入基准期。出于统计分析目的, 在筛选时收集额外血样以评估血红蛋白浓度。

[0498] 在完成筛选评估之后符合研究参与条件的患者在第一剂量之前进行基准程序和评估 (即, 第 -3 天至第 0 天)。为了证实患者的血红蛋白浓度比对于年龄和性别来说的正

常下限低至少 1g/dL,在基准时患者提供血样。分析并报道血红蛋白浓度。只有在筛选和基准时具有对于年龄和性别来说的正常下限低至少 1g/dL 的血红蛋白浓度的那些患者符合随机分组条件。出于统计分析目的,在基准中收集额外血样以评估血红蛋白浓度。在施用第一剂量之前进行额外基准程序和评估。

[0499] 在完成基准评估和证实合格性之后,使患者以 1:1 比率随机分组以经由计算机产生的随机分组方案接受维拉苷酶 α 60U/kg 或维拉苷酶 α 45U/kg。

[0500] 患者在临床地点每隔一周一次接受总共 26 次 IV 输注的双盲研究投药,历时总共 51 周。在治疗期期间以规则间隔进行安全性和功效评价。在第 51 周和第 53 周访问进行安全性和功效的最终评价。

[0501] 在整个研究中通过评价不良事件(包括输注有关的不良事件)、伴随的投药和生命体征来评价安全性。在第 13 周、第 25 周、第 37 周和第 53 周进行包括 12 导联心电图、身体检查、临床实验室检验(血液学、血清化学、尿检)的额外安全性评价。约每 6 周进行抗维拉苷酶 α 抗体和酶中和抗体的存在的测定,直至第 53 周。

[0502] 经血红蛋白浓度和血小板计数、肝和脾体积及血浆壳三糖苷酶和 CCL18 水平评价功效。额外功效评价包括生长速度和 Tanner 分期、QoL 指标、骨骼生长及肺功能检验。

[0503] 在研究期间也评估单一剂量和重复剂量维拉苷酶 α 药物动力学概况。分别在第 1 周(第 1 天)和第 37 周收集血样用于这些分析。

[0504] 向完成该研究的患者提供加入后续长期临床研究的机会。对于选择加入该长期研究中的患者,将在 TKT032 中在第 51 周和第 53 周访问的某些评价用作基准评价;在完成对于 TKT032 排定的 53 周程序之后,在第 53 周访问时患者接受其第一维拉苷酶 α 输注以便长期临床研究。因此,希望患者将横跨该 2 种研究接受连续的维拉苷酶 α 治疗。完成该研究但没有选择加入后续长期临床研究中的患者在其最后输注维拉苷酶 α 之后 30 天将通过现场访问和通电话而得到安全性评估。

[0505] 研究群的选择

[0506] 在评价的 30 个患者中,14 个不符合随机分组条件(12 个不符合包括标准;2 个不符合排除标准)。使 25 名参与者随机分组至 45U/kg($n = 13$)或 60U/kg($n = 12$)维拉苷酶 α 。将接受至少 1 次输注(或部分输注)的所有随机分组患者包括在意向治疗(ITT)患者群中。

[0507] 合格的参与者为诊断有 1 型戈谢病(白血球中葡糖脑苷脂酶活性不足和通过基因型分析)和疾病有关的贫血(血红蛋白水平比对于年龄和性别来说正常的当地实验室下限低 $\geq 1\text{g/dL}$)的 ≥ 2 岁的男性和女性。参与者也具有以下一项和多项:通过触诊至少中度脾肿大(在左肋缘以下 2 至 3cm);疾病有关的血小板减少(血小板计数 $<90 \times 10^3$ 血小板/立方毫米);和容易触知的肝肿大。参与者在进入研究之前的 30 个月内不能接受对戈谢病的治疗。

[0508] 如果参与者具有脾切除;具有(和怀疑具有)2 或 3 型戈谢病;为抗体阳性和经历对伊米苷酶的过敏性休克,则排除所述参与者。其他排除标准包括在进入研究之前的 30 天内用任何非戈谢病有关的研究药物和装置治疗;HIV 或者 B 或 C 型肝炎的阳性检验;严重贫血(维生素 B12、叶酸或缺铁有关的贫血)或可能影响研究数据的任何显著的共存疾病。排除怀孕和泌乳妇女且要求具有生育小孩潜力的妇女一直使用医学可接受的避孕方法。

[0509] 研究治疗

[0510] 治疗任务:使患者以 1:1 比率随机分组以接受:每隔一周维拉苷酶 α 60U/kg, 历时 51 周 (12 个患者, 26 次输注); 或每隔一周维拉苷酶 α 45U/kg, 历时 51 周 (12 个患者, 26 次输注)。

[0511] 随机分组:在完成基准评估并证实合格性之后, 使患者经计算机产生的随机分组方案以 1:1 的比率随机分组以接受每隔一周输注维拉苷酶 α 45U/kg 或维拉苷酶 α 60U/kg 历时 51 周。

[0512] 根据初步分析的统计有效性, 对预后因子的平衡的追求在小型试验中是重要的。为了在许多预后因子 (例如, 年龄和性别) 之间实现该平衡, 使用动态分派技术。对于动态随机化程序, 患者的分派受所讨论的患者的分层因子的当前平衡所影响。该技术使用如由 Pocock 和 Simon 提出的方法且非常符合在 ICH-9 中提供的方针 (Pocock 等, Biometrics. 1975 ;31:105-115)。

[0513] 治疗方案:患者在第 1 周接受其第一次输注。所有患者每隔一周治疗历时 12 个月 (51 周); 因此, 总共施用 26 次维拉苷酶 α 输注。

[0514] 剂量计算:研究药物的实际剂量根据基准时患者的体重计算。如果在第 13 周、第 25 周或第 37 周发现相对于先前评价的体重变化 $\geq 5\%$, 就需要重新计算研究投药的剂量。

[0515] 维拉苷酶 α 施用:维拉苷酶 α 在 60U/kg 剂量水平和 45U/kg 剂量水平下以连续 IV 输注施用。所有输注经 1 小时持续时间施用。所有输注在 4.3mL 的无防腐剂的灭菌注射水中重构, 且随后在生理盐水 (0.9%氯化钠) 中稀释以产生 100mL 总体积。研究药物输注在一周中大致相同的一天发生, 但可在排定日的每 14 天 (± 3 天) 发生以促进患者排定。

[0516] 研究程序和数据收集方法

[0517] 测定基因型:在筛选时所有患者提供血样用于测定戈谢病基因型和血浆壳三糖苷酶基因型。

[0518] 病史:在筛选时, 记录患者的完整病史。这包括身体系统的复查、当前和预先医疗程序的记载, 和当前和预先伴随投药使用的记载、和在进入研究之前的 30 个月内患者没有接受戈谢病治疗的记载。

[0519] 生命体征:记录的生命体征参数包括脉搏、血压、呼吸率和温度。遵循以下方案以记录在输注访问时的生命体征:输注开始 (在开始输注之前 10 分钟内)、输注期间 (30 分钟 (± 5 分钟))、输注之后 (在完成输注之后 5 分钟内、30 分钟 (± 5 分钟) 和 60 分钟 (± 5 分钟))。在筛选、基准时和第 53 周, 仅在一个时间点收集生命体征。

[0520] 身体检查:在筛选、基准时和在研究的第 13 周、第 25 周、第 37 周和第 53 周进行身体检查。身体检查包括以下项目:一般外观、内分泌、头颈部、心血管、眼睛、腹部、耳朵、泌尿生殖器、鼻、皮肤、喉咙、肌骨骼、胸部和肺及神经系统。另外, 在筛选期间进行肝和脾触诊以证实患者具有中度脾肿大 (在左肋缘下 2 至 3cm) 和戈谢病有关的肝肿大。

[0521] 身高和体重:在基准时和在研究的第 13 周、第 25 周、第 37 周和第 53 周记录身高和体重。

[0522] 12-导联心电图:在基准和研究的第 13 周、第 25 周、第 37 周和第 53 周进行 12-导 ECG。各 12-导联 ECG 包括 PR、QRS、QT 和 QTc 间隔及心率的评价。

[0523] 临床实验室检验:如下所述收集血样和尿样用于临床实验室检验。所有血样经静

脉穿刺收集。

[0524] **血液学**：在筛选、基准和研究的第 13 周、第 25 周、第 37 周和第 53 周收集血样用于完整的血液学检验。评估以下血液学参数：具有差异的全血细胞计数 (CBC)、活化部分凝血活酶时间 (aPTT)、网织红细胞计数（通过临床地点当地实验室分析并报道）、血小板计数和前凝血酶时间 (PT)。在筛选、基准时和在每次研究访问（除了在第 1 周访问外）时收集血样以测量血红蛋白浓度和血小板计数。

[0525] **血清化学**：在筛选、基准时和在研究的第 13 周、第 25 周、第 37 周和第 53 周收集血样用于血清化学检验。评估以下血清化学参数：钠、丙氨酸转氨酶、钾、天冬氨酸转氨酶、葡萄糖、乳酸脱氢酶、总钙、 γ -谷氨酰基转移酶、总蛋白质、肌酐磷酸激酶、白蛋白、NTx*、肌酐、CTx*、尿素氮、叶酸（仅筛选）、总胆红素、维生素 B12（仅筛选）、碱性磷酸酶*（*结果用于评价骨生物标志物）。

[0526] **尿检**：在筛选、基准时和在研究的第 13 周、第 25 周、第 37 周和第 53 周收集尿样用于尿检。评估以下尿检参数：pH 值、宏观评估、微观评估。

[0527] **血清抗伊米苷酶抗体测定**：所有患者仅在筛选期间收集血样用于测定血清抗伊米苷酶抗体。从研究中排除具有阳性结果的患者。

[0528] **血清抗维拉苷酶 α 抗体测定**：在基准时和在治疗期期间约每 6 周（第 7 周、第 13 周、第 19 周、第 25 周、第 31 周、第 37 周、第 43 周和第 49 周）和在第 53 周患者提供血样以测量在血清中的抗维拉苷酶 α 抗体。在治疗期期间，在输注之前收集这些血样。

[0529] **不良事件**：对于完成研究且没有选择从第 53 周访问之前的研究进入长期临床研究的患者，在最后输注之后的 30 天内在整个研究中根据告知同意 / 认可来监测不良事件。对于完成该研究且选择加入长期临床研究中的患者，根据告知同意 / 认可在第 53 周访问中监测不良事件。

[0530] **预先和伴随的疾病**：将在基准时存在的额外疾病视为伴随疾病且记载在病史 CRF 的适当页面上。在研究期间首先发生或检测到的疾病或在研究期间伴随疾病的恶化被视为 AE 且因而记载在 CRF 中。

[0531] **肝和脾 MRI**：患者在基准时和第 25 周和第 51 周进行肝和脾的 MRI。肝和脾的大小使用定量腹部 MRI 测量。

[0532] **血浆壳三糖苷酶水平**：在基准、第 13 周、第 25 周、第 37 周和在第 53 周时收集血样（约 2.5mL）用于评估血浆壳三糖苷酶水平。

[0533] **血浆 CCL18 水平**：在基准、第 13 周、第 25 周、第 37 周和在第 53 周时收集血样（约 2.5mL）用于评估血浆 CCL18 水平。

[0534] **生活质量检验**：在基准时和在第 53 周，使用包括 36 项短格式 (SF-36) 第 2 版（对于 ≥ 18 岁的患者）和儿童健康调查表 (CHQ) PF50（对于 5 至 17 岁的患者）的经批准的调查表来评估患者生活质量。

[0535] **生长速度和 Tanner 分期**：对于 2 至 17 岁的患者，在基准和第 13 周、第 25 周、第 37 周和第 53 周时评价生长。使用在该研究期间在规则的时间点记录的身高和体重测量结果来计算生长速度。在该研究中患者的生长率相对于从疾病控制和预防中心的身高和体重数据获得的相当年龄的正常个体的生长率来测量。在基准和第 13 周、第 25 周、第 37 周和第 53 周时记录 Tanner 分期。

[0536] 骨骼生长 :在基准和第 51 周时对在 2 至 17 岁之间的患者进行左手和腕部的放射照相以评估骨骼年龄。

[0537] 肺功能检验 :在基准和第 53 周时,在试验地点登记的具有进行肺活量测定的能力的 ≥ 18 岁的患者具有 PFT。

[0538] 根据由美国胸科学会或欧洲呼吸学会对于肺活量测定标准公布的指标进行肺活量测定 (American Thoracic Society. Standardization of Spirometry. Am J Respir Crit Care Med. 1995 ;152:1107-1136 ;Quanjer 等, Eur Respir J. 1993 ;16(增刊):5-40)。用力肺活量 (FVC) 和用力呼气容积 / 秒 (FEV_1) 两者以绝对值和正常预测值 % 来表示,其基于对于成人公布的参考值来计算 (Hankinson 等, Am J Respir. Crit Care Med. 1999 ;159:179-187)。对于该计算使用当前的身高。

[0539] 与肺活量检验同时进行肺容量和扩散能力 (DL_{CO}) 测定。肺容量测量包括总肺容量 (TLC) 和剩余容积 (RV), 其根据公布的参考值以绝对值和正常预测值 % 来记录。 DL_{CO} 也根据公布的参考值以绝对值和正常预测值 % 来表示。

[0540] 药物动力学评估 :对于 ≥ 18 岁的患者,在第 1 周 (第 1 天) 和第 37 周的以下时间收集血样 :在第一剂量之前立即,在输注期间 (在 5 分钟、10 分钟、15 分钟、20 分钟、40 分钟和 60 分钟 (输注结束) 收集样品) 和在输注之后 (在 65 分钟、70 分钟、80 分钟、90 分钟、105 分钟和 120 分钟收集样品)。

[0541] 对于 2 至 17 岁的患者,在第 1 周 (第 1 天) 和第 37 周的以下时间收集血样 :在第一剂量之前立即,在输注期间 (在 10 分钟、20 分钟、40 分钟和 60 分钟 (输注结束) 收集样品) 和在输注之后 (在 70 分钟、80 分钟和 90 分钟收集样品)。

[0542] 使用葡糖脑苷脂酶抗原免疫测定评估血清样品中施用维拉昔酶 α 的存在。评估以下 PK 参数 :AUC (曲线下的面积)、 C_{max} (最大血清浓度)、 T_{max} (达到最大血清浓度的时间)、CL (mL/min/kg) (血清清除率,对于体重正规化)、 V_{ss} (mL) (稳态时的表观分布容积)、 V_{ss} (% BW) (V_{ss} 对于体重正规化)、MRT (平均滞留时间) 和 $T_{1/2}$ (消除半衰期 (通过适当 PK 模型来分析))

[0543] 骨生物标志物 :仅在基准时,对 ≥ 18 岁的患者进行腰脊柱和股骨颈的 DXA,包括冠状面成像,以测定戈谢有关的局部和全身性骨病。骨质流失和脱矿质也通过测量血清碱性磷酸酶、NTx 和 CTx 评估。从仅在基准时收集用于临床实验室检验的血样获得这些参数的结果。

[0544] 对于 2 至 17 岁的患者,在基准时 (即,与对患者进行肝和脾的 MRI 的同时) 获得股骨颈和腰脊柱的 MRI。不出所料在该研究期间任何治疗作用对于这些参数都将是显然的 ;因此,仅在基准时收集测量结果以确立在长期临床研究期间据以监测这些生物标志物的参考点。

[0545] 不良事件

[0546] 不良事件定义 :不良事件 (AE) 为如由在临床试验的任一期中出现的身体体征、症状和 / 或实验室变化所指示的解剖学、生理或代谢功能的任何有毒、病理学或非预期变化,而不管是否被视为与研究药物有关。这包括预先存在病状的恶化。根据告知同意 / 认可在最后剂量的研究投药之后 30 天和 / 或直到不良事件已经解决 / 稳定或达到一种结果 (无论何者在先) 收集不良事件。对于完成该研究且选择加入后续长期临床研究中的患者,从

向患者提供告知同意书的时间到 TKT032 的第 53 周访问监测不良事件。

[0547] AE 包括：在研究起始时存在的病状的恶化（性质、严重性或频率方面的改变）；并发的疾病；药物相互作用；与伴随投药有关或可能与伴随投药有关的事件；异常实验室值（这包括研究人员认为临床上重要的在正常范围内的相对于基准的显著转变）；身体检查、生命体征、体重和 ECG 的临床上显著的异常。

[0548] 另外，AE 也可以包括出乎意料的实验室值，其变得显著超出范围且由研究人员测定为临床上显著的。

[0549] 输注有关的不良事件的定义：输注有关的不良事件定义为如下不良事件：1) 在输注开始之后的 12 小时期间或 12 小时内开始，和 2) 被判断为可能或者或许与研究投药有关。在输注之前发生的其他 AE 以及在输注之前进行的与实验方案限定的检验和评价（例如，实验室检验、ECG 和身体检查）相关的 AE 并不定义为输注有关的不良事件。

[0550] 严重的不良事件定义：严重的 AE (SAE) 为导致任何以下结果的以任何剂量发生的任何 AE：死亡；为危及生命的；需要患者住院治疗；需要现有住院治疗延期；持久或显著的病废 / 无能和出生缺陷 / 先天缺陷。

[0551] 当可能不导致死亡、危及生命或需要住院治疗的事件的重要医疗事件根据适当医疗判断可能危害患者且可能需要医疗或外科手术以预防以上所列结果之一时，它们可被视为 SAE。

[0552] 危及生命的 AE 定义为鉴于最初报道使患者处于随着 AE 发生而引起死亡的即时危险中的 AE（即，其不包括如果以更重度的形式出现时可能致死的 AE）。

[0553] 不良事件和严重不良事件的分类：当评价 AE 的严重性时，参考国立癌症研究院通用毒性标准 (NCI CTC) 3.0 版分级量表。如果 AE 没有描述在 NCI CTC 中，则根据以下量表记录严重性。所有 AE/SAE 的严重性在适当 CRF 页面上记录为分别对应于轻微、中度、重度或危及生命的严重性的 1 级、2 级、3 级或 4 级。1 级（轻微）定义为日常活动不受限制。2 级（中度）定义为日常活动受一定程度地限制；3 级（重度）定义为不能实施日常活动；和 4 级（危及生命的）定义为即时死亡危险。

[0554] 不良事件或严重不良事件与盲法研究投药的关系由研究人员根据以下定义测定。“无关”定义为与研究药物无关。“可能有关”定义为在施用研究药物的合理时序下的临床事件 / 实验室异常，但其也可以由同时发生的疾病或其他药物 / 化学解释。“或许有关”定义为在施用研究药物的合理时序下的临床事件 / 实验室异常，不大可能归因于同时发生的疾病或其他药物和化学且其在去激发时依循临床上合理的响应。临床事件 / 实验室异常的相关性也必须具有一定的生物学似真性，至少是在理论根据上。

[0555] 在严重和重度之间的阐明术语“重度”常用以描述具体事件的强度（严重性）（如在轻微、中度或重度心肌梗塞中）；然而，事件本身可能具有相对较少的医疗意义（诸如重度头痛）。这不同于“严重”，其基于通常与对生命或功能造成威胁的事件相关的结果或作用标准。严重（非重度）和因果性用作限定调整报告责任的指导。

[0556] 不良事件监测和观察期：对于该研究的目的，观察期从告知同意 / 认可延伸到该研究的患者最终评估。出于安全目的，对于完成研究且没有选择加入长期研究的患者，最终评估定义为在最后输注之后约 30 天进行的后研究安全评估。对于完成该研究且选择加入长期临床研究中的患者，从患者提供告知同意书的时间到 TKT032 的第 53 周访问监测不良

事件。

[0557] 统计方法

[0558] 一般统计方法论：对于所有功效变量来说，统计分析根据 ITT 原理。所述 ITT 分析基于接受至少一次输注（全部或部分输注）的所有随机分组患者。对于治疗组的各参数相对于基准的变化和变化%提供概括统计量。按照治疗组对于各终点提供相对于基准的平均变化和平均变化%的双侧 95%置信区间。

[0559] 汇总在基准时和后续研究访问时收集的连续数据，且列出各变量的平均值、标准偏差、最小值、最大值和中值以促进探求可以归因于研究药物的随时间而变的趋势。分类变量按照频率和%提供。在组内，使用配对 T 检验检查变化。以 0.05 水平限定统计显著性。

[0560] 人口统计和基准特征以频率和百分数来汇总，且数据使用描述性统计提供。特定地对于 2 至 17 岁之间的患者进行额外分析。

[0561] 一般来讲，使用描述性统计和图表提供研究结果，包括（如果有关）示出个别患者和各治疗组随时间的发展的图表。

[0562] 根据报道的 AE、临床实验室数据、ECG 记录、病史、生命体征和身体检查评估安全性。另外，分析血样来测定抗维拉昔酶 α 抗体的存在。

[0563] 假设检验：所有假设检验都为双侧的且以 0.05 的显著性水平进行。各变量量化为相对于基准的平均变化或相对于基准的平均变化%。所检验的各变量的零假设在于，在第 51 周或第 53 周，相对于基准没有变化。备选假设在于在相对于基准的任一方向存在变化。

[0564] 筛选失败：对于进入研究所筛选的所有患者的处置连同筛选失败的原因一起列出。所有随机分组患者的处置按照治疗和访问列出，且中断的原因按照治疗列出。

[0565] 样本量调整：选择用于该研究的样品量以具有检测平均血红蛋白浓度自基准到 12 个月的临床上显著的差异的高效能。对于第一分析按治疗组总共需要 12 个患者。该数量基于来自 I/II 期研究 TKT025 的结果，其检查患者内相对于基准的变化结果。观察到，在第 25 周，相对于基准的平均血红蛋白增加为 1.92g/dL，标准偏差为 0.824。假设在于平均变化的标准偏差大致相同。使用 0.05 的双侧 α 水平且假设 1- 单位血红蛋白的变化视为临床上显著的，相对于基准的变化的标准偏差为 0.824，则将需要 10 个患者用于试验以具有 90%的效能。假设 20%的退出率，则每治疗组将需要 12 个患者。为了采集额外的安全数据且为了防止可能的患者漏失，且为了获得目标患者群，在该研究中可以登记高达 30 个患者。

[0566] 分析群：用于功效数据分析的主要群体为 ITT 患者群，定义为接受至少一次维拉昔酶 α 输注（或部分输注）的所有登记并治疗的患者。可以预料由于缺乏后基准数据引起的自原始随机样本的损耗应该足够小（5%或更小）以使关于由于这类患者的排除引起的偏差的忧虑最小化。

[0567] 安全群由接受至少一次研究输注（或部分输注）的所有随机分组患者组成。根据占优势地接受输注而不是随机分派的治疗来分析安全群中不接受对其随机分派的研究输注的任何患者。这类患者自完成实验方案 (PP) 患者群排除。PP 患者群定义为接受 $\geq 80\%$ 的排定输注且具有有效的基准和第 51 周和 / 或第 53 周评估的所有随机分组患者。

[0568] 功效分析：对于进入研究所筛选的所有患者的处置连同筛选失败的原因一起列出。所有随机分组患者的处置按照治疗组和访问列出并列出中断的原因。

[0569] 在随机分组到 60U/kg 维拉昔酶 α 的患者中,第一临床活动变量为血红蛋白浓度。第一目的在于通过示出在随机分组到 60U/kg 维拉昔酶 α 的患者中血红蛋白自基准到 12 个月的平均变化来证明功效。出于分析目的,将在筛选和基准时收集的血红蛋白值取平均值以确立用以计算变化的基准。零假设在于血红蛋白浓度自基准到 12 个月没有变化。自基准到 12 个月的平均差异使用配对 T 检验或 Wilcoxon 符号秩检验来检验。还提供了该平均差异的 95% 置信区间。

[0570] 第二和第三临床活动变量为:血红蛋白浓度(对于 45U/kg 组评价自基准到 12 个月(第 53 周)的变化);血小板计数(对于两个治疗组评价自基准到 12 个月(第 53 周)的变化);脾体积(对于两个治疗组评价自基准到 12 个月(第 51 周)的变化%;除了观察值以外,脾体积按照体重正规化并且以正常的倍数提供);肝体积(对于两个治疗组评价自基准到 12 个月(第 51 周)的变化%;除了观察值以外,肝体积按照体重正规化并且以正常的倍数提供);血浆壳三糖苷酶(对于两个治疗组评价自基准到 12 个月(第 53 周)的变化;在 12 个月的治疗之后预期统计上显著的降低);血浆 CCL18(对于两个治疗组评价自基准到 12 个月(第 53 周)的变化;在 12 个月的治疗之后预期统计上显著的降低);生活质量(SF-36 和 CHQ)(对于两个治疗组评价自基准到 12 个月(第 53 周)的变化);血红蛋白响应(获得血红蛋白响应的时间,定义为对于两个治疗组评价血红蛋白浓度增加 $\geq 1\text{g/dL}$);生长速度和 Tanner 分期(对于在 2 至 17 岁之间的患者的两个治疗组评估自基准到 12 个月(第 53 周)的变化);骨骼年龄(对于两个治疗组评估自基准到 12 个月(第 51 周)的变化,如通过在 2 至 17 岁之间的患者的左手和腕部的放射照相测量);和 PFT(对于 ≥ 18 岁的患者的两个治疗组评价自基准到 12 个月(第 53 周)的变化)。

[0571] 对于用以证明在随机分组到 45U/kg 维拉昔酶 α 的患者中血红蛋白自基准到 12 个月(第 53 周)的平均变化的第二目的,零假设在于自基准到 12 个月(第 51 周或第 53 周)将没有变化。出于分析目的,将在筛选和基准时收集的血红蛋白值取平均值以确立用以计算变化的基准。自基准到 12 个月(第 51 周或第 53 周)的平均差异使用配对 T 检验或 Wilcoxon 符号秩检验来检验。还提供了平均差异的 95% 置信区间。

[0572] 对于剩余的二次参数,零假设在于对于各治疗组自基准到 12 个月(第 51 周或第 53 周)将没有变化。出于分析目的,将在筛选和基准时收集的血小板值取平均值以确立用以计算两治疗组的变化基准。自基准到 12 个月(第 51 周或第 53 周)的平均差异使用配对 T 检验或 Wilcoxon 符号秩检验来检验。还提供了平均差异的 95% 置信区间。

[0573] 对于达到血红蛋白响应的时间,对于各治疗组提供 Kaplan-Meier(乘积极限)生存曲线。获得中值时间和 95% 置信区间。在第 53 周审查在研究结束时(即,在第 53 周)没有经历事件的患者。另外,提供在该研究期间实现在正常范围内的血红蛋白水平的患者的比例。

[0574] 对于检查相对于基准的变化的剩余的三次参数,零假设在于对于各治疗组自基准到 12 个月(第 51 周或第 53 周)将没有变化。在基准到 12 个月(第 51 周或第 53 周)之间的平均差异使用配对 T 检验或 Wilcoxon 符号秩检验来检验。还提供了平均差异的 95% 置信区间。

[0575] 安全分析:评价接受至少一剂研究药物(或部分剂量)的所有患者的临床安全性和耐受性。不对安全参数进行正式统计检验。列出各患者的生命体征、12-导联 ECG、临床

化学、血液学和尿检安全监测且标记出异常值。对于诸如 AE 的分类变量,列出经历各 AE 的患者的数量和百分数。AE 以事件严重性汇总。也显示出了经历药物有关的 AE 以及不被视为与研究药物有关的 AE 的患者的数量和百分数。

[0576] 使用临床实验室评估(血液学、血清化学、尿检和抗维拉苷酶 α 抗体的测定)来评价维拉苷酶 α 的安全性。

[0577] 亚组的分析:特定地对于在 2 至 17 岁之间的患者进行额外分析。并且,在分析中关于血红蛋白基准值给出对于疾病严重性的考虑因素。

[0578] 药物动力学分析:维拉苷酶 α 的单一剂量和重复剂量的药物动力学曲线分别通过分析第 1 周和第 37 周的标准 PK 参数来确立。

[0579] 结果

[0580] 在 12 个月,在两组中平均血红蛋白浓度增加(60U/kg:增加 23.3%, $+2.4 \pm 0.3$ g/dL, $P = 0.0001$;45U/kg:增加 23.8%, $+2.4 \pm 0.5$ g/dL, $P = 0.0001$),平均血小板计数增加(60U/kg:增加 66%, $+51 \pm 12 \times 10^9$ /L, $P = 0.0016$;45U/kg:增加 66%, $+41 \pm 12 \times 10^9$ /L; $P = 0.0111$)。在两组中平均脾体积减小(60U/kg:减小 50%, -1.9 ± 0.5 体重%, $P = 0.0032$,从在基准时的 14.0 正常的倍数 [MN] 减小到 5.6MN;45U/kg:减小 40%, -1.9 ± 0.6 体重%, $P = 0.0085$;从 14.5MN 减小到 9.5MN);肝体积也减小(60U/kg:减小 17%, 0.8 ± 3 体重%, $P = 0.0282$,从 1.5MN 减小到 1.2MN;45U/kg:减小 6%, -0.3 ± 0.3 体重%, $P = 0.3149$,从 1.4MN 减小到 1.2MN)。

[0581] 在两组中,在第 15 周四分之三患者实现血红蛋白浓度增加 ≥ 1 g/dL;在 60U/kg 组中,所有患者在第 27 周实现血红蛋白浓度增加 ≥ 1 g/dL,在 45U/kg 组中,所有患者在第 37 周实现血红蛋白浓度增加 ≥ 1 g/dL。

[0582] 如果患者具有 2 个壳三糖苷酶突变拷贝(患者 6,45U/kg)或如果他们具有小于 5700 的基准壳三糖苷酶活性(患者 4,60U/kg;患者 15,45U/kg),则将患者从壳三糖苷酶分析中排除。在治疗 12 个月之后,对于两治疗组,平均血浆壳三糖苷酶活性相对于基准减小:在 60U/kg 组中,减小 83% (95% CI:-91.15, -74.08%; $N = 11$; $p < 0.001$)和在 45U/kg 组中,减小 60% (95% CI:-73.26, -46.63%; $N = 11$; $p < 0.001$)。经过用维拉苷酶 α 60U/kg 和 45U/kg 治疗 1 年,平均 CCL18 水平也分别降低 66% (95% CI:-77.81, -54.22; $p < 0.001$)和 47% (95% CI:-63.37, -30.15%; $p < 0.001$)。

[0583] 维拉苷酶 α 通常耐受性良好,没有药物有关的严重 AE 且没有由于 AE 引起的患者退出。最常见的 AE 为头痛、鼻咽炎、损伤、关节痛、咳嗽和发热。单个患者发展出了抗体。

[0584] 总之,在该全局多中心研究中,维拉苷酶 α 60U/kg 和 45U/kg 通常耐受性良好且有效作为患有 1 型戈谢病的成人和儿童的一线治疗。两种剂量与血红蛋白值的迅速改善相关,大多数患者早在 15 周就响应。所测量的所有临床参数在 12 个月之后证明临床上有意义的改善,用维拉苷酶 α 60U/kg 见到更大的响应。

[0585] 实施例 3:HGT-GCB-039(9M, 60U/kg 维拉苷酶 α 或伊米苷酶)

[0586] 概述

[0587] 该实施例描述设计用来比较酶替代疗法维拉苷酶 α 与伊米苷酶在患有 1 型戈谢病的患者的治疗中的安全性和功效的多中心 III 期随机分组双盲平行组研究。

[0588] 该实施例的第一目的在于证明如由血红蛋白浓度自基准到第 41 周(9M)的变化所

测量维拉昔酶 α 不亚于伊米昔酶。关键的第二目的在于证明在两组中到第 41 周血小板计数增加或肝 / 脾体积减小没有差异。

[0589] 使患者以 1:1 的比率随机分组以接受 60U/kg 维拉昔酶 α (N = 17) 或伊米昔酶 (N = 17)。患者的基准特征列于表 13 和表 14 中。分层因子包括年龄 (2-17 ; ≥ 18) 和脾切除状况 (Y ; N)。第一目的和第二目的两者都满足。第一功效评价和第二功效评价的结果分别示于表 15 和表 16 中。图 11 和图 12 示出在用 60U/kg 维拉昔酶 α 或伊米昔酶治疗 41 周的患者中平均血红蛋白浓度和血小板计数分别相对于基准的可比较的增加。图 13 示出在用 60U/kg 维拉昔酶 α 或伊米昔酶治疗 41 周的无脾的患者中平均血小板计数相对于基准的可比较的增加。图 14 示出在用 60U/kg 维拉昔酶 α 或伊米昔酶治疗 41 周的患者中平均正规化肝体积相对于基准的可比较的减小。在安全性方面在维拉昔酶 α 和伊米昔酶之间没有显著差异。治疗突现的不良事件汇总于表 17 中。接受维拉昔酶 α 的患者没有发展出抗体 (表 18)。四个接受伊米昔酶的患者发展出了抗伊米昔酶抗体 (表 18)。

[0590] 表 13 ITT 的 HGT-GCB-039 基准特征

[0591]

基准因子	维拉昔酶 α 60 U/kg N = 17	伊米昔酶 60 U/kg N = 17
2 至 17 岁 n (%)	4 (23.5)	5 (29.4)
2 至 4 岁 n (%)	0	4 (23.5)
5 至 17 岁 n (%)	4 (23.5)	1 (5.9)
> 18 岁 n (%)	13 (76.5)	12 (70.6)
男性 n (%)	8 (47.1)	8 (47.1)
女性 n (%)	9 (52.9)	9 (52.9)
脾切除	10 (58.8)	10 (58.8)
具有完整的脾	7 (41.2)	7 (41.2)
BL 血红蛋白浓度 (g/dL) 中值 [Min, Max]	11.40 [9.65, 14.35]	10.60 [8.10, 13.05]
BL 血小板计数 ($\times 10^9/L$) 中值 [Min, Max]	172.00 [44.0, 310.5]	188.00 [63.0, 430.5]

[0592] 表 14 按照年龄组的 HGT-GCB-039 基准特征

[0593]

基准因子	2-4 岁 N = 4	>5 岁 N = 30
男性 n (%)	4 (100.0)	12 (40.0)
女性 n (%)	0	18 (60.0)
脾切除	1 (25.0)	19 (63.3)
具有完整的脾	3 (75.0)	11 (36.7)
BL 血红蛋白浓度 (g/dL) 中值 [Min, Max]	9.275 [8.10, 9.70]	11.300 [8.95, 14.35]
BL 血小板计数 ($\times 10^9/L$) 中值 [Min, Max]	70.75 [63.0, 188.0]	176.25 [44.0, 430.5]
BL 脾体积 (体重%) 中值 [Min, Max]	8.90 [7.3, 8.9]	1.70 [0.6, 6.3]
BL 肝体积 (体重%) 中值 [Min, Max]	5.8 [5.4, 7.0]	3.85 [1.7, 12.2]

[0594] 表 15 HGT-GCB-039 第一功效评价 - 在对于非劣性的 Hgb 单侧 CI 中自基准到第 41 周的平均变化 (维拉昔酶 α - 伊米昔酶)

[0595]

自基准到第 41 周的变化的治疗差异						
ITT 群				每个实验方案群		
参数/ 终点	n	平均治疗 差异	97.5%单侧 CI 的下界	n	平均治疗差异	97.5%单侧 CI 的下界
血红蛋白浓度 (g/dL)						
	34	0.135	-0.596	30	0.157	-0.599

[0596] 表 16 HGT-GCB-039 第二功效评价 - 自基准到 41 周的平均变化的差异 (维拉昔酶 α - 伊米昔酶)

[0597]

自基准到第 41 周的变化			
参数	n	平均治疗 差异	95% CI
血小板 ^a ($\times 10^9/L$)	34	-38.71	(-88.42, 10.99)
正规化肝体积 ^a (体重%)	34	-0.07	(-0.43, 0.29)
正规化脾体积 ^a (体重%)	14	0.08	(-0.52, 0.68)
壳三糖苷酶 ^a (nmol/mL/h)	21	-703.6	(-11762.3, 10355.1)
趋化因子 (C-C 基元) 配体 18 (ng/mL)	34	145.7	(-188.6, 480.0)

[0598] a- 根据在告知同意年龄、脾切除状况和基准值下调整的混合模型。

[0599] b- 有 20 个脾切除患者被排除 (10 个患者, 维拉昔酶 α 60U/kg; 10 个患者, 伊米昔酶)

酶 60U/kg)。

[0600] c- 根据在告知同意年龄和基准值下调整的混合模型。

[0601] d- 有 13 个缺乏壳三糖苷酶活性的患者被排除 (7 个患者, 维拉苷酶 α 60U/kg ;6 个患者, 伊米苷酶 60U/kg)。

[0602] 表 17 治疗突现不良事件的 HGT-GCB-039 总概述

[0603]

描述	患者 n (%)	
	维拉苷酶 α 60 U/kg N = 17	伊米苷酶 60 U/kg N = 17
没有经历不良事件	1 (5.9)	1 (5.9)
经历至少 1 件不良事件	16 (94.1)	16 (94.1)
经历至少 1 件药物有关的不良事件	8 (47.1)	6 (35.3)
经历至少 1 件输注有关的不良事件	5 (29.4)	4 (23.5)
经历至少 1 件重度或危及生命的不良事件	3 (17.6)	2 (11.8)
经历至少 1 件严重的不良事件	3 (17.6)	0
经历至少 1 件药物有关的 SAE	1 (5.9)	0

[0604] 表 18 HGT-GCB-039 抗体

[0605]

	维拉昔酶 α 60 U/kg N = 17 n (%)	伊米昔酶 60 U/kg N = 17 n (%)
抗伊米昔酶抗体结果		
阴性	17 (100.0)	13 (76.5)
阳性 ^a	0	4 (23.5)
IgG	0	4 (23.5)
IgA	0	0
IgM	0	0
IgE	0	0
中和抗体	0	1 (5.9)
抗维拉昔酶 α 抗体结果		
阴性	17 (100.0)	16 (94.1)
阳性 ^a	0	1 (5.9)
IgG	0	1 (5.9)
IgA	0	0
IgM	0	0
IgE	0	0
中和抗体	0	1 (5.9)

[0606] 研究目的

[0607] 该研究的第一目的在于比较维拉昔酶 α 和伊米昔酶对患有1型戈谢病的患者的血红蛋白浓度的影响。

[0608] 该研究的第二目的为：比较维拉昔酶 α 和伊米昔酶对血小板计数的影响；比较维拉昔酶 α 和伊米昔酶对肝体积和脾体积的影响（通过MRI）；比较维拉昔酶 α 和伊米昔酶对戈谢病特异性生物标志物（血浆壳三糖苷酶和CCL18水平）的影响；评估维拉昔酶 α 和伊米昔酶在患有1型戈谢病的患者中的安全性，如对于各治疗组通过标准临床实验室评价（包括抗体形成率和酶中和抗体活性）和安全评价（包括输注有关的不良事件的比率和需要用以控制输注有关的不良事件的预投药的患者的比例）所测量；和比较维拉昔酶 α 和伊米昔酶对血红蛋白响应（定义为血红蛋白水平相对于基准改善 $\geq 1\text{g/dL}$ ）的最早时间的影响。

[0609] 该研究的第三目的为：评估维拉昔酶 α 和伊米昔酶对在2至17岁之间的患者的生长速度和Tanner分期的影响；通过左手和腕部的放射照相评估维拉昔酶 α 和伊米昔酶对在2至17岁之间的患者的骨骼年龄变化的影响；评估如通过 ≥ 18 岁的患者的SF-36和5至17岁的患者的CHQ PF-50所测量的维拉昔酶 α 和伊米昔酶对总体QoL变化的影响；评估如通过选择的细胞因子评价（TNF- α 、IL6、IL1b、IL8、IL13、CD14和GM-CSF）所测量的维拉昔酶 α 和伊米昔酶对 ≥ 18 岁的患者的免疫和炎症响应的影响；确立基准，据以通过腰脊柱和股骨的MRI来评估在2岁至17岁之间的患者中的骨病；和确立基准，据以通过以

下项目来评估维拉昔酶 α 疗法对在 ≥ 18 岁的患者中戈谢有关的局部和全身性骨病的长期影响：腰脊柱和股骨颈的双能量 X 线吸收测定法 (DXA)，包括冠状面成像；和血清碱性磷酸酶，N-末端肽交联 (NTx) 和 C-末端肽交联 (CTx)。

[0610] 研究终点

[0611] 该研究的第一终点在于测量自基准至第 41 周 / 研究结束 (EOS) 时在两个治疗组之间血红蛋白浓度的平均变化。

[0612] 该研究的第二终点为：评估如由不良事件和输注有关的不良事件（和需要预投药以控制输注有关的不良事件的比例）、临床实验室值、生命体征、12-导联心电图 (ECG)、抗体形成和酶中和抗体活性所评价的维拉昔酶 α 和伊米昔酶的安全性；比较在治疗组之间血小板计数相对于基准的平均变化和变化%；比较在治疗组之间根据 MRI 的肝体积和脾体积相对于基准的平均变化和变化%；比较在治疗组之间血浆壳三糖苷酶和血浆 CCL18 水平相对于基准的平均变化和变化%；和比较在治疗组之间达到血红蛋白浓度响应的时间（定义为血红蛋白水平相对于基准的改善 $\geq 1\text{g/dL}$ ）。

[0613] 该研究的第三终点为：评估在各治疗组内在 2 至 17 岁之间的患者的生长速度和 Tanner 分期相对于基准的变化；评估在各治疗组内 ≥ 18 岁的患者的 SF-36 参数相对于基准的变化；评估如由选择的细胞因子评价 (TNF- α 、IL6、IL1b、IL8、IL13、CD14 和 GM-CSF) 测量的维拉昔酶 α 和伊米昔酶对 ≥ 18 岁的患者的免疫和炎性响应的影响；评估在各治疗组内 5 至 17 岁的患者的 CHQ (PF-50) 参数相对于基准的变化；和评估在各治疗组内对于在 2 至 17 岁之间的患者如通过左手和腕部的放射照相测量的骨骼年龄相对于基准的变化。

[0614] 总体研究设计

[0615] 该研究由以下 5 期组成：筛选：第 -21 天至第 -4 天；基准：第 -3 天至第 0 天（通过患者随机分组）；治疗：第 1 周（第 1 天，即，第一剂量的那天）至第 39 周；研究访问结束：第 41 周；

[0616] 跟踪接触：在最后输注之后 30 天（对于在第 41 周评估之前中断 / 退出的患者，或对于没有选择登记在长期临床研究中的患者）。

[0617] 在筛选时，提供书写的告知同意书以参与该研究的患者相对于研究加入标准复查以测定研究合格性。患者提供血样以测量血红蛋白浓度。仅具有低于对于年龄和性别来说的正常下限的血红蛋白浓度的那些患者符合登记条件。出于统计分析目的，在筛选时收集额外血样以评估血红蛋白浓度。

[0618] 在完成筛选评估之后符合研究参与条件的患者进行基准程序和评估（即，第 -3 天至第 0 天）。为了证实患者的血红蛋白浓度低于对于年龄和性别来说的正常下限，在基准时提供血样。分析并报道血红蛋白浓度。只有在筛选和基准二者时具有低于对于年龄和性别来说的正常下限的血红蛋白浓度的那些患者符合登记条件。出于统计分析目的，在基准时收集额外血样以评估血红蛋白浓度。在施用盲法研究投药的第一剂量之前进行额外基准程序和评估。

[0619] 在完成筛选和基准程序并证实患者合格性后，使患者以 1:1 比率随机分组以接受双盲研究投药（维拉昔酶 α 60U/kg 或伊米昔酶 60U/kg）。随机分组经集中程序实现。利用计算机产生的随机分组方案来分派患者到治疗组。进行尝试以得到在诸如年龄、血红蛋白浓度的某些预后变量方面可比较的治疗组，而不管患者已进行脾切除。在研究之前制定随

机分组方案。

[0620] 患者在临床地点每隔一周一次接受总共 20 次 IV 输注的双盲研究投药, 总共 39 周。在治疗期期间以规则间隔进行安全性和功效评价。在第 41 周访问 (在最后一输注之后 2 周) 进行安全性和功效的最终评价。

[0621] 贯穿研究通过评价不良事件 (包括输注有关的不良事件)、伴随的投药和生命体征来评价安全性。在第 13 周、第 25 周和第 41 周进行包括 12- 导联心电图、身体检查、临床实验室检验 (血液学、血清化学、尿检) 的额外安全性评价。约每 6 周进行抗维拉昔酶 α 抗体或抗伊米昔酶抗体和酶中和抗体的存在的测定直至第 41 周。

[0622] 经血红蛋白浓度和血小板计数、肝和脾体积及血浆壳三糖苷酶和 CCL18 水平评价功效。额外功效评价包括生长速度和 Tanner 分期、QoL 指标、骨骼生长。测量在进入研究时 ≥ 18 岁的患者中的免疫和炎症响应 (如通过选择的细胞因子参数所测量)。

[0623] 在该研究中治疗的持续时间为 39 周且在该研究中患者参与的持续时间高达 11 个月 (从筛选到跟踪)。

[0624] 提供给完成该研究的患者机会以登记在后续开放标签的长期临床研究中, 其中所有患者将接受维拉昔酶 α 。对于选择加入后续开放标签的长期临床研究的患者来说, 将来自第 41 周访问的某些评价用作用于临床研究的标准评价; 在完成对于该研究排定的 41 周程序和评估之后患者将接受其第一次维拉昔酶 α 输注以便长期临床研究。因此, 希望患者跨越该两种研究接受连续治疗。完成该研究但没有选择加入后续长期临床研究中的患者在该研究中在其最后输注之后 30 天将通过现场访问和通电话而得到安全性评估。

[0625] 研究群的选择

[0626] 登记 34 个患者 (17 个患者分配给各治疗组)。

[0627] 合格的参与者为诊断有 1 型戈谢病 (白血球中葡糖脑苷脂酶活性不足和通过基因型分析) 和疾病有关的贫血 (血红蛋白水平比对于年龄和性别来说正常的当地实验室下限低) 的 ≥ 2 岁的男性和女性。参与者也具有以下一项和多项: 通过触诊至少中度脾肿大 (在左肋缘以下 2 至 3cm); 疾病有关的血小板减少 (血小板计数 $<120 \times 10^3$ 血小板 / 立方毫米); 和容易触知的肝肿大。参与者在进入研究之前的 12 个月内不能接受对戈谢病的治疗。

[0628] 如果参与者具有脾切除; 患有 (或怀疑患有) 2 或 3 型戈谢病; 为抗体阳性或经历对伊米昔酶的过敏性休克, 则排除所述参与者。其他排除标准包括在进入研究之前的 30 天内用任何非戈谢病有关的研究药物和装置治疗; HIV 或者 B 或 C 型肝炎为阳性检验; 严重贫血 (维生素 B₁₂、叶酸或缺铁有关的贫血) 或能够影响研究数据的任何显著的共存疾病。排除怀孕和泌乳妇女且要求具有生育小孩潜力的妇女一直使用医学可接受的避孕方法。

[0629] 研究治疗

[0630] 治疗任务: 在施用第一剂量的以下物质之前使患者以 1:1 的比率随机分组: 每隔一周施用维拉昔酶 α 60U/kg 历时 39 周 (高达 16 个患者, 20 次输注), 或每隔一周施用伊米昔酶 60U/kg 历时 39 周 (高达 16 个患者, 20 次输注)。所有研究投药通过 IV 输注经 1 小时施用以保持治疗盲目。

[0631] 治疗施用

[0632] 研究投药输注: 在临床地点以连续 1- 小时 IV 输注施用双盲研究投药输注, 以保持

对治疗盲目。研究投药输注在一周中大致相同的一天发生,但每 14 天(± 3 天)发生以促进患者排定。

[0633] 剂量计算:双盲研究投药的第一剂量根据基准时患者的体重计算。自基准或自最新记录的测量值(第 13 周或第 25 周)体重变化 $\geq 5\%$ 将需要重新计算研究投药的剂量。

[0634] 研究投药的描述

[0635] 维拉苷酶 α :维拉苷酶 α 为在 2 至 8°C 下供应并运输到临床试验地点以便储存的冻干产品。

[0636] 伊米苷酶(Cerezyme®):伊米苷酶(Cerezyme)作为无菌无热原的白色至灰白色冻干产品供应。

[0637] 研究程序和数据收集方法

[0638] 研究加入标准:在筛选时,复查患者对于研究加入标准的合格性。不符合研究加入标准的患者被视为筛选失败。

[0639] 合格性的证实:在筛选时,患者提供血样以测量血红蛋白浓度,从而测定研究合格性。仅具有低于对于年龄和性别来说正常的当地实验室下限的血红蛋白浓度的那些患者符合登记条件。

[0640] 在基准时,患者提供血样以证实其血红蛋白浓度低于对于年龄和性别来说正常的当地实验室下限。只有在筛选和基准二者时具有低于对于年龄和性别来说正常的下限的血红蛋白浓度的那些患者被证实符合该研究条件。

[0641] 测定基因型:在筛选时所有患者提供血样用于测定戈谢病基因型和血浆壳三糖苷酶基因型。

[0642] 病史:在筛选时,记录患者的完整病史。这包括身体系统的复查、当前和预先医疗程序的记载、和当前和预先伴随投药使用的记载、和在进入研究之前的 12 个月内没有对戈谢病治疗的患者的记载。

[0643] 生命体征:记录的生命体征参数包括脉搏、血压、呼吸率和温度。遵循以下方案以记录在输注访问时的生命体征:输注开始(在开始输注之前 10 分钟内)、输注期间(30 分钟(± 5 分钟))、输注之后(在完成输注之后 5 分钟内、30 分钟(± 5 分钟)和 60 分钟(± 5 分钟))。在筛选、基准时和第 41 周,仅在一个时间点收集生命体征。

[0644] 身体检查:在筛选、基准时和在研究的第 13 周、第 25 周和第 41 周进行身体检查。身体检查包括以下项:一般外貌、内分泌、头颈部、心血管、眼睛、腹部、耳朵、泌尿生殖器、鼻、皮肤、喉咙、肌骨胳、胸部和肺及神经学。身体发现的任何异常变化在适当 CRF 页面上记录为不良事件。

[0645] 身高和体重:在基准时和在研究的第 13 周、第 25 周和第 41 周记录身高和体重。对于儿科患者(即,2 至 17 岁),使用身高和体重评价来测定生长速度。

[0646] 12-导联心电图:在基准和研究的第 13 周、第 25 周和第 41 周进行 12-导 ECG,且所述 12-导 ECG 包括 PR、QRS、QT 和 QTc 间隔及心率的评价。

[0647] 临床实验室检验:如下所述收集血样和尿样用于临床实验室检验。

[0648] 血液学:在筛选时和在基准时收集血样以测量血红蛋白水平用于统计分析。也在筛选、基准和研究的第 13 周、第 25 周和第 41 周收集血样用于完整的血液学检验。评估以下血液学参数:具有差异的全血细胞计数(CBC)、血小板计数、活化部分凝血活酶时间

(aPPT)、网织红细胞计数（通过临床地点当地实验室分析并报道）和前凝血酶时间（PT）。在筛选时、基准时和在每次研究访问（除了在第1周访问外）时收集血样以测量血红蛋白浓度和血小板计数。

[0649] 血清化学：在筛选、基准时和在研究的第13周、第25周和第41周收集血样用于血清化学检验。评估以下血清化学参数：钠、丙氨酸转氨酶、钾、天冬氨酸转氨酶、葡萄糖、乳酸脱氢酶、总钙、 γ -谷氨酰基转移酶、总蛋白质、肌酐磷酸激酶、白蛋白、NTx*、肌酐、CTx*、尿素氮、叶酸（以测定研究合格性）、总胆红素、维生素B₁₂（仅筛选）、碱性磷酸酶*（*结果用于评价骨生物标志物）。在筛选时具有叶酸和/或维生素B₁₂缺乏有关的贫血且如此不符合研究进入标准的患者被视为筛选失败。

[0650] 尿检：在筛选、基准时和在研究的第13周、第25周和第41周收集尿样用于尿检。评估以下尿检参数：pH、微观评估和宏观评估。

[0651] 血清抗维拉昔酶 α 抗体：在筛选时和在治疗期期间约每6周（第7周、第13周、第19周、第25周、第31周和第37周）和第41周患者提供血样以测量在血清中的抗维拉昔酶 α 抗体。在治疗期期间，在双盲研究投药的输注之前收集这些血样。

[0652] 评估收集用于抗维拉昔酶 α 抗体测定的血样。这些样品使用酶联免疫吸附测定（ELISA）筛选，且所有阳性实例使用放射免疫沉淀测定（RIP）证实为阳性。阳性实例为同种型的（IgG、IgA、IgM或IgE）。另外，使用体外测定检验阳性实例的酶中和活性。

[0653] 血清抗伊米昔酶抗体测定：在筛选时患者提供血样以测量抗伊米昔酶抗体。使用为抗维拉昔酶 α 抗体分析获得的相同样品进行抗伊米昔酶抗体分析。

[0654] 评估这些血样以测定抗伊米昔酶抗体的存在。这些样品使用酶联免疫吸附测定（ELISA）筛选，且所有阳性实例使用放射免疫沉淀测定（RIP）证实为阳性。阳性实例为同种型的（IgG、IgA、IgM或IgE）。另外，使用体外测定检验阳性实例的酶中和活性。

[0655] 在筛选时检验抗伊米昔酶抗体为阳性的患者不符合该研究的条件。

[0656] 对于在该研究期间发展出抗伊米昔酶抗体的患者进行抗体交叉反应性检验（与维拉昔酶 α ）。

[0657] 免疫和炎性响应检验：在基准时和在第13周、第25周和第41周 ≥ 18 岁的患者提供血样用于免疫和炎性响应检验。在基准时获得一个样品。在第13周、第25周和第41周，在用研究药物的各输注之前、在紧接着跟踪和在用研究药物的各输注之后的1小时获得样品。

[0658] 不良事件：贯穿该研究从患者提供告知同意书的时间至在最后输注之后的30天对于完成研究且没有选择加入后续开放标签长期临床研究中的患者或对于在第41周访问之前从研究中中断或退出的患者监测不良事件。对于完成该研究且选择加入后续开放标签长期临床研究中的患者，从告知同意到完成第41周访问监测不良事件。

[0659] 输注有关的不良事件的管理：蛋白质的输注可能与对该输注的反应相关。输注有关的不良事件定义为如下不良事件：1) 在输注开始之后的12小时期间或12小时内开始，和2) 被判断为可能或或许与研究盲法研究投药有关。

[0660] 肝和脾MRI：在基准时和在第25周和第41周/EOS对患者进行肝和脾的MRI。肝和脾的大小使用定量腹部MRI测量。

[0661] 血浆壳三糖苷酶水平：在基准时和在第1周、第5周、第9周、第13周、第17周、第

21 周、第 25 周、第 29 周、第 33 周、第 37 周和第 41 周收集血样以评估血浆壳三糖苷酶水平。使用酶活性测定分析壳三糖苷酶。

[0662] 血浆 CCL18 水平:在基准时和在第 1 周、第 5 周、第 9 周、第 13 周、第 17 周、第 21 周、第 25 周、第 29 周、第 33 周、第 37 周和第 41 周收集血样以评估血浆 CCL18 水平。通过在市售试剂盒中酶联免疫吸附测定 (ELISA) 测量 CCL18 水平。

[0663] 生活质量检验:在基准时和在第 41 周,使用包括 36 项短格式 (SF-36) 第 2 版 (对于 ≥ 18 岁的患者) 和儿童健康调查表 (CHQ) PF50 (对于 5 至 17 岁的患者) 的批准的调查表评估 (Ware Arch Phys Med Rehabil Vol 84, 增刊 2, 2003 年 4 月:43-51;SF-36v2 Health Survey© 1996, 2000 by QualityMetric Incorporated and Medical Outcomes Trust. 版权所有;Landgraf 等, Child Health Questionnaire: 使用手册。第 2 次印刷, Health Act, Inc., Boston MA, 1999;Landgraf 等, Quality of Life Research. 1998; 7(5):433-445)。

[0664] 生长速度和 Tanner 分期:对于 2 至 17 岁的患者,在基准和第 13 周、第 25 周和第 41 周时评价生长。使用在该研究期间在规则的时间点记录的身高和体重测量结果计算生长速度且使其与 Tanner 分期相关。在基准和第 13 周、第 25 周和第 41 周时记录 Tanner 分期。将在各治疗组中在 2 至 17 岁之间的各患者相对于基准的变化评估为三次功效参数。

[0665] 骨骼生长:在基准和第 41 周时对在 2 至 17 岁之间的患者进行左手和腕部的放射照相以评估骨骼年龄。

[0666] 骨生物标志物:在基准时和第 41 周,对 ≥ 18 岁的患者进行腰脊柱和股骨颈的 DXA,包括冠状面成像,以测定戈谢有关的局部和全身性骨病。在基准时和第 41 周,这些患者的骨质流失和脱矿质通过测量血清碱性磷酸酶、NTx 和 CTx 评估。

[0667] 对于 2 至 17 岁的患者,在基准时和第 41 周 (与对这些患者进行肝和脾的 MRI 的同时) 获得股骨颈和腰脊柱的 MRI。

[0668] 不出所料在该研究期间任何治疗作用对于这些参数都将是显然的;然而,在基准时和第 41 周收集的测量结果将用以确立在后续开放标签长期临床研究期间自其监测这些生物标志物的参考点。

[0669] 预先和伴随的疾病:将在基准时存在的额外疾病视为伴随疾病且记载在病史 CRF 的适当页面上。在研究期间首先发生或检测到的疾病或在研究期间伴随疾病的恶化被视为 AE 且因而记载在 CRF 中。

[0670] 患者在该研究期间的任一点或在最后输注之后的 30 天内没有接受用红血球生长因子或研究药物或装置的治疗。

[0671] 在该研究的治疗期期间,患者可以接受作为预投药的皮质类固醇以减轻潜在的输注有关的不良事件。

[0672] 不良事件

[0673] 不良事件定义:不良事件 (AE) 为如由在临床试验的任一期中出现的身体体征、症状和 / 或实验室变化所指示的解剖学、生理或代谢功能的任何无毒、病理学或无意识的变化,而不管是否被视为研究药物有关。这包括预先存在病状的恶化。自患者提供告知同意的时间至在最后剂量的盲法研究投药之后的 30 天和 / 或直到不良事件已经解决 / 稳定或达到一种结果 (无论何者在先) 收集不良事件。对于在第 41 周访问之前被中断或退出的

患者,跟踪 AE 直至在其最后输注之后的 30 天。对于完成该研究且选择加入长期临床研究中的患者,从患者提供告知同意书的时间到第 41 周访问监测不良事件。

[0674] AE 包括:在研究起始时存在的病状的恶化(性质、严重性或频率方面的改变);并发的疾病;药物相互作用;与伴随投药有关或可能与伴随投药有关的事件;异常实验室值(这包括自在研究人员认为临床上重要的正常范围内的基准的显著转变);身体检查、生命体征、体重和 ECG 的临床上显著的异常。

[0675] 另外,AE 也可以包括出乎意料的实验室值,其变得显著超出范围且由研究人员测定为临床上显著的。如果存在出乎意外的溢出值,则重复实验室检验,直至其恢复到正常或可被解释且患者的安全不在保险范围内。

[0676] 输注有关的不良事件的定义:输注有关的不良事件定义为如下不良事件:1) 在输注开始之后的 12 小时期间或 12 小时内开始,和 2) 被判断为可能或或许与盲法研究投药有关。在输注之前发生的其他 AE 以及在输注之前进行的与实验方案限定的检验和评价(例如,实验室检验、ECG 和身体检查)相关的 AE 并不定义为输注有关的不良事件。

[0677] 严重的不良事件定义:严重的 AE(SAE) 为导致任何以下结果的以任何剂量发生的任何 AE:死亡,为危及生命的,需要患者住院治疗,需要现有住院治疗延期、持久或显著的病废/无能和出生缺陷/先天缺陷。

[0678] 可能不导致死亡、危及生命或需要住院治疗的重要医疗事件可被视为 SAE,当根据适当医疗判断,它们可能危害患者且可能需要医疗或外科手术以预防以上所列结果之一。

[0679] 危及生命的 AE 定义为鉴于最初报道使患者处于随着 AE 发生而引起的死亡即时危险中的 AE(即,其不包括使其以更严重的形式出现或可能致死的 AE)。

[0680] 不良事件和严重不良事件的分类:当评价 AE 的严重性时,参考国立癌症研究院通用毒性标准(NCI CTC) 3.0 版分级量表。如果 AE 没有描述在 NCI CTC 中,则根据以下量表记录严重性。所有 AE/SAE 的严重性在适当 CRF 页面上记录为分别对应于轻微、中度、重度或危及生命的严重性的 1 级、2 级、3 级或 4 级。1 级(轻微)定义为日常活动不受限制。2 级(中度)定义为日常活动受一定程度地限制;3 级(重度)定义为不能实施日常活动;和 4 级(危及生命的)定义为即时死亡危险。

[0681] 不良事件或严重不良事件与盲目研究投药的关系由研究人员根据以下定义测定。“无关”定义为与研究药物无关。“可能有关”定义为在施用研究药物的合理时序下的临床事件/实验室异常,但其也可以由同时发生的疾病或其他药物/化学解释。“或许有关”定义为在施用研究药物的合理时序下的临床事件/实验室异常,不大可能归因于同时发生的疾病或其他药物和化学且其接着是对再挑战的临床上合理的响应。临床事件/实验室异常的相关性也必须具有一定的生物学似真性,至少是在理论根据上。

[0682] 在严重和重度之间的阐明:术语“重度”常用以描述具体事件的强度(严重性)(如在轻微、中度或重度心肌梗塞中);然而,事件本身可能具有相对较少的医疗意义(诸如重度头痛)。这与“严重”没有不同,其根据通常与造成对生命或功能的威胁的事件通常相关的结果或作用标准。严重(非重度)和因果性用作限定调整报告责任的指导。

[0683] 不良事件监测和观察期:对于该研究的目的,观察期从患者提供告知同意书的时间延伸到该研究的患者最终评估。出于安全目的,最终评估定义为对完成研究且没有选择加入长期研究的患者或对在第 41 周访问之前从研究中中断或退出的患者在最后输注之后

约 30 天进行的后研究安全评估。对于完成该研究且选择加入长期临床研究中的患者,从患者提供告知同意书的时间到第 41 周访问监测不良事件。如果研究人员认为必须在观察期结束之后记录研究患者中的 AE,他或她可联系主办者来测定应该如何记下或报道 AE。

[0684] 统计方法

[0685] 一般统计方法论:考虑两组数据以统计分析功效:1) 意向治疗 (ITT) 数据集和 2) 完成实验方案 (PP) 数据集。所述 ITT 数据集由接受至少一完整或部分剂量的研究药物的所有随机分组患者组成。所述 PP 数据集为 ITT 数据集的亚组,其包括完成 41 周研究、具有所收集的第一功效变量的基准测量结果和 41 周测量结果并接受至少 80% 的其排定输注剂量的患者。

[0686] 对于跟踪连续分布的变量,表格汇总由 n、平均值、标准偏差、最小值、最大值和中值组成。关键功效变量的图表由治疗组提供。对于分类变量,表格汇总由由治疗组在各分类中提供的频率和百分比组成。第一功效变量由治疗组提供,且包括:原始值:以最初报道的标度的变量的未转化值;该值相对于基准的绝对变化,即 $X-B$ (其中 B 为基准值且 X 为后基准值);和该值相对于基准的变化%,即 $100*(X-B)/B$ (其中 B 为基准值且 X 为后基准值)。

[0687] 假设检验:该研究比较维拉苷酶 α 与伊米苷酶的作用。意图在于示出维拉苷酶 α 在临床上以 0.025 的显著性水平与伊米苷酶至少一样好。

[0688] 对于第一功效终点的零假设在于对于维拉苷酶 α 来说血红蛋白浓度自基准至第 41 周的平均变化比对于伊米苷酶来说血红蛋白浓度自基准至第 41 周的平均变化低至少 1g/dL。待检验的假设可陈述为:

[0689] $H_0: \mu_{\text{vela}} - \mu_{\text{IMIG}} \leq -1$, 相对地, $H_A: \mu_{\text{vela}} - \mu_{\text{IMIG}} > -1$

[0690] 或

[0691] H_0 : 关于平均血红蛋白响应维拉苷酶 α 较差

[0692] H_A : 关于平均血红蛋白响应维拉苷酶 α 不差

[0693] 筛选失败和患者处置:对于进入研究筛选的所有患者的处置连同筛选失败的原因一起列出。所有随机分组患者的处置通过治疗组和访问列出,且中断的原因通过治疗组列出。

[0694] 样本量调整:当各治疗组中的样本量为 14 时,两组 0.025 单侧 t- 检验将具有 80% 的效能以拒绝血红蛋白的平均差值 $\leq -1\text{g/dL}$ 的零假设、支持平均差值大于 -1 的备选假设,假定预期平均差值为 0 且常见标准偏差为 0.90。

[0695] 假设 15% 漏失,总共 32 个患者 (每治疗组有 16 个患者) 登记到研究中。

[0696] 功效分析

[0697] 分析群:考虑两组数据用于统计分析功效 (意向治疗 (ITT) 数据集和完成实验方案 (PP) 数据集)。

[0698] 第一功效分析:第一功效终点为在两个治疗组之间血红蛋白浓度自基准到第 41 周的平均变化。第一分析使用 ITT 群进行。这是设计用来证明就治疗患有 1 型戈谢病的患者的功效而言维拉苷酶 α 不亚于伊米苷酶的非劣性随机分组控制的试验。

[0699] 使用单侧 97.5% 置信区间。非劣性由单侧置信区间或假设检验证明,所述假设检验用于检验治疗差异在血红蛋白方面小于或等于等效界值 (-1g/dL) 的零假设相对伊米苷酶治疗差异大于所述较低等效界值的备选假设。换句话说,刚好集中在置信区间的一端且

忽略置信区间的另一端产生单侧 97.5% 置信区间 $[(a, \infty)]$, 其中 a 为单侧置信区间的下界, 可以绘出功效结论。

[0700] **第二功效分析:** 对于比较在治疗组之间相对于基准的变化的第二功效分析 (血小板计数、肝和脾体积、壳三糖苷酶和 CCL18), 如果在两个治疗组之间自基准到第 41 周的平均变化为统计上显著的 (统计上显著的将定义为 p - 值小于 0.05), 则评估统计检验。使用协方差分析 (ANCOVA) 模型对在两个治疗组之间相对于基准的平均变化的差异提供 95% 置信区间, 其包括例如作为共向变量的基准年龄。

[0701] 对于得到事件结果量度的时间 (即, 自基准达到第一血红蛋白响应 $\geq 1\text{g/dL}$ 的时间), 对各治疗组提供 Kaplan-Meier (乘积极限) 生存曲线且使用对数秩检验比较在治疗组之间的曲线。对各治疗组提供中值时间和 95% 置信区间。在第 41 周审查到研究结束 (即, 到第 41 周) 没有经历事件的患者。在第 41 周评估之前退出或被中断的患者和在退出或中断的时候没有达到响应的患者在对于该患者的最后已知评估的时候审查。另外, 使用费雪精确检验提供并比较在治疗组之间响应的患者与未响应者的比例。

[0702] **安全分析:** 评价接受至少一剂研究药物 (或部分剂量) 的所有患者的临床安全性和耐受性。对安全参数不进行正式统计检验。概述了生命体征、12- 导 ECG、临床化学、血液学和尿检安全监测。对于诸如 AE 的分类变量, 列出经历各 AE 的患者的数量和百分数。AE 以事件严重性汇总。也将显示出经历药物有关的 AE 和输注有关的 AE 以及不被视为与研究药物有关的 AE 的患者的数量和百分数。使用临床实验室评估 (血液学、血清化学、尿检和抗维拉昔酶 α 抗体的测定) 来评价维拉昔酶 α 的安全性。

[0703] 实施例 4: TKT034 研究 (在从用伊米昔酶治疗转换的患者中的多中心开放标签研究)

[0704] **概述**

[0705] 该实施例描述检查在预先接受伊米昔酶的患有 1 型戈谢病的患者中维拉昔酶 α 的安全性和功效的全局开放标签 12 个月研究。 ≥ 2 岁的患者接受剂量等于其预先伊米昔酶剂量的维拉昔酶 α , 输注每隔一周经 1 小时施用。

[0706] 40 个患者接受维拉昔酶 α (18 个男性, 4 个预先脾切除; 年龄范围: 9-17 岁)。中值预先伊米昔酶使用为 67 个月 (范围: 22-192 个月)。维拉昔酶 α 剂量为 15-22.5U/kg ($n = 14$) (“15U/kg 组”)、22.5-37.5U/kg ($n = 12$) (“30U/kg 组”)、37.5-52.5U/kg ($n = 7$) (“45U/kg 组”) 和 $>52.5\text{U/kg}$ ($n = 7$) (“60U/kg 组”)。维拉昔酶 α 耐受性通常良好, 大多数不良事件 (AE) 为轻微或中度严重性。11 个患者 (28%) 经历视为可能或或许与研究药物有关的 AE, 大多数 AE 被视为与输注有关。患者没有经历危及生命的 AE。一种严重的 AE 被视为或许与治疗有关: 1 个患者在首次输注期间具有 2 级过敏性反应, 且被选择以中断研究。在输注时和 2 周后检验该患者的 IgE、IgM、IgG、IgA 和中和抗体为阴性。患者没有发展出维拉昔酶 α 的 IgG 抗体。血红蛋白浓度、血小板计数、肝和脾体积在治疗水平下维持 1 年。

[0707] 总之, 先前用伊米昔酶治疗 ≥ 22 个月的患有 1 型戈谢病的成人和儿科患者成功转换到维拉昔酶 α , 经 12 个月测量到临床疾病的稳定性。

[0708] **研究目标**

[0709] 该研究的第一目的在于评估在先前用伊米昔酶治疗的患有 1 型戈谢病的患者中

每隔一周给予维拉苷酶 α 的安全性。

[0710] 第二目的为：评估在每隔一周给予维拉苷酶 α 之后血红蛋白浓度相对于基准的变化，评估在每隔一周给予维拉苷酶 α 之后血小板计数相对于基准的变化，和通过腹部MRI 评估在每隔一周给予维拉苷酶 α 之后肝体积和脾体积相对于基准的变化。

[0711] 第三/探索目的为：评估在每隔一周给予维拉苷酶 α 之后血浆壳三糖苷酶和趋化因子 (C-C 基元) 配体 18 (CCL18) 水平相对于基准的变化，通过左手和腕部的放射照相评估在每隔一周给予维拉苷酶 α 之后在 2 至 17 岁之间的患者中的骨骼年龄的变化，评估在每隔一周给予维拉苷酶 α 之后在 2 至 17 岁之间的患者中的生长速度和 Tanner 分期的变化，确立如下基准，自其监测维拉苷酶 α 疗法对在 ≥ 18 岁的患者中戈谢有关的局部和全身性骨病的长期影响，如通过腰脊柱和股骨颈的骨密度 (DXA) (包括冠状面成像)、血清碱性磷酸酶、N-末端肽交联 (NTx) 和 C-末端肽交联 (CTx) 所测量和确立以下基准，自其通过腰脊柱和股骨颈的 MRI 评估在 2 岁至 17 岁之间的患者中的骨病。

[0712] 总体研究设计

[0713] 这是设计用来评估维拉苷酶 α 疗法对当前接受对 I 型戈谢病的伊米苷酶疗法的患者的安全性的多中心 II/III 期开放标签研究。登记 41 个患者以接受与其伊米苷酶剂量相同单位数的维拉苷酶 α 。剂量在 15U/kg 至 60U/kg 范围内。在研究登记之前在 6 个月期间患者接受相同剂量的伊米苷酶。对各患者的研究的总持续时间为约 14 个月 (视情况从筛选到研究和 / 或跟踪结束)。

[0714] 所述研究由以下 5 期组成：筛选 (第 -14 天到第 -4 天)；基准 (第 -3 天至第 0 天 (在第一剂量之前)；治疗期：第 1 周 (第 1 天；第一剂量) 至第 51 周 (每个患者施用总共 26 次输注)；研究访问结束：第 53 周；跟踪：在最后输注之后的 30 天 (对于在第 53 周评估之前中断 / 退出的患者，或对于完成该研究但没有选择加入后续长期临床研究中的患者)。

[0715] 在患者第一剂量之前的两周内对提供书写的告知同意书的患者 (或父母 / 法定监护人) 进行筛选评估以测定符合登记条件。为了测定研究合格性，在筛选期间自各患者收集血样以评估血红蛋白浓度和血小板计数。

[0716] 出于统计分析目的，在筛选期间收集额外血样以评估血红蛋白浓度和血小板计数。

[0717] 将第一剂量的研究药物的施用定义为第 1 周 (第 1 天)。维拉苷酶 α 输注每隔一周施用历时 12 个月 (51 周)，总共 26 次输注。患者接受与其伊米苷酶剂量相同单位数的维拉苷酶 α 。剂量在 15U/kg 至 60U/kg 范围内。输注时间为 60 分钟 (1 小时)。在源文件和适当 CRF 中记载了增加的输注持续时间 (例如，2 小时)。输注的持续时间不小于 1 小时。

[0718] 在临床地点对各患者施用前 3 次维拉苷酶 α 输注。没有经历治疗有关的严重不良事件或维拉苷酶 α 输注有关的不良事件的患者可以按研究人员的判断和指导在家接受由有资格和经培训的医务人员进行的其后续输注。在研究期间可以在随后的时间点重新评估经历输注有关的不良事件的患者以考虑转换到家庭输注。要求接受作为家庭疗法的维拉苷酶 α 的患者在第 7 周、第 13 周、第 19 周、第 25 周、第 31 周、第 37 周、第 45 周和第 51 周及第 53 周返回临床地点。

[0719] 研究完成的访问定义为第 53 周。只要患者 1) 完成 51- 周治疗期和 2) 完成第 51 周和第 53 周的研究访问，则将患者视为完成了该研究。

[0720] 提供给完成该研究的患者机会以登记在后续长期开放标签的临床研究中。对于选择加入后续长期开放标签的临床研究的患者来说,将来自该研究 (TKT034) 的第 51 周和第 53 周访问的某些评价用作该研究的基准评价;在患者完成对第 53 周访问的所有评价并提供书写的告知同意书以参与后续长期开放标签的临床研究之后,患者将在第 53 周访问时接受其后续长期开放标签临床研究的第一次输注。因此,希望患者将横跨该 2 种研究接受连续维拉昔酶 α 治疗。完成该研究且没有选择加入后续长期开放标签的临床研究的患者将在其最后输注之后通过现场访问或打电话 30 天而得到安全评价(收集不良事件和伴随的投药)。

[0721] 研究群的选择

[0722] 将接受至少一次输注(或部分输注)的所有登记的患者包括在 ITT 患者群中。

[0723] 合格的参与者为诊断有 1 型戈谢病(白血球中葡糖脑苷脂酶活性不足或通过基因型分析)的 ≥ 2 岁的男性或女性,其接受与伊米昔酶一致的治疗历时最少连续 30 个月;允许 1 个患者参与得到连续 22 个月的使用伊米昔酶的先前治疗。

[0724] 如果参与者的血红蛋白浓度 $\leq 10\text{g/dL}$ 且血小板计数 $\leq 80 \times 10^3$ 血小板/立方毫米;在筛选之前的 6 个月期间具有不稳定的血红蛋白浓度(超过筛选值的 $\pm 1\text{g/dL}$ 的范围)或血小板计数(超过筛选值的 $\pm 20\%$);患有(或怀疑患有)2 或 3 型戈谢病;经历对伊米昔酶的过敏性休克;在进入研究之前的 6 个月内具有与伊米昔酶不一致的治疗或接受美格鲁特;或在筛选的 12 个月内具有放射证实的活性、临床上显著的脾梗塞或恶化骨坏死,则排除这些参与者。

[0725] 其他排除标准包括在进入研究之前的 30 天内用任何研究性药物或装置治疗;HIV、B 或 C 型肝炎为阳性检验;在筛选时有非戈谢病有关的贫血;或可能影响研究数据的任何显著的共存疾病。排除怀孕和泌乳妇女且要求具有生育小孩潜力的妇女一直使用医学可接受的避孕方法。

[0726] 研究治疗

[0727] 治疗任务:患者每隔一周以与其伊米昔酶剂量相同的单位数接受维拉昔酶 α 输注。在基准时记录患者的当前伊米昔酶剂量。维拉昔酶 α 剂量在 15U/kg 至 60U/kg 范围内。

[0728] 治疗方案:患者在第 1 周(第 1 天)接受其第一次输注。所有患者每隔一周接受一次维拉昔酶 α 历时 12 个月(51 周),因此,施用总共 26 次输注。

[0729] 所有剂量的维拉昔酶 α 作为连续 IV 输注以 1U/kg/min 的最大流速施用。输注时间为 60 分钟(1 小时)。在源文件和适当 CRF 中记载了增加的输注持续时间(例如,2 小时)。输注的持续时间可不小于 1 小时。患者在进入研究之前最多 30 天且在进入研究之前最少 14 天接受其最终伊米昔酶剂量。

[0730] 剂量计算:用以计算在第 13 周、第 25 周或第 37 周的剂量的相对于基准或先前记录的体重的体重变化 $\geq 5\%$ 需要重新计算研究投药的剂量。

[0731] 剂量调节:贯穿治疗期监测患者的临床参数(即,血红蛋白浓度、血小板计数和肝体积及脾体积)的变化。如果患者证明了这些参数的临床上显著的改变,则研究人员评估患者的剂量增加 15U/kg 的选择。如果经两次连续评估满足且符合以下四项标准中的两项或更多项,则考虑剂量调节:血红蛋白浓度相对于基准减小 $>1\text{g/dL}$;血小板计数相对于基

准减小 >20% ;如通过器官触诊指示且如通过 MRI 测量证实的肝体积增大 >15% ;和如通过器官触诊指示且如通过 MRI 测量证实相对于基准的脾体积增大 >15%。

[0732] 如果临床参数值在 3 个月内没有回到基准水平,则研究人员选择使剂量以 15U/kg 的增量增加。对接受 60U/kg 剂量的患者没有提供剂量增加且不允许剂量高于 60U/kg。如果患者对最大剂量 60U/kg 未能作出响应,如果根据研究者的临床判断认为适当,则可以使患者退出。

[0733] 维拉昔酶 α 施用

[0734] 维拉昔酶 α 施用的总论:静脉内施用维拉昔酶 α 。研究药物输注在一周中大致相同的一天发生,但可在目标日的每 14 天 (± 3 天) 发生以有助于患者排定。如果完全可能的话,则应该避免缺失输注。如果患者在从其排定给药的 17 天内没有给药,则患者将在批准其在研究中继续之后尽快地接受下一次输注。可能可以接受在先前输注之后的早至 7 天给出下一次输注。后续输注将回到原始方案。

[0735] 维拉昔酶 α 施用的家庭输注指南:前三次维拉昔酶 α 输注在临床地点施用。在前三次给药之后,没有经历治疗有关的严重不良事件或输注有关的不良事件的患者可以在家接受其后续输注。在研究期间可以在随后的时间点重新评估经历输注有关的不良事件的患者以考虑转换到家庭输注。要求接受作为家庭疗法的维拉昔酶 α 的患者在第 7 周、第 13 周、第 19 周、第 25 周、第 31 周、第 37 周、第 45 周、第 51 周及第 53 周返回临床地点。

[0736] 在家庭环境中,在各次访问时收集生命体征和不良事件的记载。

[0737] 输注有关的不良事件的管理:输注有关的不良事件定义为如下不良事件:1) 在输注开始之后的 12 小时期间或 12 小时内开始,和 2) 被判断为可能或或许与研究药物有关。

[0738] 研究药物的描述:维拉昔酶 α 为在 2°C 至 8°C 下供应并由合格的分配器运输到临床试验地点以便储存的冻干产品。

[0739] 戈谢病特异性治疗史:在筛选时,记录所有戈谢病特异性治疗,包括患者的当前伊米昔酶剂量。患者的初始维拉昔酶 α 剂量根据所记录的当前伊米昔酶剂量。

[0740] 历史血红蛋白和血小板值:收集并复查在进入研究之前的 30 个月内血红蛋白浓度和血小板计数的所有评估以测定患者合格性。

[0741] 测定戈谢病和壳三糖苷酶基因型:仅在筛选时,所有患者收集血样用于测定戈谢病和血浆壳三糖苷酶基因型。

[0742] 生命体征:记录的生命体征参数包括脉搏、血压、呼吸率和温度。

[0743] 遵循以下方案以记录在所有输注访问时的生命体征:输注开始(在开始输注之前 10 分钟内)、在输注期间(30 分钟 (± 5 分钟));输注之后(在完成输注之后 5 分钟内、在完成输注之后 30 分钟 (± 5 分钟)和在完成输注之后 60 分钟 (± 5 分钟))。

[0744] 身体检查:在基准访问时和在第 13 周、第 25 周、第 37 周、第 51 周和第 53 周进行身体检查。身体检查包括以下项:一般外貌、内分泌、头颈部、心血管、眼睛、腹部、耳朵、泌尿生殖器、鼻、皮肤、喉咙、肌骨骼、胸部和肺及神经学。身体发现的任何异常变化在适当 CRF 页面上记录为不良事件。

[0745] 身高和体重:在基准访问时和在第 13 周、第 25 周、第 37 周和第 51 周记录身高和体重。使用身高和体重测量结果计算生长速度且使其与 Tanner 分期相关。

[0746] 12-导联心电图:在基准访问时和在第 13 周、第 25 周、第 37 周和第 51 周进行

12-导 ECG。各 12-导 ECG 包括 PR、QRS、QT 和 QTc 间隔及心率的评价。

[0747] 临床实验室检验：如下所述收集血样和尿样用于以下评估。

[0748] 血液学：在筛选和基准访问时和在第 7 周、第 13 周、第 19 周、第 25 周、第 31 周、第 37 周、第 45 周、第 51 周和第 53 周收集血样用于血液学检验。评估以下血液学参数：具有差异的全血细胞计数 (CBC)、活化部分凝血活酶时间 (aPPT)、网织红细胞计数（通过就地的当地实验室进行）、血小板计数和前凝血酶时间 (PT)。在筛选、基准时和在每次研究访问时（除了在第 1 周访问外）收集血样以测量血红蛋白浓度和血小板计数。

[0749] 出于统计分析目的，在筛选期间收集额外血样以测量血红蛋白浓度和血小板计数。

[0750] 血清化学：在基准访问时和在第 13 周、第 25 周、第 37 周、第 51 周和第 53 周收集血样用于血清化学检验。

[0751] 评估以下血清化学参数：钠、总胆红素、钾、碱性磷酸酶*、葡萄糖、丙氨酸转氨酶、总钙、天冬氨酸转氨酶、总蛋白质、乳酸脱氢酶、白蛋白、 γ -谷氨酰基转移酶、肌酐、肌酐磷酸激酶、尿素氮、CTx、NTx*（* 结果用于评价骨生物标志物）。在筛选时具有叶酸和 / 或维生素 B₁₂ 缺乏有关的贫血且如此不符合研究加入标准的患者被视为筛选失败。根据临床地点的标准惯例，可以使这些患者在研究人员的判断下对其叶酸和 / 或维生素 B₁₂ 缺乏有关的贫血治疗高达 12 周。在这些患者完成叶酸和 / 或维生素 B₁₂ 治疗方案之后可以针对该研究进行重新筛选。

[0752] 尿检：在基准访问时和在第 13 周、第 25 周、第 37 周、第 51 周和第 53 周收集尿样用于尿检。评估以下尿检参数：pH、微观评估和宏观评估。

[0753] 血清抗伊米苷酶抗体测定：所有患者仅在基准时给出血样用于测定血清抗伊米苷酶抗体。患者符合在该研究中登记的条件，而与其抗伊米苷酶抗体状况无关。将允许抗伊米苷酶抗体为阳性的患者进入该研究。评估这些血样以测定抗伊米苷酶抗体的存在。

[0754] 血清抗维拉苷酶 α 抗体测定：在基准访问时和在第 7 周、第 13 周、第 19 周、第 25 周、第 31 周、第 37 周、第 45 周和第 51 周收集血样用于测定抗维拉苷酶 α 抗体。评估收集用于抗维拉苷酶 α 抗体测定的血样。使用酶联免疫吸附测定 (ELISA) 筛选这些样品。

[0755] 不良事件：贯穿其研究从患者提供签字的告知同意书的时间至在最后输注之后的 30 天对于完成研究且没有选择加入后续长期开放标签的临床研究中的患者或对于在第 53 周访问之前从研究中中断或退出的患者监测不良事件。对于完成该研究且选择加入后续长期开放标签的临床研究中的患者，从告知同意到该研究 (TKT034) 的第 53 周访问监测不良事件。

[0756] 预先和伴随的疾病：将在基准时存在的额外疾病视为伴随疾病且记载在 CRF 的适当病史页面上。在研究期间首先发生或检测到的疾病或在研究期间伴随疾病的恶化被视为 AE 且因而记载在 CRF 中。

[0757] 功效评估的研究程序

[0758] 血红蛋白浓度：在本文所述的时间点测量血红蛋白浓度。血红蛋白浓度自基准到 12 个月的变化为该研究的第二终点。

[0759] 血小板计数：在本文所述的时间点测量血小板计数。血小板计数自基准到 12 个月的变化为该研究的第二终点。

[0760] 通过腹部 MRI 测量的肝体积和脾体积 :在基准时和第 25 周及第 51 周对患者进行肝和脾的定量腹部 MRI。肝体积和脾体积自基准到 12 个月的变化为该研究的第二终点。

[0761] 血浆壳三糖苷酶和 CCL19 水平 :在基准访问时和在第 13 周、第 25 周、第 37 周、第 51 周和第 53 周收集血样用于评估血浆壳三糖苷酶和 CCL18 水平。壳三糖苷酶体积和 CCL18 水平自基准到 12 个月的变化为该研究的第三终点。

[0762] 生长速度和 Tanner 分期 :对于 2 至 17 岁的患者,在本文中限定的时间点评价生长。使用在该研究期间在规则的时间点记录的身高和体重测量结果计算生长速度,且使其与 Tanner 分期相关。生长速度和 Tanner 分期自基准到 12 个月的变化为该研究的第三终点。

[0763] 骨骼生长 :在基准和第 51 周时对在 2 至 17 岁之间的患者进行左手和腕部的放射照相以评估骨骼年龄。在 2 至 17 岁的患者中的骨骼生长自基准到 12 个月的变化为该研究的第三终点。

[0764] 额外研究程序

[0765] 骨生物标志物 :仅在基准时,对 ≥ 18 岁的患者进行腰脊柱和股骨颈的 DXA,包括冠状面成像,以测定戈谢有关的局部和全身性骨病。骨质流失和脱矿质也通过测量血清碱性磷酸酶、NTx 和 CTx 评估。从仅在基准时收集用于临床实验室检验的血样获得这些参数的结果。

[0766] 对于 2 至 17 岁的患者,在基准时(与对患者进行肝和脾的 MRI 的同时)获得股骨颈和腰脊柱的 MRI。

[0767] 不出所料在该研究期间任何治疗作用对于这些参数都将是显然的,因此,仅在基准时收集的测量结果用以确立在后续长期开放标签临床研究期间自其监测这些生物标志物的参考点。

[0768] 不良事件

[0769] 不良事件定义 :不良事件(AE)为如由在临床试验的任一期中出现的身体体征、症状和/或实验室变化所指示的解剖学、生理或代谢功能的任何无毒、病理学或无意识的变化,而不管是否被视为研究药物有关。这包括预先存在病状的恶化。自告知同意至在最后剂量的研究药物之后 30 天和/或直到不良事件已经解决/稳定或达到一种结果(无论何者在先)收集不良事件。对于在第 53 周访问之前被中断或退出的患者,跟踪 AE 直至在其最后输注维拉苷酶 α 之后的 30 天。

[0770] AE 包括 :在研究起始时存在的病状的恶化(性质、严重性或频率方面的改变);并发的疾病;药物相互作用;与伴随投药有关或可能与伴随投药有关的事件;异常实验室值(这包括自在研究人员认为临床上重要的正常范围内的基准的显著转变);身体检查、生命体征、体重和 ECG 的临床上显著的异常。

[0771] 另外,AE 也可以包括出乎意料的实验室值,其变得显著超出范围且由研究人员测定为临床上显著的。如果存在出乎意外的溢出值,则将重复实验室检验,直至其恢复到正常或可被解释且患者的安全不在保险范围内。

[0772] 输注有关的不良事件的定义 :输注有关的不良事件定义为如下不良事件 :1) 在输注开始之后的 12 小时期间或 12 小时内开始,和 2) 被判断为可能或或许与研究药物有关。在输注之前发生的其他 AE 以及在输注之前进行的与实验方案限定的检验和评价(例如,实

验室检验、ECG 和身体检查) 相关的 AE 并不定义为输注有关的不良事件。如上定义管理输注有关的不良事件。

[0773] 严重的不良事件定义:严重的 AE (SAE) 为导致任何以下结果的以任何剂量发生的任何 AE:死亡,为危及生命的,需要患者住院治疗,需要现有住院治疗延期、持久或显著的病废/无能和出生缺陷/先天缺陷。

[0774] 可能不导致死亡、危及生命或需要住院治疗的重要医疗事件可被视为 SAE,当根据适当医疗判断,它们可能危害患者且可能需要医疗或外科手术以预防以上所列结果之一。

[0775] 危及生命的 AE 定义为鉴于最初报道使患者处于随着 AE 发生而引起的死亡即时危险中的 AE (即,其不包括使其以更严重的形式出现或可能致死的 AE)。

[0776] 不良事件和严重不良事件的分类:当评价 AE 的严重性时,参考国立癌症研究院通用毒性标准 (NCI CTC) 3.0 版分级量表。如果 AE 没有描述在 NCI CTC 中,则根据以下量表记录严重性。所有 AE/SAE 的严重性在适当 CRF 页面上记录为分别对应于轻微、中度、重度或危及生命的严重性的 1 级、2 级、3 级或 4 级。1 级(轻微)定义为日常活动不受限制。2 级(中度)定义为日常活动受一定程度地限制;3 级(重度)定义为不能实施日常活动;和 4 级(危及生命的)定义为即时死亡危险。

[0777] 不良事件或严重不良事件与盲目研究投药的关系由研究人员根据以下定义测定。“无关”定义为与研究药物无关。“可能有关”定义为在施用研究药物的合理时序下的临床事件/实验室异常,但其也可以由同时发生的疾病或其他药物/化学解释。“或许有关”定义为在施用研究药物的合理时序下的临床事件/实验室异常,不大可能归因于同时发生的疾病或其他药物和化学且其接着是对再挑战的临床上的合理的响应。临床事件/实验室异常的相关性也必须具有一定的生物学似真性,至少是在理论根据上。

[0778] 在严重和重度之间的阐明:术语“重度”常用以描述具体事件的强度(严重性)(如在轻微、中度或重度心肌梗塞中);然而,事件本身可能具有相对较少的医疗意义(诸如重度头痛)。这与“严重”没有不同,其根据通常与造成对生命或功能的威胁的事件通常相关的结果或作用标准。严重(非重度)和因果性用作限定调整报告责任的指导。

[0779] 不良事件监测和观察期:对于该研究的目的,观察期从患者提供签字的告知同意书的时间延伸到该研究的患者最终评估。出于安全目的,最终评估定义为对完成研究且没有选择加入后续长期开放标签的临床研究中的患者或对在第 53 周之前从研究中中断或退出的患者在最后输注之后约 30 天进行的后研究安全评估。对于选择加入后续长期开放标签的临床研究中的患者,监测不良事件直至第 53 周;将到该研究的第 53 周为止还没有解决的不良事件记录在后续长期开放标签的临床研究的患者病史中。

[0780] 统计方法

[0781] 一般统计方法论:意向治疗 (ITT) 患者群定义为接受至少一次输注(全部或部分输注)的所有登记的患者。对所述 ITT 群进行统计数据进行分析。使用描述性统计(n、平均值、中值、最小值、最大值和标准偏差)概述在基准时和在后续研究访问时收集的连续数据。分类数据作为频率和百分数汇总。根据人口统计和基准特征对在 ITT 群中的所有患者提供描述性统计。

[0782] 第二终点(即,临床参数)的分析根据如下所述的非劣性假设。

[0783] 样本量调整:这是安全性试验且难以鉴定该研究的单一第一安全性结果变量。然

而,根据功效参数的选择的样本量适合评估不太常见的副作用。

[0784] 纳入至少 26 个患者提供关于安全性和耐受性的基本信息。根据 TKT025 研究,对于患者在 6 个月的平均变化没有指示不良事件 (AE) 相对于基准的任何恶化。一些患者将具有被解决的 AE,一些患者具有恶化的 AE。假设患者自然变化,则对于单一患者见到恶化的机会为 11%。这 11% 的失效率属于 1 个患者。该试验中所有 26 个患者不具有 SAE 的可能性等于患者 1 不具有 SAE 的可能性乘以患者 2 不具有 SAE 的可能性乘以 (... 等...) 患者 26 不具有 SAE 的可能性。也就是说,在 26 个患者中至少一个患者将显示 AE 相对于基准的恶化的 95% 可能性 [$1 - (.89)^{26} = 0.95$]。换句话说,对于 26 个的样本量来说,当事件的概率为 0.11 时,观察到至少 1 件的概率将为 0.95。Or, 或者,在没有观察到事件时,为了获得 95% 置信区间上对罕见事件的概率的上界 0.11,将需要 26 个的样本量。

[0785] 零假设为对于选择的临床参数 (血红蛋白浓度、血小板计数、肝体积和脾体积) 中的每一个相对于基准 (即,伊米苷酶治疗结束时) 到 12 个月的平均变化在预指定的临床上显著的值内。对于血红蛋白来说自基准到第 53 周的临床上显著的变化定义为变化小于 1gm/dL,且对于血小板计数来说自基准到第 53 周的临床上显著的变化定义为变化 20%。对于正规化肝体积和正规化脾体积,自基准到第 53 周的变化定义为至多增加 15%。26 个患者的样本量评估值根据配对 T 检验的平均值,0.671 的标准偏差,0.05 的双侧 α 水平和 80% 效能。

[0786] 第一分析:评价接受至少一剂全部或部分剂量的研究药物的所有患者的临床安全性和耐受性。列出收集的各患者的生命体征、临床化学和血液学用于安全监测且标记出异常值。对于诸如 AE 的分类变量,列出经历各 AE 的患者的数量和百分数。AE 以事件的严重性汇总。也显示出经历药物有关的 AE 和不被视为与研究药物有关的 AE 的患者的数量和百分数。也汇总了输注有关的不良事件反应和抗维拉昔酶 α 抗体形成率。

[0787] 第一临床变量在于评估每隔一周施用维拉昔酶 α 给临床上对伊米苷酶稳定的患有 I 型戈谢病的患者的安全性。通过在各研究访问时评价生命体征和记载的不良事件 (按类型、频率和严重性) 以及在需求访问时身体检查的进行和实验室评价方面的变化来评估安全性。

[0788] 所有 AE 使用 MedDRA 编码词典编码。AE 汇总通常根据在患者第一次输注研究药物之后发生的所有 AE (治疗突现的)。

[0789] 第二分析:该研究的第二终点为:血红蛋白浓度自基准到 12 个月的变化;血小板计数自基准到 12 个月的变化;根据腹部 MRI 的脾体积自基准到 12 个月的变化 (评估为变化%) (脾体积通过体重正规化);和根据腹部 MRI 的肝体积自基准到 12 个月的变化 (评估为变化%) (肝体积通过体重正规化)。

[0790] 对于各临床活动参数,备选假设在于相对于基准 (即,伊米苷酶治疗结束时) 到 12 个月的平均变化在对待评估参数指定的临床上显著的水平内 (其中血红蛋白相对于基准的总体平均变化在 1g/dL 内,血小板计数在 20% 内且肝体积和脾体积在 15% 内)。这使用对于这些临床参数相对于基准的真实差值的 2-侧 90% 置信区间评估。例如,如果血红蛋白相对于基准的变化的置信区间在 -1 至 1g/dL 的区间内,则推断出维拉昔酶 α 的功效。

[0791] 主办者的期望在于平均血红蛋白浓度经 12 个月的时间基本恒定。例如,对于血红蛋白的第二功效分析,代替使用 90% 的置信区间,可以使用以下成对的统计假设检验,各自

在 0.05 的 α 水平下。

[0792] $H_{01}: \mu_d \leq 1$ 相对 $H_{11}: \mu_d < 1$

[0793] $H_{02}: \mu_d \leq -1$ 相对 $H_{21}: \mu_d > -1$

[0794] 通过拒绝第一零假设 (H_{01}), 支持第一备选假设 (H_{11}), 在 0.05 的显著水平下推断出血红蛋白相对于基准的治疗平均变化小于比基准值高的 1g/dL。通过拒绝第二零假设 (H_{02}), 支持第二备选假设 (H_{21}), 在 0.05 的显著水平下推断出血红蛋白相对于基准的治疗平均变化大于比基准值低的 1g/dL。因为 H_{01} 和 H_{02} 不能同时为真, 所以对于以上成对的假设检验, 总 I 型出错率为 0.05。因此, 通过拒绝两种零假设, 支持备选假设, 在 0.05 的显著水平下推断出治疗 (维拉苷酶 α) 血红蛋白浓度在 -1 至 1g/dL 的区间内。

[0795] 主办者认为置信区间方法将比使用假设检验的响应方法更容易解释。因此, 所述置信区间方法将用于第二推理。

[0796] 第三分析: 该研究的第三终点为: 血浆壳三糖苷酶和 CCL18 水平自基准到 12 个月的变化; 在 2 至 17 岁的患者中骨骼年龄自基准到 12 个月的变化; 及生长速度和 Tanner 分期自基准到 12 个月的变化。

[0797] 在各时间点使用描述性统计概述第三终点 (平均值、中值、标准偏差、最小值和最大值)。对于在该研究期间在基准时和其他时间点收集数据的终点, 检查组内变化。

[0798] 亚组的分析: 特定地对于在 2 至 17 岁之间的患者进行额外分析。并且, 在分析中关于血红蛋白基准值给出对于疾病严重性的考虑因素。

[0799] **结果**

[0800] 将 40 个患者包括在意向治疗 (ITT) 分析中 (表 19) 且 38 个患者 (93%) 完成该分析。1 个患者在接受研究药物之前中断; 在 15U/kg 组中的两个患者中断, 其中 1 个患者是在其用维拉苷酶 α 第一次输注期间的类过敏反应而中断, 且 1 个患者是在第 31 周由于在戈谢有关的症状方面缺乏察觉的改善而中断。

[0801] 患者接受与其预先伊米苷酶方案相同单位数的维拉苷酶 α 。中值预先伊米苷酶使用为 67 个月 (范围: 22-192 个月)。维拉苷酶 α 剂量归合成四个范围: ≤ 22.5 U/kg ($n = 14$)、22.5-37.5U/kg ($n = 12$)、37.5-52.5U/kg ($n = 7$) 和 >52.5 U/kg ($n = 7$)。如果患者被证明了血红蛋白或血小板计数的临床上显著的变化, 则研究者选择增加维拉苷酶 α 剂量 (到每隔一周的最大值 60U/kg)。在研究期间不进行剂量调节。

[0802] 表 19 在基准时 TKT034 患者特征

[0803]

	ITT 群(n = 40)
年龄, 平均(范围)	36 岁(9-71 岁); 25% <18 年
性别, n (%)	18 (45%) 男性/22 (55%) 女性
临床参数, 中值(范围)	
血红蛋白	13.8 g/dL (10.4-16.5 g/dL)
血小板计数	$162 \times 10^9/L$ ($29-399 \times 10^9/L$)
肝体积*	0.8 MN (0.6-1.6 MN)
脾体积**	2.5 MN (1.0-16.0 MN)
生物标志物, 中值	
壳三糖苷酶	3071.3 nmol/mL/h
C-C 基元配体 18 趋化因子 (CCL18)	325.0 ng/mL
预先伊米苷酶使用, 中值(范围)	67 个月(22-192 个月)
在接受维拉苷酶 α 之前抗伊米苷酶抗体为阳性, n (%)	3 (8%)

[0804] * 正常肝体积为体重的 2.5%。

[0805] ** 在具有完整脾的 36 个患者中:在登记之前对 4 个患者进行脾切除。正常脾体积为体重的 0.2%。MN = 正常的倍数。

[0806] 临床参数在治疗水平下维持 1 年(表 20)。

[0807] 表 20

[0808]

	n	基准中值	自基准到 12 个月的平均变化或变化%	90% CI	临床上显著的截止点
血红蛋白浓度(g/dL)	40	10.8	-0.1	-0.3, 0.1	-1, 1
血小板计数($\times 10^9/L$)	40	162	7.0%	0.5%, 13.5%	-20%, 20%

[0809]

正规化肝体积(体重%)	40	1.9	0.0%	-2.6%, 2.6%	-15%, 15%
正规化脾体积(体重%)	36	0.5	-5.6%	-10.8%, -0.4%	-15%, 15%

[0810] 维拉苷酶 α 耐受性通常良好,大多数不良事件(AE)为轻微或中度严重性(表21)。最常报道的AE为鼻咽炎(8/40个患者)、关节痛(9/40个患者)和头痛(12/40个患者)。总体上,40个患者中有11个(28%)经历被视为可能或或许与研究药物有关的AE,这些事件中的大多数被视为与输注有关。患者没有经历危及生命的AE。一件重度不良事件被视为或许与治疗有关且出现在具有重度过敏性反应的患者中。在输注时和两周以后,检验该患者的所有4种同种型(IgE、IgM、IgG、IgA)(包括中和抗体)为阴性。1个患者(在15U/kg组中)经历导致中断的类过敏性反应;其他患者没有由于AE而被中断。患者没有发展出维拉苷酶 α 的IgG抗体,包括在筛选时检验抗伊米苷酶 α 抗体为阳性的三个患者。

[0811] 表21 TKT034 安全性汇总

[0812]

	患者, n (%)				
	总计 (n = 40)	15 U/kg (n = 15)	30 U/kg (n = 12)	45 U/kg (n = 6)	60 U/kg (n = 7)
经历 ≥ 1 件治疗突现的 AE*	34 (85)	12 (80)	11 (92)	5 (83)	6 (86)
经历 ≥ 1 件药物有关的 AE	11 (28)	6 (40)	3 (25)	1 (17)	1 (14)
经历 ≥ 1 件输注有关的 AE	9 (23)	6 (40)	2 (17)	0	1 (14)
经历 ≥ 1 件重度 AE	5 (13)	0	2 (17)	1 (17)	2 (29)
经历 ≥ 1 件危及生命的 AE	0	0	0	0	0
经历 ≥ 1 件严重的 AE	4 (10)	1 (7)	1 (8)	2 (33)	0
由于 AE 引起中断	1 (3)	1 (7)	0	0	0
死亡	0	0	0	0	0
发展出的抗维拉苷酶 α 抗体	0	0	0	0	0

[0813] * 治疗突现的AE定义为在第一次输注的那天或那天之后直至患者最后输注之后的30天发生的AE。

[0814] 输注有关的AE定义为如下AE:1)在输注开始之后的12小时期间或12小时内开始,和2)被判断为可能或或许与研究药物有关。

[0815] 各患者的前三次输注在临床地点施用,此后没有经历药物有关的严重AE或输注有关的AE的患者符合在家接受后续输注的条件。在研究期间,40个合格患者中的25个(63%)接受至少一次家庭疗法,10个患者(67%)在15U/kg组中,6个患者(50%)在30U/kg组中,5个患者(83%)在45U/kg组中且4个患者(57%)在60U/kg组中。

[0816] 对于血红蛋白浓度,相对于基准的平均变化为 -0.1g/dL ,其中 90% 置信区间为 -0.3 至 0.1g/dL ,在 $\pm 1\text{g/dL}$ 的预定功效标准内。对于血小板计数,相对于基准的变化%为 $+7.0\%$,其中 90% 置信区间为 0.5 至 13.5% ,在 $\pm 20\%$ 的预定功效标准内。对于肝体积,相对于基准的变化%为 -0.0% ,其中 90% 置信区间为 -2.6 至 2.6% ,在 $\pm 15\%$ 的预定功效标准内。对于脾体积,相对于基准的变化%为 -5.6% ,其中 90% 置信区间为 -10.8 至 -0.4% ,在 $\pm 15\%$ 的预定功效标准内。如通过对于临床上显著的变化的预指定的功效标准所证明,血红蛋白浓度、血小板计数和肝体积及脾体积在治疗水平下维持至维拉苷酶 α 治疗的 1 年。血红蛋白的平均变化及血小板计数和器官体积的平均变化%示于图 15- 图 18 中。对于各参数,在四个计量组中见到类似结果。

[0817] 血浆壳三糖苷酶和血浆 CCL18 的变化%示于图 19 和图 20 中。经 12 个月的治疗期维持且可能降低两种生物标志物的水平。

[0818] 实施例 5 :HGT-GCB-058 研究

[0819] 概述

[0820] HGT-GCB-058 为用以观察维拉苷酶 α 在被新近诊断(治疗雏期)或从伊米苷酶转换到维拉苷酶 α 的患有 1 型戈谢病的患者中的安全性的多中心开放标签治疗研究。该研究设计用于两岁或更大的男性或女性患者。维拉苷酶通过 1 小时静脉内(IV) 输注每隔一周(EOW) 以 $15\text{--}60\text{U/kg}$ 的剂量施用。患者接受与其预先伊米苷酶剂量相同单位数的维拉苷酶 α (EOW 接受 $<15\text{U/kg}$ 伊米苷酶的患者接受 15U/kg 维拉苷酶 α)。输注速率的最大值为 1U/kg/min 。

[0821] 患者和方法

[0822] 开始 HGT-GCB-058 以提供给可能另外受限或由于供应限制而无法使用伊米苷酶的患者备选的治疗选择。第一终点为观察维拉苷酶 α 的安全性。

[0823] 对于 HGT-GCB-058,在第一地点开始的 3 个月内,跨越美国的 20 个临床地点登记患者。在 2009 年 9 月 1 日和 2010 年 1 月 31 日之间,超过 150 个患者登记到 HGT-GCB-058 上且接受至少一次维拉苷酶 α 输注。仅 3 个患者为治疗雏期,所有其他患者先前用伊米苷酶治疗。

[0824] 初步安全性结果

[0825] 中断: $<10\%$; 同意和其他原因退出 ($<10\%$); 经历包括 SAE 的 AE ($<2\%$)。

[0826] 治疗突现的不良事件 (TE-AE):

[0827] 治疗雏期患者 ($n = 3$): 没有严重的 AE; 没有重度 AE; 2 个患者经历中度 AE: 头痛(中度)和背痛(中度)(输注有关 - 可能有关)

[0828] 先前用伊米苷酶治疗 ($n > 150$): 35.8% - 至少一件 TE-AE; 18.2% - 至少一件可能/或许有关的 TE-AE; 13.8% - 至少一件输注有关的反应; $<1\%$ - 无关的严重 AE (69 岁的女性经历需要住院治疗的重度脑血管意外); 3.1% - 至少一件重度 AE: 关节痛(无关)、疲劳(或许有关)、骨痛(无关)、手足痛(无关)、白细胞减少(可能有关)、脑血管意外(无关)。

[0829] 实施例 6 :比较研究

[0830] 该研究的目的在于比较 **Ceredase®**、**Cerezyme®**、维拉苷酶 α 、Genz-112638 和 **Zavesca®** 在治疗 1 型戈谢病中的功效。在治疗 6 个月、9 个月或 12 个月

之后测量血红蛋白浓度、血小板计数、肝体积和脾体积。

[0831] 剂量:酶替代疗法(ERT):60U/kg EOW;Genz112638:50mg 和 100mg BID;Zavesca:100mg TID

[0832] 男性与女性的比率 Cerezyme(8 比 7);Ceredase(3 比 2);维拉昔酶 I/II 期 TKT025 研究(5 比 7);维拉昔酶-TKT032 研究-45U/kg 剂量(8 比 5);维拉昔酶-TKT032 研究-60U/kg 剂量(7 比 5);Zavesca(1 比 1);Genz-112638(3 比 4)

[0833] 纳入标准:

[0834] Cerezyme 和 Ceredase:贫血和脾肿大

[0835] 维拉昔酶 TKT025:贫血和血小板减少

[0836] Genz-112638:贫血、血栓形成和脾肿大

[0837] Zavesca 剂量:器官异常肿大和 <100/nL P1 或 <11.5Hb

[0838] 维拉昔酶 HGT-GCB-039 和 TKT032:贫血和 1 项其他参数表现

[0839] 在雏期患者中的基准比较示于表 22 中。

[0840] 表 22

[0841]

	血红蛋白 (g/dL)	血小板 (细胞/nL)	肝体积 MN (L)	脾体积 MN (L)
Ceredase®	10.8	71	1.83	24
Cerezyme®	10.7	72	1.65	19
维拉昔酶 (I/II 期 TKT025)	11.6	57	1.95 (2.4L)	19 (2.1L)
维拉昔酶 (TKT032-45 U/kg 剂量)	10.9	58	3.50 (体重%)	2.90 (体重%)
维拉昔酶 (TKT032) 60 U/kg 剂量	10.83	66	3.60 (体重%)	2.90 (体重%)
Genz-112638	11	70	1.7	19
Zavesca®	11.9	77	(2.4L)	(1.6L)

[0842] MN = 正常的倍数

[0843] 来自用 **Ceredase®**、**Cerezyme®**、维拉昔酶 a 或 Genz-112638 治疗 6 个月的患者比较结果示于表 23 中。

[0844] 表 23

[0845]

	平均脾体积减小 (%)	平均肝体积减小 (%)	平均血小板增加 (%) [实际]	平均血红蛋白增加 (g/dL)
Ceredase®¹ 6mo	32.1 ± 8.8	11.4 ± 9.4	34 [16/nL]	1.60
Cerezyme®¹ 6mo	37.3 ± 13.6	13.4 ± 13.1	22 [23/nL]	1.82
维拉昔酶² 6mo (n = 11)	41.3 ± 9.8	14.7 ± 12.0	38 [23.4 ± 24.6/nL]	1.92 ± 0.82
Genz-112638³ 6mo (n = 17-21)	27	7	18 [12.5/nL]	0.9

[0846] 1 Cerezyme 和 Ceredase 资料如由 Grabowski 等, (1995) Ann. Intern. Med. 122, 33-39 报道。

[0847] -Cerezyme 和 Ceredase 的血小板计数平均增加 (以细胞 /nL 计) 来自 Cerezyme SBA。

[0848] 2 维拉昔酶 α I/II 期结果

[0849] 3 Oppenheimer 分析员报告:2008 年 5 月 8 日, 来自 Genzyme Analyst Day 2008

[0850] 4 由基准乘以变化%计算

[0851] ERT 以 60U/kg EOW 施用, Genz112638 以 50mg 和 100mg BID 施用

[0852] 来自用 **Ceredase®**、**Cerezyme®**、维拉昔酶 α 或 Genz-112638 治疗 9 个月和 12 个月的患者的比较结果示于表 24 中。

[0853] 表 24

[0854]

	平均脾体积减小 (%)	平均肝体积减小 (%)	平均血小板增加 (%) [实际]	平均血红蛋白增加 (g/dL)
Ceredase¹ 9mo (n = 15)	42.2 ± 6.9	16.4 ± 8.4	53.2 [25.1/nL]	2.28
Cerezyme¹ 9mo (n = 15)	47.1 ± 13.7	21.4 ± 10.8	43.5 [30.9/nL]	2.54
维拉昔酶² 9mo (n = 11)	49.5 ± 12.6	18.2 ± 8.2	67.6 [40.6 ± 30.7/nL]	2.24 ± 0.89
维拉昔酶³ 1yr (n = 10)	不可用	不可用	95.3 ± 21.2	2.35 ± 0.3

[0855]

			[54.9 ± 11.1]	
维拉昔酶 ¹ 1yr (n = 12)	50.4 ± 5.3	17.0 ± 4.5	65.9 ± 16.9 [50.88 ± 12.2]	2.43 ± 0.3
维拉昔酶 ¹ 1yr (n = 10)			40.92	2.44
Genz-1126383 ¹ 1yr (n = 40 11-13)		17	40 [26/nL4]	1.6

[0856] 1 Cerezyme 和 Ceredase 9-Mo 资料如由 Grabowski 等, (1995) Ann. Intern. Med. 122, 33-39 报道。剂量为每隔一周 (EOW) 60U/kg。

[0857] - 平均血小板计数增加 (以细胞 /nL 计) 由原始数据计算

[0858] 2 维拉昔酶 α 9-Mo 数据来自 TKT025 (I/II 期) -60U/kg 剂量 EOW

[0859] 3 维拉昔酶 α 12-Mo 数据来自 TKT025 扩展 -60U/kg 剂量 EOW

[0860] 4 维拉昔酶 α 12-Mo 数据来自 TKT032-60U/kg 剂量 EOW

[0861] 5 维拉昔酶 afa 12 Mo 数据来自 TKT032-45U/kg 剂量 EOW

[0862] 3 WORLD 符合外观, 2009 年 2 月 20 日 - 以 50mg 和 100mg BID 施用

[0863] 结论: 该研究显示, 根据在开始治疗之后 6 个月、9 个月或 12 个月的血红蛋白浓度、血小板计数、肝体积和脾体积的测量结果, 在治疗 1 型戈谢病方面维拉昔酶 α 至少与 **Ceredase®**、**Cerezyme®**、Genz-112638 和 **Zavesca®** 一样有效。

[0864] 实施例 7: 抗药物抗体 (ADA) 测定

[0865] 概述

[0866] 治疗蛋白质的抗体的发展可以影响患者安全、功效和药物动力学。发展并批准了一组抗药物抗体 (ADA) 和中和抗体 (NAbs) 测定以评估并比较在三种维拉昔酶 α III 期研究之一中接受维拉昔酶 α 或伊米昔酶的患者抗体响应。

[0867] 诸如酶替代疗法 (ERT) 的生物学疗法的潜在免疫原性的评价通过以下步骤进行:

[0868] 1. 筛选 ERT 的抗体

[0869] i. 允许假阳性

[0870] ii. 对所有同种型的广泛特异性

[0871] iii. 对药物存在的耐受性

[0872] 2. 验证性步骤

[0873] i. 排除假阳性

[0874] ii. 同种型特异性

[0875] 3. 滴度步骤

[0876] i. 相对浓度

[0877] 4. 对中和抗体的检验

[0878] i. 体外活性

[0879] ii. 体外细胞吸收

[0880] 该评价可以对任何 ERT 进行。

[0881] 该评价的实例示于图 21 中。

[0882] 免疫测定方法

[0883] 抗维拉苷酶 α 和抗伊米苷酶抗体使用桥免疫测定和免疫球蛋白 (Ig) 亚类特异性间接免疫测定同等评估,所有都根据电致化学发光平台以及 RIP 测定。桥电致化学发光免疫测定检测所有免疫球蛋白亚类且被视为抗体筛选测定。Ig 亚类电致化学发光免疫测定为 IgA、IgM 和 IgE 抗体的验证性测定,而 RIP 测定验证 IgG 抗体的存在。抗体筛选测定和 IgG 测定为校准的、定量的且利用人类抗体阳性对照。IgA、IgM 和 IgE 测定为半定量的且利用混合 (人类-绵羊) 阳性对照。

[0884] 所有抗维拉苷酶 α 免疫测定和抗伊米苷酶免疫测定为相同的,包括正截止标准,不同之处在于使用维拉苷酶 α 或伊米苷酶来询问样品。这些测定为高吞吐量的,提供增加的检测表面积,允许使用具有最小非特异性结合的高血清样品浓度且检测所有抗体亚类。

[0885] 抗体筛选测定

[0886] 如在图 22 中所示,抗药物抗体筛选可以使用电致化学发光 (ECL) 免疫测定进行。

[0887] 抗维拉苷酶 α (抗伊米苷酶) 抗体使用在抗生蛋白链菌素涂覆的微孔板上固定的生物素结合的维拉苷酶 α (或伊米苷酶) 检测。固定的生物素化的维拉苷酶 α (或伊米苷酶) 捕获在患者血清中存在的抗维拉苷酶 α (抗伊米苷酶) 抗体且未结合的蛋白质通过洗涤除去。将钆配合物标记的维拉苷酶 α (或伊米苷酶) 加到各微孔中,导致与结合的抗维拉苷酶 α (抗伊米苷酶) 抗体形成复合物。这接着是第二洗涤步骤,其中除去未结合的标记蛋白质。

[0888] 在微孔表面附近结合的标记分子在通过电致化学发光反应触发的过程中发光,这通过 MSD SECTOR 成像器 2400 仪测量 (http://www.mesoscale.com/CatalogSystemWeb/WebRoot/products/imager_2400.aspx)。将维拉苷酶 α 和伊米苷酶的具有交叉反应性的小鼠单克隆抗体用作在各测定板内的定标器且将可与维拉苷酶 α 交叉反应的人类抗伊米苷酶抗体用作正测定对照。在检验样品中抗维拉苷酶 α (抗伊米苷酶) 抗体的浓度通过将未知的测量的电致化学发光信号内插在校准曲线上来评估。

[0889] 检验最少 67 个戈谢病患者基准以设定这些测定的抗体阳性切割点。该检验设计包括经至少 14 天的时间使用不同板批号检验重复样品的至少 3 种分析。使用至少三批不同的微孔板。对于各测定总共 1269 次测定随机使用两种可用 MSD 仪器。将抗维拉苷酶 α (抗伊米苷酶) 抗体的测定切割点确立为如在 Mire-Sluis, AR 等, Journal of Immunological Methods 289 (2004), 第 1-16 页中推荐获得的电致化学发光值的平均值加 1.645 标准偏差。对于抗维拉苷酶 α 和抗伊米苷酶抗体的测定敏感性分别评估为 33.4ng/mL 和 65.6ng/mL。

[0890] 该测定的筛选特征 (使用维拉苷酶作为药物) 示于表 25 中。

[0891] 各参数的规格显示高敏感性且批准可重现的 ADA 筛选测定来评估在接受维拉苷酶 α 或伊米苷酶的患者中的抗体响应。

[0892] 表 25

[0893]

参数	规格
不精确性, % RSD	
内部测定	≤ 6.2
中间测定	≤ 8.5
准确性, %	93.5-107.7
敏感性, 1 ng/mL	100
LOD, 2 ng/mL	5
LOQ, ng/mL	15
阳性对照	人类和小鼠 ADA
定标器	小鼠单克隆 ADA

- [0894] ADA = 抗药物抗体 ;LOD = 检测极限 ;LOQ = 量化极限 ;RSD = 相对标准偏差
- [0895] 维拉昔酶的筛选测定剂量响应曲线示于图 23 中。
- [0896] 在 **BIACORE®** 平台上单克隆 ADA 的亲合性和结合动力学的实例示于表 26 中。对于 ADA 测定定标器观察到了类似配体亲和性和结合动力学。
- [0897] 表 26
- [0898]

配体	ka(1/Ms)	kd(1/s)	KD(M)
维拉昔酶 α	9.2E+05	2.7E-04	3.0E-10
伊米昔酶	2.8E+06	1.0E-04	3.7E-10
生物素化维拉昔酶 α	4.0E+05	2.2E-04	5.4E-10
生物素化伊米昔酶	2.0E+06	9.5E-04	4.8E-10

- [0899] 放射免疫沉淀测定
- [0900] 如果在样品中检测到抗药物抗体,则可以进行用以测定抗体的 Ig 同种型的验证性测定。使用放射免疫沉淀检测免疫球蛋白 G(IgG) 抗体。放射免疫沉淀 (RIP) 测定示于图 24 中。
- [0901] 在放射免疫沉淀测定中,患者血清中存在的抗维拉昔酶 α (抗伊米昔酶) IgG 抗体在溶解期结合 ¹²⁵I- 维拉昔酶 α (或伊米昔酶) 且形成使用蛋白质 G 微柱捕获的抗原 / 抗体复合物。洗涤所述微柱以除去游离标记物且在 γ 计数器中直接量化。保留在所述微柱中的放射性计数与在检验样品中的抗维拉昔酶 α (抗伊米昔酶) IgG 抗体的浓度成比例。在检验样品中抗维拉昔酶 α (抗伊米昔酶) IgG 抗体的浓度使用与以上讨论相同的单克隆抗体定标器自校准曲线评估。在该测定中使用与上述相同的人类抗体阳性对照。

[0902] 使用充分表征的已知浓度的抗体,最小平方法适合根据高纯度单克隆抗体的校准曲线,提供用于计算在分析测定中的不可靠性的可靠且一致的方法。该工具允许自试剂放射性标记衰减、放射性自分解和 / 或测定处理变量发生的计数的中间测定变化以及允许非特异性结合的变化和可以随时间发生的测定读出数据的变化的切割点的正规化。

[0903] 检验总共 59 个戈谢病患者基准以设定该测定的抗体阳性切割点。抗维拉苷酶 α (伊米苷酶) IgG 的测定切割点如上所述确立且在 Mire-Sluis 等 2 中推荐。抗维拉苷酶 α IgG 和抗伊米苷酶 IgG 测定的测定敏感性分别评估为 28.3 ng/mL 和 64.5 ng/mL。

[0904] 用这样的测定使用维拉苷酶作为药物获得的结果的实例示于表 27 中。

[0905] 批准高敏感性且可重现的 IgG ADA 验证性测定来评估在接受维拉苷酶 α 或伊米苷酶的患者中的抗体响应。

[0906] 表 27

[0907]

参数	规格
不精确性, % RSD	
内部测定	≤ 8.7
中间测定	≤ 12.0
准确性, %	90.5-132.8
敏感性, 1 ng/mL	80
LOD, 2 ng/mL	4
LOQ, ng/mL	13
阳性对照	人类和绵羊 ADA
定标器	小鼠单克隆 ADA

[0908] ADA = 抗药物抗体 ; LOD = 检测极限 ; LOQ = 量化极限 ; RSD = 相对标准偏差

[0909] 对于 RIP 测定使用维拉苷酶获得的剂量响应曲线的实例示于图 25 中。

[0910] 间接电致化学发光免疫测定

[0911] 平行于 IgG 抗体的筛选,进行测定以筛选 IgE 抗体的存在。也可以进行测定来检测 IgA 和 IgM 抗体的存在。

[0912] 使用 ECL 测定检测 IgA、IgM 和 IgE ADA。这种检测的实施例示于图 26 中。

[0913] 使用间接电致化学发光免疫测定分析抗维拉苷酶 α (伊米苷酶) 抗体的 Ig 亚类。通过在抗生蛋白链菌素涂覆的微孔板上固定生物素化维拉苷酶 α (伊米苷酶) 检测在血清中的抗体。将稀释的血清样品加到固定的维拉苷酶 α (伊米苷酶) 中,其捕获在样品中存在的任何抗维拉苷酶 α (抗伊米苷酶) 抗体。在样品孵育之后,洗涤微孔以除去未结合的蛋白质。接着,单独加入相对 IgA、IgM 或 IgE 的钆配合物标记的抗人类二次抗体且将其进一步孵育导致与任何结合的抗维拉苷酶 α 或伊米苷酶抗体形成 Ig 种类特异性复合物。这接着是另一洗涤步骤,其中除去未结合的标记的二次抗体。在微孔表面附近结合的标记分子随后如上所述发光。

[0914] 制备人造抗体阳性对照用于这些测定,因为不可得到抗维拉苷酶 α (抗伊米苷酶) IgA、IgM 或 IgE 抗体。人类 IgA⁻、IgM⁻ 和 IgE⁻ 抗体混合物通过化学交联纯化的非特异性人类 IgA、IgM 或 IgE 碎片到在用维拉苷酶 α 超免疫的绵羊中产生且可与伊米苷酶交叉

反应的抗体合成。IgA-、IgM- 和 IgE- 抗体混合体因此经绵羊抗体结构域与维拉昔酶 α (或伊米昔酶) 结合, 且分别使用相对人类 IgA、IgM 或 IgE 结构域的钆配合物标志的抗人类二次抗体检测。

[0915] 如上所述且在 Mire-Sluis 等²中推荐确立抗维拉昔酶 α (伊米昔酶) IgA、IgM 和 IgE 的测定切割点。抗维拉昔酶 α IgE 和抗伊米昔酶 IgE 测定的测定敏感性分别评估为 10.6ng/mL 和 11.0ng/mL。对于抗维拉昔酶 α (伊米昔酶) IgA 和 IgM 抗体, 除了时间点信号与预输注基准信号的比率大于或等于 2.0 以外, 必须符合测定阳性切割点。

[0916] 对于这些测定, 可以使用混合阳性对照。例如:

[0917] - 处理绵羊 ADA IgG 和人类 IgA、IgM 和 IgE 以产生吡啶硫醇活化的蛋白质 (参见 Gu M.L.、Feng S.L. 和 Glenn J.K. Development of an animal-human antibody complex for use as a control in ELISA. J. Pharmaceutical and Biomedical Analysis, 32(2003), 523-529)

[0918] - 降低并淡化活化的 IgA、IgM 和 IgE

[0919] - 将活化的 IgG 与降低的 IgA、IgM 或 IgE 混合

[0920] - 形成的混合抗体通过尺寸排阻色谱和 ECL ELISA 表征

[0921] - 获得 IgA 和 IgM 的人类 / 绵羊摩尔比为 5 且 IgE 的人类 / 绵羊摩尔比为 2

[0922] IgA、IgM 和 IgE 验证性测定特征的实例 (当将维拉昔酶用作药物时) 示于表 28 中。

[0923] 批准高敏感性且可重现的 IgA、IgM 和 IgE ADA 同种型测定来评估在接受维拉昔酶 α 或伊米昔酶的患者中的抗体响应。

[0924] 表 28

[0925]

参数 ¹	IgA 测定规格	IgM 测定规格	IgE 测定规格
不精确性, % RSD			
内部测定	< 3.1	< 6.2	< 6.6
中间测定	< 4.8	< 3.7	< 13.3
特异性	α -链 Fc	μ -链 Fc	ϵ -链 Fc
线性度, ¹ ng/mL	156-2000	156-2500	156-10000
LOD, ¹ ECL 计数	260	460	240
阳性对照 ³	混合 ADA	混合 ADA	混合 ADA

[0926] ¹根据混合对照响应; ²阳性切割点; 至基准的时间点的比率必须 ≥ 2.0 ; ³人类 / 绵羊 ADA 混合对照; ⁴批准高敏感性且可重现的 IgA、IgM 和 IgE ADA 同种型测定来评估在接受维拉昔酶 α 或伊米昔酶的患者中的抗体响应。

[0927] ADA = 抗药物抗体; LOD = 检测极限; LOQ = 量化极限; RSD = 相对标准偏差

[0928] 体外酶活性的抗体抑制

[0929] 使用检测并量化抑制维拉昔酶 α (伊米昔酶) 活性的抗体的测定检验通过抗维拉昔酶 α (伊米昔酶) 抗体实现的体外酶活性的抑制。该方法根据比色活性测定, 其测量维拉昔酶 α (伊米昔酶) 水解合成底物 4-硝基苯基- β -D-果聚糖为对硝基苯酚和 D-果聚

糖的能力。

[0930] 在 37℃ 下将抗维拉苷酶 α (伊米苷酶) 抗体阳性血清样品分别用固定量的维拉苷酶 α 或伊米苷酶预孵育 30 分钟。将已知抑制维拉苷酶 α 和伊米苷酶体外活性的绵羊多克隆抗体用作阳性对照。随后加入 4-硝基苯基- β -D-果聚糖底物溶液且在 37℃ 下将其用血清样品 / 酶混合物孵育 1 小时。酶促反应通过加入甘氨酸 / 碳酸钠缓冲剂 (pH 10.7) 停止且在 405nm 的吸收波长下测量产物 (对硝基苯酚)。1 单位的维拉苷酶 α (伊米苷酶) 活性定义为在 37℃ 下在 1 分钟内水解 1 μ mol 的底物 4-硝基苯基- β -D-果聚糖需要的酶的量。酶活性通过比较在相同测定板中测量的在试验样品和测定对照物中的释放的对硝基苯酚量化为对硝基苯酚的校准曲线。检验样品的结果相对于在缺乏血清样品的情况下测量的维拉苷酶 α (伊米苷酶) 的活性表达且报道为抑制 %。

[0931] 测定切割点由个别健康人类供体血清 (N = 52) 和酶替代疗法维期戈谢病患者 (N = 35) 测定。维拉苷酶 α 和伊米苷酶中和抗体测定的切割点定义为根据这些 87 个样本计抑制 >20.0 %。因此, 如果观察到的抑制水平 $\leq$ 20.0 %, 则将患者样本的抑制抗体视为阴性, 且如果观察到的抑制水平 >20.0 %, 则将患者样本的抑制抗体视为阳性。

[0932] 体外基于细胞的测定

[0933] 使用体外基于细胞的测定来评价抗药物抗体, 从而测定抗体是否为中和抗体。

[0934] 表 29 示出抗药物抗体的中和水平的测定特征的规格 (将维拉苷酶用作药物)。

[0935] 表 29

[0936]

参数	规格
不精确性, % RSD	
内部测定	≤ 12.9
中间测定	≤ 6.1
准确性, %	86.7-92.7
线性度, mU/mL	0.3-33.3
阳性切割点, ¹ 抑制%	>20
LOD, mU/mL	0.3

[0937]

LOQ, mU/mL	1.0
阳性对照	人类和绵羊 ADA

[0938] ¹n = 104NHS 和 70 种 ERT 维期戈谢血清

[0939] LOD = 检测极限 ; LOQ = 量化极限 ; RSD = 相对标准偏差

[0940] 关于在诸如对戈谢病 ERT 的 ERT 中的中和抗体, 考虑的要点包括 :

[0941] ●受体介导的细胞吸收对于治疗剂的体内功能是关键

[0942] ●由 ADA 引起的受体结合干扰可以妨碍酶运输

[0943] ●细胞吸收的抑制是评估成功 ERT 的重要工具, 因为

[0944] ●其密切模仿 NAb 可以通过其在体内施加作用的机制, 和

[0945] ●NAb 可以降低或消除治疗剂的生物活性

[0946] ●基于细胞的测定提供评价 NAb 的最适当的生物模型

[0947] 评估来自在戈谢病的临床试验中进行 ERT 的患者的样品的维拉苷酶或伊米苷酶的中和抗体。结果示于表 30 中。

[0948] 表 30

[0949]

患者 ID	治疗	伊米苷酶吸收的抑制, %平均值				维拉苷酶α吸收的抑制, %平均值			
		访问周				访问周			
		0	13	19	41	0	13	19	41
032-191-0002	维拉苷酶	NS ²				NS			
039-009-0001	伊米苷酶								
039-194-0001	伊米苷酶								
039-194-0002	伊米苷酶								
039-167-0001	伊米苷酶	NT3	38	38	33	NT	4	5	5
034-027-0002	伊米苷酶 ¹	NS	NT ³			NS	NT		
034-154-0001	伊米苷酶 ¹	5				10			
034-164-0001	伊米苷酶 ¹	38				10			
在两个 ADA-阳性患者中观察到伊米苷酶吸收的显著的基于细胞的抑制									
在检验的任何 ADA-阳性患者中可以忽略或没有观察到维拉苷酶α吸收的基于细胞的抑制									

[0950] ¹先前接受伊米苷酶治疗且在试验开始时转换到维拉苷酶 α ; ²不显著 (小于量化极限) ; ³至今没有检验 ; 对于抗维拉苷酶 α 抗体为阴性的时间点

[0951] 体外基于细胞的测定 I

[0952] 主要目的 : 进行实验以测定人类抗体与维拉苷酶 α 和 / 或伊米苷酶反应以通过工程化以表达 CD206 的人类细胞系 (HT1080) 抑制 (阻断或中和) 重组酶的 CD206 介导的吸收的能力, 和比较在这方面响应伊米苷酶产生的抗药物抗体 (ADA) 与响应维拉苷酶 α 产生的抗药物抗体 (ADA) 。假设为在维拉苷酶 α 和伊米苷酶之间存在抗原差异 ; 关于 ADA 对治疗剂的细胞结合、细胞内化和 / 或细胞内运输的功能作用, 这些表位将区别维拉苷酶 α 与伊米苷酶。

[0953] 材料和设备 :

[0954] 关键材料

[0955] 1. 细胞系 MRC1-18 源自于 HT1080 系且用人类 CD206 (巨噬细胞甘露糖受体 ; MMR, 也称作 MRC1, 1 型甘露糖受体 C) 稳定转染。HT1080 (saf) 细胞通过电穿孔并直接涂到 96- 孔板中而用携带编码 MMR 的基因的表达载体 (从人类肝 cDNA 文库中分离) 转染。稳定的克隆使用含有 0. 4mg/mL G418 的培养基选择。MRC1 表达使用 FITC 抗 MRC1 染色和分析通过荧光位移分析。MMR 在 MRC1-18 上的表达通过用抗 -MMR Ab 表面染色而证实。另外, MRC1-18 通过免疫染色和流式细胞术确定 Fc (γ) 受体的表达为阴性。

[0956] 2. Alexa **FLUOR**® 488 结合的维拉苷酶 α 和伊米苷酶 : 维拉苷酶 α 或伊米苷酶

根据制造商实验方案 (Molecular Probes, 目录号 A 10235) 使用 Alexa **FLUOR®** 488 蛋白质标记试剂盒与 Alexa **FLUOR®** 488 结合。

[0957] 3. 来自临床试验 TKT-032、TKT-034 和 HGT-GCB-039 的伊米苷酶或维拉苷酶 α ADA- 阳性患者血清 (见表 31, 样品 ID)

[0958] 4. 测定阳性对照 (PC) : 在正常人类血清中的 250 μ g/mL 纯化多克隆绵羊抗维拉苷酶 α 抗体 (G140) (NHS ;BRH127439)

[0959] 5. 阴性对照样品 : 来自正常健康供体的人类血清样品 (Bioreclamation, 目录号 HMSM, BRH127438, BRH127439) 或来自登记在临床试验 TKT-032 (N = 25) 中的患者的基准血清样品。

[0960] 6. 聚甘露糖 :Sigma 目录号 M7054

[0961] 7. D- 甘露糖 -6- 磷酸 :Sigma 目录号 M3655

[0962] 8. 生长培养基 :50 % CD-CHO (Invitrogen 目录号 10743) 和 50 % CD-293 (Invitrogen 目录号 11913), 补充有 4mM L- 谷氨酰胺 (Invitrogen 目录号 25230) 和 0.4mg/mL Geneticin (G418, Invitrogen 目录号 11811-031)

[0963] 9. 0.05% 胰蛋白酶 -EDTA :Invitrogen 目录号 25300

[0964] 10. 洗涤缓冲液 :PBS/0.5% BSA

[0965] 11. BD 细胞计数机构和跟踪珠粒 :BD Bioscience 目录号 641319

[0966] 设备的部分目录

[0967] 1. 具有 5% CO₂ 的 37°C 孵育箱 :Forma Scientific Model 3033

[0968] 2. 离心机 :Thermo Scientific Model Sorvall Legend T+

[0969] 3. 细胞计数器 :Mexcelom Bioscience LLC, Model Cellometer Auto T 4

[0970] 4. 流式细胞仪 :BD Bioscience, FACSCanto II

[0971] 方法 :通过抗体筛选和验证性测定检验抗药物抗体为阳性的患者血清利用工程化以表达人类巨噬细胞甘露糖受体 (MMR) 的 HT1080 细胞系通过体外细胞吸收测定进一步检查。简要地讲, MRC1-18 细胞保持在补充有 0.5mg/mL G418 的 CD 培养基中。对于各测定, 在平底 96- 孔板中加入在具有 G418 的 CD 培养基中的 1.5×10^5 细胞 / 孔, 且在 37°C 下将其用 1:20 稀释的检验患者血清样品、1:20 稀释的正常人类血清 (NHS) 或在 1:20 稀释的 NHS 中的测定阳性对照 (G140 抗体, 5mg/mL 聚甘露糖) 预孵育 15 分钟。随后, 加入 5nM Alexa **FLUOR®** 488 标记的维拉苷酶 α 或伊米苷酶, 且在 37°C 下孵育额外 2 小时。在各实验中通过在 37°C 下用在 1:20 稀释的 NHS 中的 Alexa **FLUOR®** 488 标记的维拉苷酶 α 或伊米苷酶 (0-10nM) 孵育 2 小时而包括 Alexa **FLUOR®** 488 标记的维拉苷酶 α 或伊米苷酶的校准曲线。

[0972] 在孵育 2 小时之后, 通过离心分离除去培养基, 将细胞用胰蛋白酶 -EDTA 处理 3 分钟以除去表面结合的维拉苷酶 α 或伊米苷酶, 随后通过加入相等体积的具有 10% FBS 的培养基中和。将细胞用 PBS/0.5% BSA 洗涤一次, 使其再悬浮在 PBS/0.5% BSA 中并通过具有固定仪器安装的 BD FACS Canto II 分析。在各分析之前该 Canto II 仪器用 BD 细胞计数机构和跟踪珠粒装配。结果用 FlowJo 软件分析, 且记录各样品的平均荧光强度 (MFI)。调节的 MFI 通过从各样品的 MFI 中减去背景样品 (具有 0nM 药物的细胞) 的 MFI 计算。相对

于正常人类血清样品 (NHS) 或患者自己的雏期基准样品 (在可得到的情况下) 测定患者样品的维拉昔酶 α 和伊米昔酶吸收的抑制。抑制百分数可以使用以下方程式计算:

[0973] $\text{抑制}\% = 1 - (\text{检验样品的调节的 MFI} / \text{患者基准或 NHS 的调节的 MFI}) \times 100$

[0974] 结果:

[0975] 测定发展:

[0976] 以下初始结果根据该测定的发展获得 (数据未示出):

[0977] MRC118 细胞系的维拉昔酶 α 和伊米昔酶的内化具有剂量依赖性。

[0978] MRC1-18 细胞系的维拉昔酶 α 和伊米昔酶的内化由甘露糖受体介导, 因为该内化由聚甘露糖 (5mg/mL, >89%) 抑制, 但不由 M6P (5mM) 抑制。

[0979] 在 1:20 稀释的 NHS (正常人类血清) 中示踪的 G140 抗体抑制 MRC1-18 细胞系的维拉昔酶 α 和伊米昔酶吸收。该抑制具有剂量依赖性。

[0980] 以 1:20 稀释度检验的 12 批 NHS 没有抑制 MRC1-18 细胞系的维拉昔酶 α 吸收。

[0981] 测定变量:

[0982] 来自临床研究的 25 种个别雏期戈谢血清样品经 3 天检验 ($N = 75$) 以确立对 MRC1-18 细胞系中维拉昔酶 α 或伊米昔酶的吸收的基准影响。

[0983] 对维拉昔酶 α 吸收的平均基准血清影响类似于对伊米昔酶吸收的基准血清影响。然而, 与维拉昔酶 α 相比, 伊米昔酶的计算为 CV% 的变量大得多 (28% 对 15%) (数据未示出)。

[0984] ADA- 阳性患者血清样品结果和测定的可重现性

[0985] 检验由先前测定具有抗伊米昔酶或维拉昔酶 α 的抗体的患者血清样品的维拉昔酶 α 和伊米昔酶吸收的抑制。平行检验各血清样品阻断伊米昔酶吸收以及维拉昔酶 α 吸收的能力, 这与酶制剂最初引起抗体产生无关。表 31 列出患者血清样品和接受的蛋白质治疗 (仅一个抗体阳性患者、ENU、接受用维拉昔酶 α 治疗)。

[0986] 表 31 :ADA- 阳性患者血清样品

[0987]

患者	患者原始细胞	接受的治疗
032-191-0002	ENU	维拉昔酶 α
039-009-0001	RW	伊米昔酶
039-194-0001	GAJ	伊米昔酶
039-194-0002	AVL	伊米昔酶
039-167-0001	KM	伊米昔酶
034-027-0002	SB	伊米昔酶
034-154-0001	JMS	伊米昔酶

034-164-0001	MPQ	伊米苷酶

[0988] 来自患者 ENU、RW、GAJ、AVL 和 SB 的样品没有显示伊米苷酶或维拉苷酶 α 吸收的抑制（数据未示出）。相对于正常人类血清样品或患者自己的雏期基准样品（在可得到的情况下），经 3 天测定患者样品 JMS、KM、MPQ 和 SB（作为阴性对照）的维拉苷酶 α 和伊米苷酶吸收的抑制的可重现性（表 32）。

[0989] 患者 KM(039-167-0001) 在基准时为雏期且随后在后续研究中用伊米苷酶治疗。相对于维拉苷酶 α 吸收的等效抑制（0-15%，其在测定变量的范围内），来自所有三次访问的样品显示伊米苷酶吸收的显著抑制（相对于其基准抑制 24-52%）。

[0990] 患者 SB(034-027-0002)、JMS(034-154-0001) 和 MPQ(034-164-0001) 先前用伊米苷酶治疗。相对于正常人类血清样品，来自患者 SB 的血清没有显示伊米苷酶吸收的抑制（0%）或维拉苷酶 α 吸收的抑制（0%）。来自患者 JMS 的血清的伊米苷酶吸收的抑制（0-14%）与观察到的维拉苷酶 α 吸收的抑制（0-20%）一致。来自患者 MPQ 的血清的伊米苷酶吸收的抑制（32-45%）显著大于维拉苷酶 α 吸收的等效抑制（0-15%，其在测定变量的范围内）。

[0991] 这些初步结果提出在来自患者 KM 和 MPQ 的血清中存在的中和抗体抑制细胞中伊米苷酶的吸收，而不是抑制维拉苷酶 α 的吸收。

[0992] 表 32 :ADA- 阳性患者样品的伊米苷酶或维拉苷酶 α 吸收的抑制

[0993]

患者编号	原始细胞	访问	治疗	伊米苷酶吸收的抑制				维拉苷酶 α 吸收的抑制
				第 1 天	第 2 天	第 3 天	平均值	
039-167-0001	KM	第 13 周	基准时为雏	38%	24%	52%	38%	0-8%
		第 19 周	期。随后用伊	29%	45%	40%	38%	
		提前中止	米苷酶治疗	28%	38%	33%	33%	
034-027-0002	SB	基准访问	先前用伊米	0%	0%	0%	0%	0-14%
034-154-0001	JMS	基准访问	先前用伊米	14%	0%	2%	5%	0-20%
034-164-0001	MPQ	基准访问	先前用伊米	45%	32%	40%	38%	0-15%

[0994] 也经 3 天测定由甘露糖抑制剂聚甘露糖和多克隆绵羊抗维拉苷酶 α 抗体 G140（阳性对照）引起的伊米苷酶或维拉苷酶 α 吸收的抑制（表 33）。聚甘露糖抑制 MRC1-18 细胞中伊米苷酶和维拉苷酶 α 经甘露糖受体的吸收。G140 抗体抑制 MRC1-18 细胞中相等量的伊米苷酶和维拉苷酶 α 的吸收。

[0995] 表 33 :由阳性对照引起的伊米苷酶或维拉苷酶 α 吸收的抑制

[0996]

		伊米苷酶吸收的抑制		维拉苷酶 α 吸收的抑制	
		平均值	CV%	平均值	CV%
G140	5 $\mu\text{g/mL}$	52%	37%	42%	14%
G140	10 $\mu\text{g/mL}$	78%	11%	71%	7%
聚甘露糖	5 mg/mL	92%	3%	91%	3%

[0997] **结论:**关于蛋白质序列(例如,在伊米苷酶中的 R495H 突变)和碳水化合物结构两者,维拉苷酶 α 和伊米苷酶在结构上不同。进行实验以阐明以下假设:抗原差异存在于维拉苷酶 α 和伊米苷酶之间,且关于抗药物抗体(ADA)阻断各治疗剂的细胞结合和/或细胞内化的能力,这些表位区别维拉苷酶 α 与伊米苷酶。使用新近发展的 HT1080 细胞系进行实验,其中治疗剂的吸收主要由 MMR 变得更方便,且由其他已知的细胞吸收机制最低限度地混淆。测量患者血清样品的伊米苷酶或维拉苷酶 α 吸收相对于彼此的抑制。该数据显示,在总共 7 种取自响应伊米苷酶治疗产生 ADA 的患者的血清样品和 1 种取自响应维拉苷酶 α 治疗产生 ADA 的患者的血清样品之中,2 种来自响应伊米苷酶产生 ADA 的血清(2/7 = 29%)引起伊米苷酶的细胞吸收抑制 24% - 52%,但维拉苷酶 α 的细胞吸收抑制仅 0-15%,其在测定变量内。相比之下,至今已经鉴定仅一个患者响应维拉苷酶 α 治疗产生抗体;该血清不抑制吸收。

[0998] 平行检验各血清样品阻断伊米苷酶吸收和/或维拉苷酶 α 吸收的能力,这与酶制剂最初引起抗体产生无关。维拉苷酶 α 吸收的抑制与伊米苷酶吸收的抑制的这种比较允许粗略“绘出”各治疗剂的生物学相关部分的表位(警告:在本文中检验的抗血清为多克隆的且因此各抗血清可能含有也许识别多个表位的抗原特异性的混合物)。在考虑这些预防措施的情况下,用抗伊米苷酶抗血清观察到的吸收的抑制似乎限于伊米苷酶,因为相同的抗血清不抑制平行检验的维拉苷酶 α 的内化。这些数据提出响应伊米苷酶产生的一些 ADA 经特别暴露于伊米苷酶的表位抑制细胞内化。此外,就翻译后修饰而言,在对伊米苷酶吸收相对维拉苷酶 α 吸收的血清影响观察到的变量差异可能归因于在维拉苷酶 α 制剂中的均质性比在伊米苷酶制剂中的均质性更大。

[0999] 使用基于细胞的测定检验由抗维拉苷酶 α (伊米苷酶)抗体引起的酶吸收的抑制,该基于细胞的测定检测并量化妨碍巨噬细胞甘露糖受体(MMR)介导的维拉苷酶 α (伊米苷酶)吸收的抗体。该方法根据在限定条件下由 MRC1-18 细胞内化的荧光标记的维拉苷酶 α (伊米苷酶)的量化。MRC1-18 为在 Shire HGT 自用 1 型人类巨噬细胞甘露糖受体 C 稳定转染的 HT1080 细胞工程化的细胞系。

[1000] 简要地讲,将抗维拉苷酶 α (伊米苷酶)抗体阳性血清样品在 37°C 下在平底 96-孔板中在培养基中用 MRC1-18 细胞预孵育 15 分钟。将池正常人类血清(NHS)用作阴性对照。已知绵羊多克隆抗体抑制维拉苷酶 α 和伊米苷酶吸收以及聚甘露糖 MMR 特异性配体(Sung SJ 等, J. Cell Biol. 1983 ;96:160-166)用作阻断酶吸收的阳性对照。

[1001] 在预孵育之后,加入 Alexa Fluor-488 标记的维拉苷酶 α 或伊米苷酶,且将其在 37°C 下孵育额外 2 小时。在各实验中包括由用 MRC1-18 细胞孵育 Alexa Fluor-488 标记的酶组成的校准曲线。在孵育之后,通过离心分离除去培养基,将细胞用胰蛋白酶-EDTA 处理

3 分钟以除去表面结合的酶,且最后通过加入等体积的培养基使反应 pH 回到中性。将细胞洗涤一次且使用含有 0.5% BSA 的磷酸盐缓冲盐水再悬浮。使用在各分析之前使用仪器细胞计数机构和追踪珠粒校准的 Becton Dickinson FACS Canto II 仪器分析细胞。

[1002] 结果用仪器的 FlowJo 软件分析,且记录各样品的平均荧光强度 (MFI)。调节的 MFI 通过从各未知且对照样品的 MFI 中减去来自不含酶的样品孔的背景 MFI 计算。根据以下方程相对于 NHS 控制评估患者血清样品引起的酶的抑制:

[1003] $\text{抑制}\% = [1 - [\text{检验样品的调节的 MFI} / \text{NHS 的调节的 MFI}]] \times 100$

[1004] 测定切割点通过分析从治疗雏期戈谢病患者收集的 25 种个别血清来测定。在单独的四天对总共 100 个值检验各血清且将阳性切割点定义为抑制作用大于这些值的平均值加 1.645 标准偏差。

[1005] 体外基于细胞的测定 II

[1006] 发展该测定以比较维拉昔酶 α 的吸收与伊米昔酶的吸收。

[1007] 方法:对于实验设计 (DOE) 测定,全因子设计由 Statease DESIGN EXPERT 软件辅助。DOE 利用来源于佛波醇豆蔻酸酯乙酸酯 (PMA) 诱发的 U937 细胞的巨噬细胞,且在 5mM 甘露糖 -6- 磷酸 (M6P) 存在下进行。对于内化比较,源自 U937 的巨噬细胞用在 pH 7.5 下具有 10mM 钙的 GCB 孵育 3 小时。内化药物通过活性测定用在裂解时发荧光的合成底物 (4-MU-glc) 测量。

[1008] 结果:在 U937 细胞中维拉昔酶 α 和伊米昔酶的内化率的比较显示维拉昔酶 α 比伊米昔酶更有效地内化,高达 2.5 倍。使用 MMR- 表达鼠细胞系 J774 也观察到在细胞内化方面的该差异。在具体测定条件下,钙的加入适度抑制伊米昔酶的细胞吸收,同时其增强维拉昔酶 α 的吸收。两种酶的内化可以通过向培养基中加入聚甘露糖而抑制,尽管与伊米昔酶相比, J774 细胞的维拉昔酶 α 吸收的抑制更彻底。DOE 测定揭示:i) 钙与 pH 的相互作用大大影响吸收;和 ii) 生物测定样品比较需要存在钙,符合 MMR 的已知钙依赖性。在 DOE 实验中甘露糖 -6- 磷酸 (M6P) 的存在保证在 U937 细胞上的 M6P 受体不促进测量的内化。

[1009] 这些数据提出维拉昔酶 α 比伊米昔酶更有效地内化。虽然两种酶主要经 MMR 内化,一小部分 (与维拉昔酶 α 相比,伊米昔酶更多) 通过备选机制内化。这些数据可以证明在区别维拉昔酶 α 、伊米昔酶和其他未来疗法方面有价值。

[1010] 概述

[1011] 根据这些研究,抗体检测方法的概述如下:

[1012] ●按 ICH 和 FDA 准则批准用于维拉昔酶 α 和伊米昔酶 ADA 的等效测定。参见,例如 <http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM128049.pdf> 和 <http://www.ich.org/LOB/media/MEDIA417.pdf>

[1013] ●筛选

[1014] ●验证

[1015] ●滴度

[1016] ●同种型

[1017] ●中和

[1018] ●平行评估隐匿性患者试样的维拉昔酶 α ADA 和伊米昔酶 ADA

[1019] ●对照和定标器涵盖大量抗体亲和性

- [1020] ●在没有发现人类阳性血清时发展同种型特异性混合对照
- [1021] 在 III 期研究中患者的免疫原性状况：
- [1022] ● TKT032 :使患者对维拉昔酶 α 60U/kg 或 45U/kg EOW 随机分组
- [1023] ●基准时 ADA- 阴性的 1 个患者响应维拉昔酶 α 发展出 NAb
- [1024] ● TKT034 :将先前对伊米昔酶稳定的患者转换到维拉昔酶 α 15-60U/kg
- [1025] ●基准时抗伊米昔酶阳性的 3 个患者经 12 个月治疗为抗维拉昔酶阴性。
- [1026] ● HGT-GCB-039 :使患者对维拉昔酶 α 60U/kg 或伊米昔酶 60U/kg EOW 随机分组
- [1027] ●基准时 ADA- 阴性的 4 个患者响应伊米昔酶血清转化
- [1028] ●其中, 1 个患者相对于伊米昔酶和维拉昔酶 α 两者发展出 NAb 反应性, 而 3 个患者仅相对于伊米昔酶发展出非抑制性 IgG ADA 反应性
- [1029] 患者血清转化汇总示于表 34 中。在检验的 99 个患者中, 82 个患者接受维拉昔酶 α 且 17 个患者接受伊米昔酶。在试验期间, 接受维拉昔酶 α 的 82 个患者中的 1 个患者和接受伊米昔酶的 17 个患者中的 4 个患者发展出抗体。
- [1030] 表 34
- [1031]

研究	月数	治疗	U/kg EOW	n	血清转化
TKT032					
12 个月		维拉昔酶 α	60	12	0/12 个患者
		维拉昔酶 α	45	13	1/13 个患者
TKT034					
12 个月		维拉昔酶 α	15-60	40	到此为止: 0 个患者 (3 个患者在基准时伊米昔酶抗体阳性)
HGT-GCB-039					
9 个月		维拉昔酶 α	60	17	0/17 个患者
		伊米昔酶	60	17	4/17 个患者

- [1032] 在该实施例中论述的研究的临床含义如下：
- [1033] ●发展、优化并批准了高度灵敏且等效的方法以直接评价并比较患者对维拉昔酶 α 和伊米昔酶治疗的抗体响应
- [1034] ●结果显示在用维拉昔酶 α 治疗的 1% 患者和用伊米昔酶治疗的 23% 患者中的血清转化, 提出在维拉昔酶 α 和伊米昔酶之间的显著抗原差异
- [1035] ●另外, 存在由伊米昔酶的 ADA 而不是维拉昔酶 α 的 ADA 引起的显著细胞吸收抑制。
- [1036] 实施例 8 :湿含量对冻干维拉昔酶产品的稳定性的影响
- [1037] 且的:评价湿含量对冻干维拉昔酶产品的稳定性的影响。
- [1038] 方法:将蛋白质配制成含蔗糖的溶液且使用 FTS 实验室规模冻干器 (Lyostar II) 冻干。在初次干燥之后用取样器不时移出冻干的小瓶以产生具有不同湿含量的样品。通过

FT-IR 检查冻干蛋白质的二级结构。这些样品的热稳定性通过物理外观、湿含量、尺寸排阻和反相 HPLC 和根据肽作图方法的氧化来表征。

[1039] 在用灭菌注射水重构后,维拉昔酶产品含有约 2.5mg/mL(40U/mL) 维拉昔酶 α 、50mg/mL 蔗糖、12.9mg/mL 柠檬酸钠二水合物、1.3mg/mL 柠檬酸单水化物和 0.11mg/mL 聚山梨醇酯 20。

[1040] 结果:在冻干产品中实现 1.3%至 6.2%的湿含量范围。在具有高湿含量($\geq 5\%$)的药物产品中在加速的温度(40℃)下观察到饼坍塌。然而,重构溶液的化学稳定性检验结果证明,根据 SE-HPLC 方法和 RP-HPLC 方法两者,与较干燥的样品相比,具有较高湿含量($\geq 3\%$)的样品的降解程度显著较低,以及显示氧化量较低。该稳定性趋势与通过干燥产品的 FT-IR 在二级结构中观察到的变化相关。

[1041] 结论:湿含量越高使冻干蛋白质的化学稳定性越好。该稳定性趋势通过较少地变为在具有较高湿含量的固相中的二级结构来解释。冻干制品中恰当湿含量的选择需要平衡饼的结构稳定性与蛋白质的化学稳定性。

[1042] 实施例 9:维拉昔酶 α 的治疗目标的分析

[1043] 已经描述了治疗目标来监测接受 ERT 的患有 1 型戈谢病的患者中治疗响应的实现、维持和连续性 (Pastores G 等, (2004) Seminars in Hematology, 41(增刊 5):4-14)。

[1044] 对于维拉昔酶 α 治疗对关于 1 型戈谢病的临床参数(贫血、血小板减少、肝肿大、脾肿大和骨骼病理)的治疗目标的影响的基准,将基准时处于贫血、血小板减少、肝肿大和脾肿大的目标的患者与在 9 个月或 1 年实现这些目标中的每一个的比例相比较。

[1045] 不使用数据归责。仅包括在两个时间点对各目标具有数据的患者。对于血液学参数,基准为筛选值和基准值的平均值;1 年治疗目标值对于 TKT032 和 TKT034 为第 51 周值和第 53 周值的平均值,且对于 HGT-GCB-039 为第 39 周值和第 41 周值的平均值。对于器官体积,在 HGT-GCB-039 中,将第 41 周值应用到 1 年治疗目标标准。

[1046] 在表 35-40 中描述具有完整脾的 TKT032、HGT-GCB-039、HGT-GCB-039 患者、HGT-GCB-039 的脾切除患者、TKT034 和 TKT025 的治疗目标。

[1047] 表 35

[1048]

治疗目标	维拉昔酶 α 60 U/kg N = 12		维拉昔酶 α 45 U/kg N = 13		维拉昔酶 α 总共 N = 25	
	基准	1 年	基准	1 年	基准	1 年
血红蛋白浓度	2/12 (17%)	11/12 (92%)	3/13 (23%)	12/13 (92%)	5/25 (20%)	23/25 (92%)
血小板计数	2/11 (18%)	6/11 (55%)	4/13 (31%)	9/13 (69%)	6/24 (25%)	15/24 (63%)
肝	5/11 (45%)	11/11 (100%)	7/12 (58%)	8/12 (67%)	12/23 (52%)	19/23 (83%)
脾	3/11 (27%)	11/11 (100%)	4/12 (33%)	11/12 (92%)	7/23 (30%)	22/23 (96%)
>3 个目标	1/11 (9%)	10/11 (91%)	5/12 (42%)	9/12 (75%)	6/23 (26%)	19/23 (83%)
所有 4 个目标	1/10 (10%)	6/10 (60%)	0/12 (0%)	7/12 (58%)	1/22 (5%)	13/22 (59%)

[1049] 表 36

[1050]

治疗目标	维拉昔酶 α 60 U/kg N = 17		伊米昔酶 60 U/kg N = 17	
	基准	9 个月	基准	9 个月
血红蛋白浓度	10/16 (63%)	15/16 (94%)	4/15 (27%)	12/15 (80%)
血小板计数	8/16 (50%)	16/16 (100%)	9/15 (60%)	15/15 (100%)
肝	8/16 (50%)	15/16 (94%)	4/16 (25%)	16/16 (100%)
脾	1/6 (17%)	6/6 (100%)	3/6 (50%)	6/6 (100%)
> 3 个目标	1/16 (6%)	14/16 (88%)	1/15 (7%)	12/15 (80%)
所有 4 个目标	0/6 (0%)	6/6 (100%)	0/6 (0%)	6/6 (100%)

[1051] 表 37

[1052]

治疗目标	维拉昔酶 α 60 U/kg N = 7		伊米昔酶 60 U/kg N = 7	
	基准	9 个月	基准	9 个月
血红蛋白浓度	5/7 (71%)	7/7 (100%)	2/6 (33%)	6/6 (100%)
血小板计数	0/7 (0%)	7/7 (100%)	0/6 (0%)	6/6 (100%)
肝	6/7 (86%)	7/7 (100%)	1/6 (17%)	6/6 (100%)
脾	1/6 (17%)	6/6 (100%)	3/6 (50%)	6/6 (100%)
> 3 个目标	1/7 (14%)	7/7 (100%)	1/6 (17%)	6/6 (100%)
所有 4 个目标	0/6 (0%)	6/6 (100%)	0/6 (0%)	6/6 (100%)

[1053] 表 38

[1054]

治疗目标	维拉昔酶 α 60 U/kg N = 10		伊米昔酶 60 U/kg N = 10	
	基准	9 个月	基准	9 个月
血红蛋白浓度	5/9 (56%)	8/9 (89%)	2/9 (22%)	6/9 (67%)
血小板计数	8/9 (89%)	9/9 (100%)	9/9 (100%)	9/9 (100%)
肝	2/9 (22%)	8/9 (89%)	3/10 (30%)	10/10 (100%)
> 2 个目标	6/9 (67%)	9/9 (100%)	5/9 (56%)	9/9 (100%)
所有 3 个目标	0/9 (0%)	7/9 (78%)	0/9 (0%)	6/9 (67%)

[1055] 表 39

[1056]

治疗目标	维拉昔酶 α 总 N = 40	
	基准	1 年
血红蛋白浓度	37/38 (97%)	37/38 (97%)
血小板计数	29/36 (81%)	29/36 (81%)
肝	37/37 (100%)	37/37 (100%)
脾	30/34 (88%)	31/34 (91%)
> 3 个目标	32/37 (86%)	33/37 (89%)
所有 4 个目标	26/32 (81%)	26/32 (81%)

[1057] 表 40

[1058]

治疗目标	TKT025 维拉昔酶 α 60 U/kg N = 12		TKT032 维拉昔酶 α 60 U/kg N = 12	
	基准	第 9 个月	基准	第 1 年
血红蛋白浓度	5/10 (50%)	10/10 (100%)	2/12 (17%)	11/12 (92%)
血小板计数	0/10 (0%)	7/10 (70%)	2/11 (18%)	6/11 (55%)
肝	5/10 (50%)	7/10 (70%)	5/11 (45%)	11/11 (100%)
脾	0/10 (0%)	9/10 (90%)	3/11 (27%)	11/11 (100%)
> 3 个目标	0/10 (10%)	8/10 (80%)	1/11 (9%)	10/11 (91%)
所有 4 个目标	0/10 (10%)	5/10 (50%)	1/10 (10%)	6/10 (60%)

[1059] 实施例 10. 维拉昔酶 α 在患有 III 型戈谢病儿童和青少年中的功效和安全性研究

[1060] 戈谢病是因葡萄糖脑苷脂酶 (GCB) 的缺乏而引起的罕见溶酶体贮积症。戈谢病已基于神经学症状的存在或不存在和神经学症状的严重程度而被分为 3 个临床亚型。患有 2 型戈谢病的患者呈现急性神经功能恶化, 患有 3 型戈谢病的患者通常显示更加亚急性的神经学过程; 1 型戈谢病这种占据超过 90% 的所有病例的最常见的形式, 并不牵涉中枢神经系统。

[1061] 维拉苷酶 α 是被批准用于患 1 型戈谢病的儿科和成年患者的酶替代疗法 (ERT)。ERT 已被证明能减轻器官巨大症, 改善血液学参数和积极影响健康相关生活质量。未曾显示 ERT 穿过血脑屏障, 因此其显示改善与戈谢病相关的神经学 (中枢神经系统; CNS) 表现的能力很有限。

[1062] 本研究提供了研究维拉苷酶 α 在患有 3 型戈谢病的患者中的功效和安全性的基础。此研究将是在患有 3 型戈谢病的儿童和青少年中进行的维拉苷酶 α 酶替代疗法的干预性多中心、标签公开的疗效和安全性研究。主要结果测量将包括: 血红蛋白浓度从基准的变化、血小板计数从基准的变化、使用磁共振成像 (MRI) 测量的标准化肝体积从基准的变化、使用磁共振成像 (MRI) 测量的标准化脾体积从基准的变化和神经学症状从基准的变化。安全性评估可包括不利事件和输注相关不利事件的评估、严重的不利事件、临床实验室值、尿分析、生命体征、12-导联心电图 (ECG) 记录、体格检查和抗-维拉苷酶 α 抗体形成。该研究的实验部分将每隔一周通过静脉内输注接受维拉苷酶 α 。

[1063] 若要包括在研究中, 则每一个患者必须满足所有下列标准: 3 型戈谢病的确认诊断; 招募时 ≥ 2 岁和 < 18 岁; 首次进行治疗或在进入研究之前 12 个月内未曾接受戈谢病的治疗 (研究的或批准的); 患者必需具有戈谢病相关贫血, 定义为低于相应年龄和性别的正常值下限的血红蛋白浓度。患者还必须满足下列标准的一项或多项: 通过触诊发现的至少中度脾肿大 (左肋缘下方 2 至 3cm); 戈谢病相关血小板减少症, 定义为血小板计数 $< 120 \times 10,000$ 血小板 / mm^3 ; 戈谢病相关的易于触知的大肝。已经历脾切除术的患者仍然可适合加入研究。具有生育可能性的女性患者必须同意在研究过程中在所有时间使用医学上可接受的避孕方法。将在招募时以及在整个研究参与过程中按需进行妊娠测试。男性患者必须同意在研究过程中在所有时间上使用医学上可接受的避孕方法并向研究人员报告伴侣的妊娠。患者的父母或患者的法律上授权的代表必须提供已被伦理委员会 / 独立伦理委员会 (IRB/IEC) 批准的书面知情同意书。

[1064] 满足下列标准中的任何标准的患者将排除出本研究: 患者怀疑患有 2 型或 I 型戈谢病; 患者 < 2 岁; 患者已经历严重的 (3 级或更高级) 对戈谢病的任何酶替代疗法 (批准的或研究的) 的输注相关的超敏反应 (过敏性或过敏样反应); 患者已在进入研究之前 30 天内接受利用研究药物的任何非戈谢病相关治疗; 患者为孕妇和 / 或哺乳期的女性。

[1065] 已经描述了本发明的许多实施方案。尽管如此, 应当理解的是可以在不脱离本发明的精神和范围的情况下进行各种改进。因此, 其他实施方案在以上权利要求书的范围内。

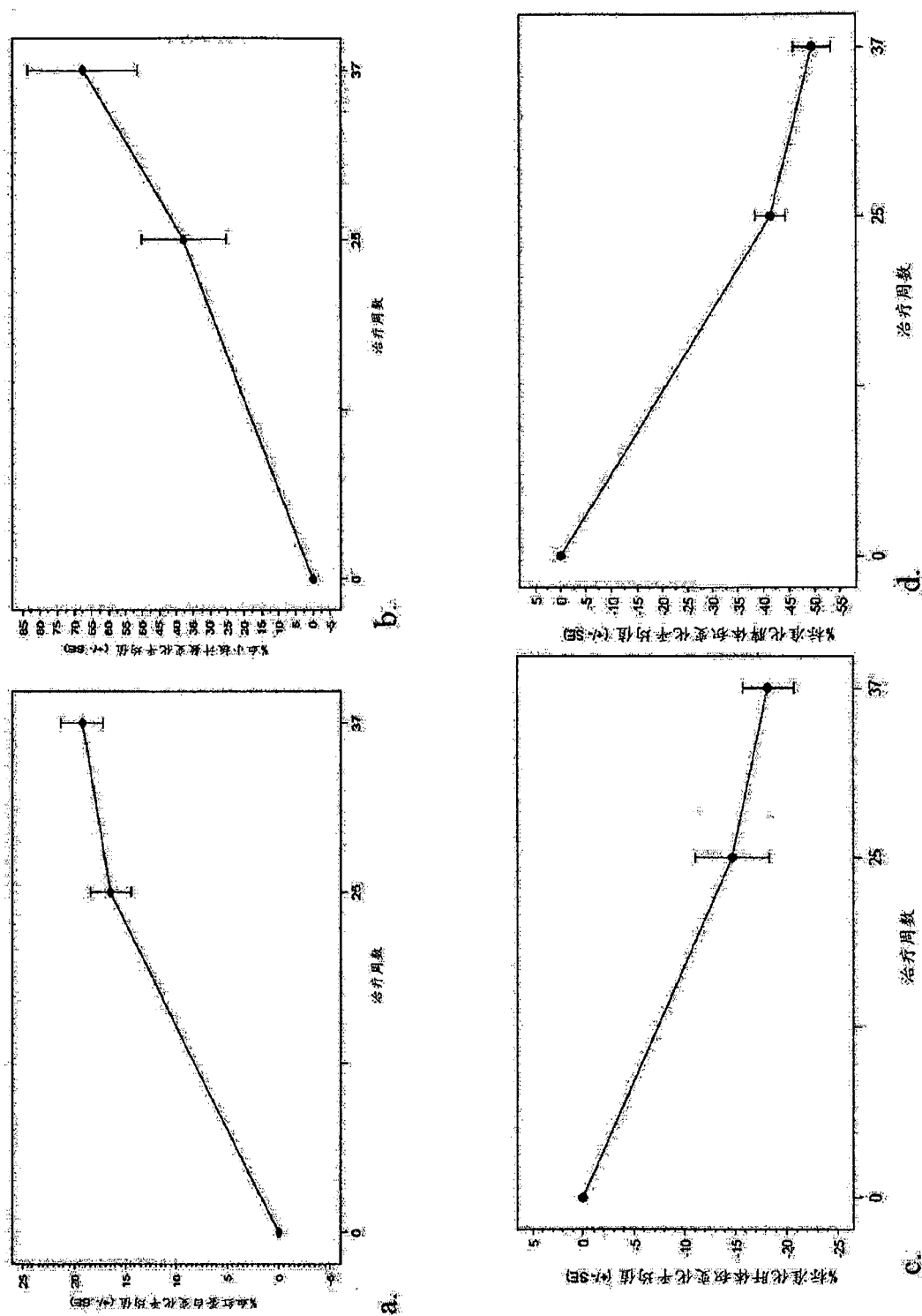


图 1(续)

图 1

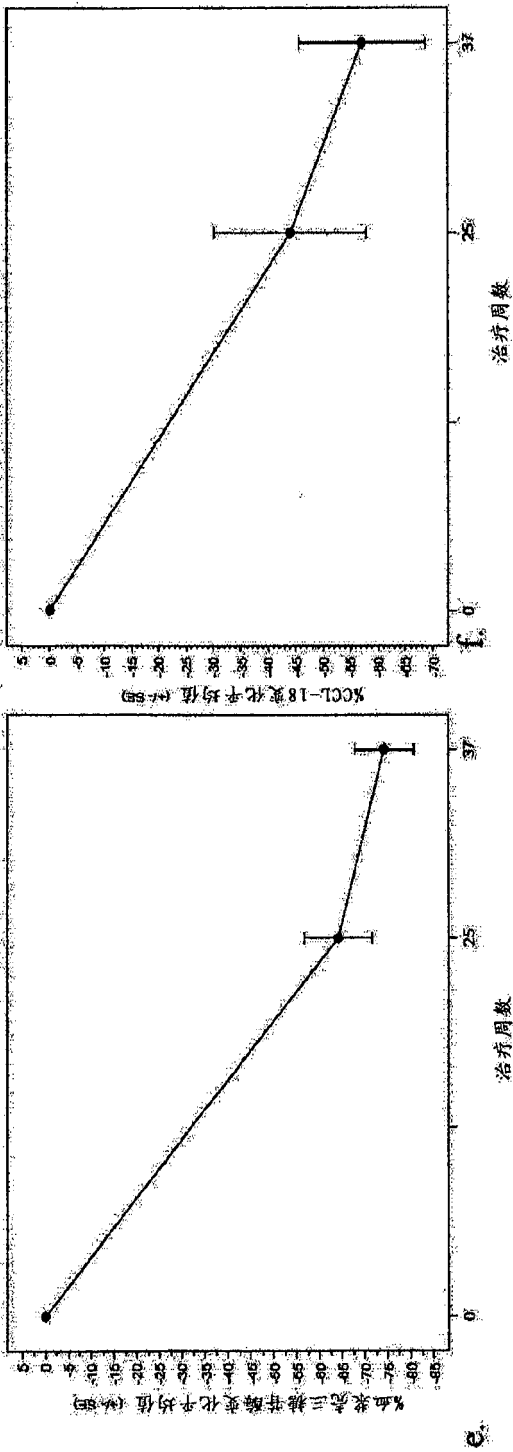


图 1(续)

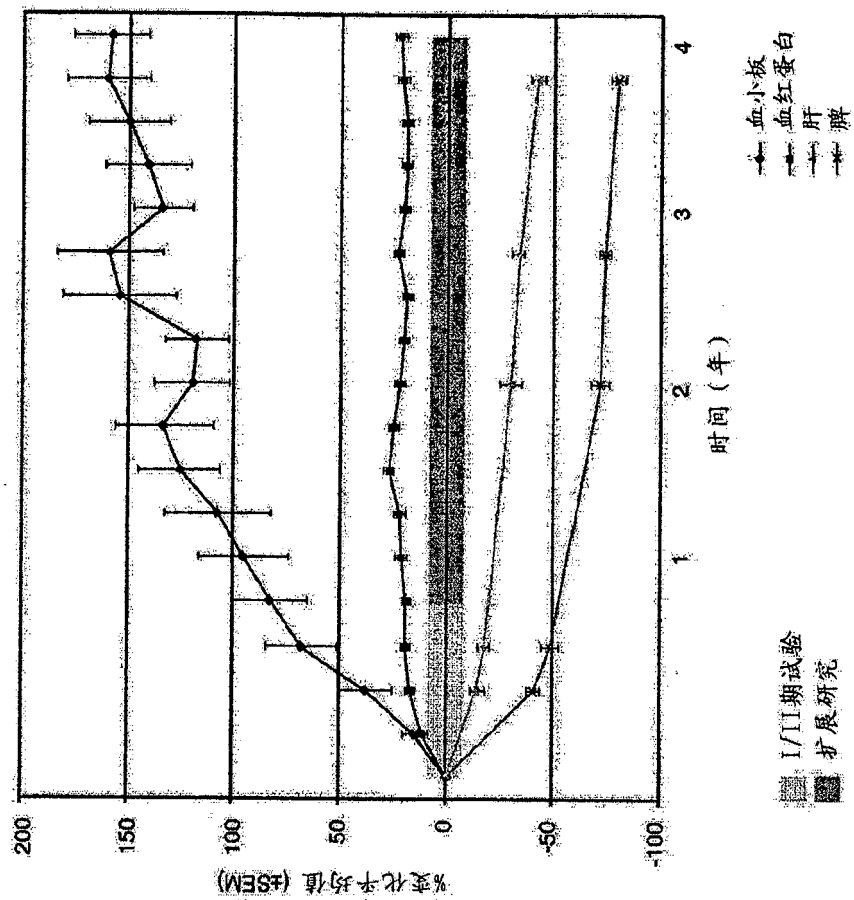
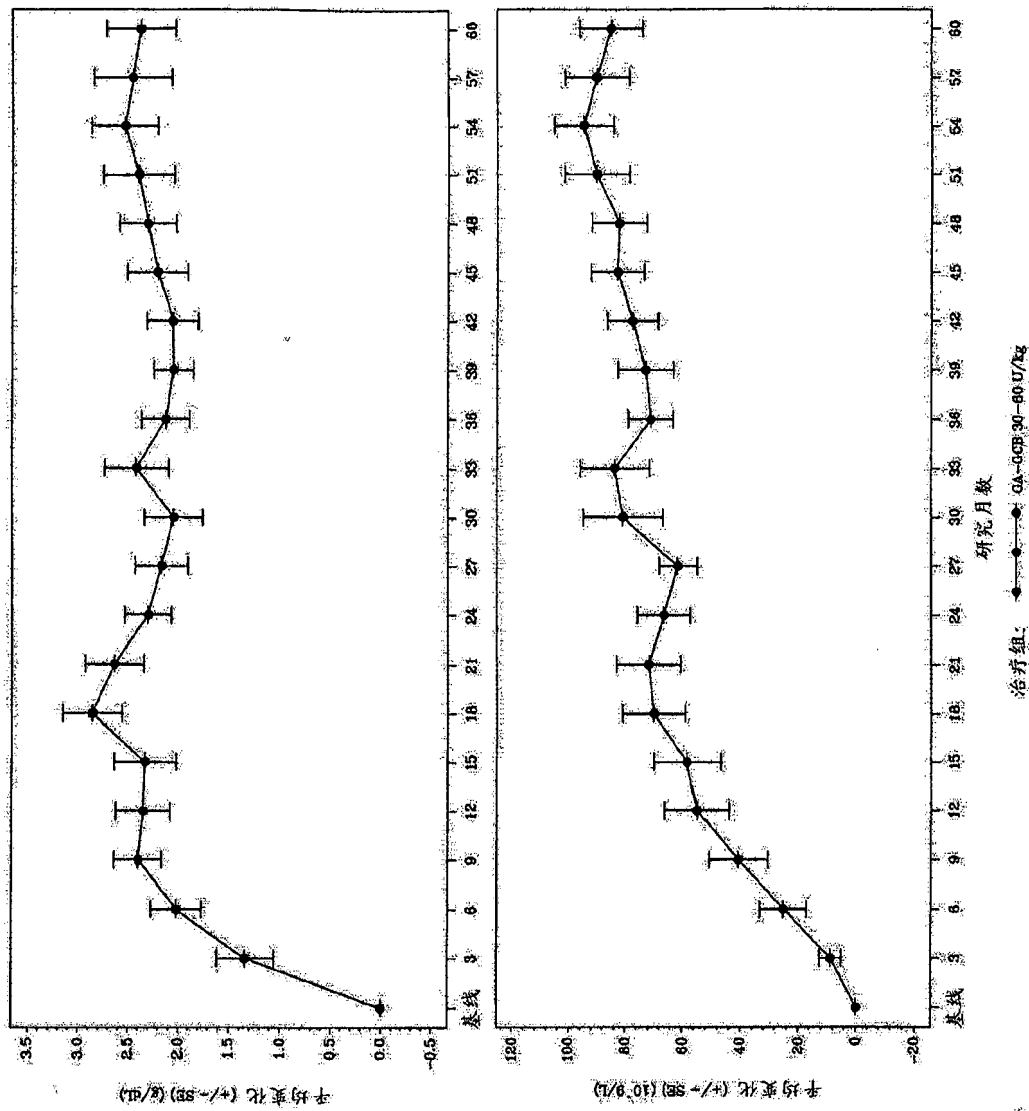


图 2



备注: 对缺失的数据进行扣减

图 3

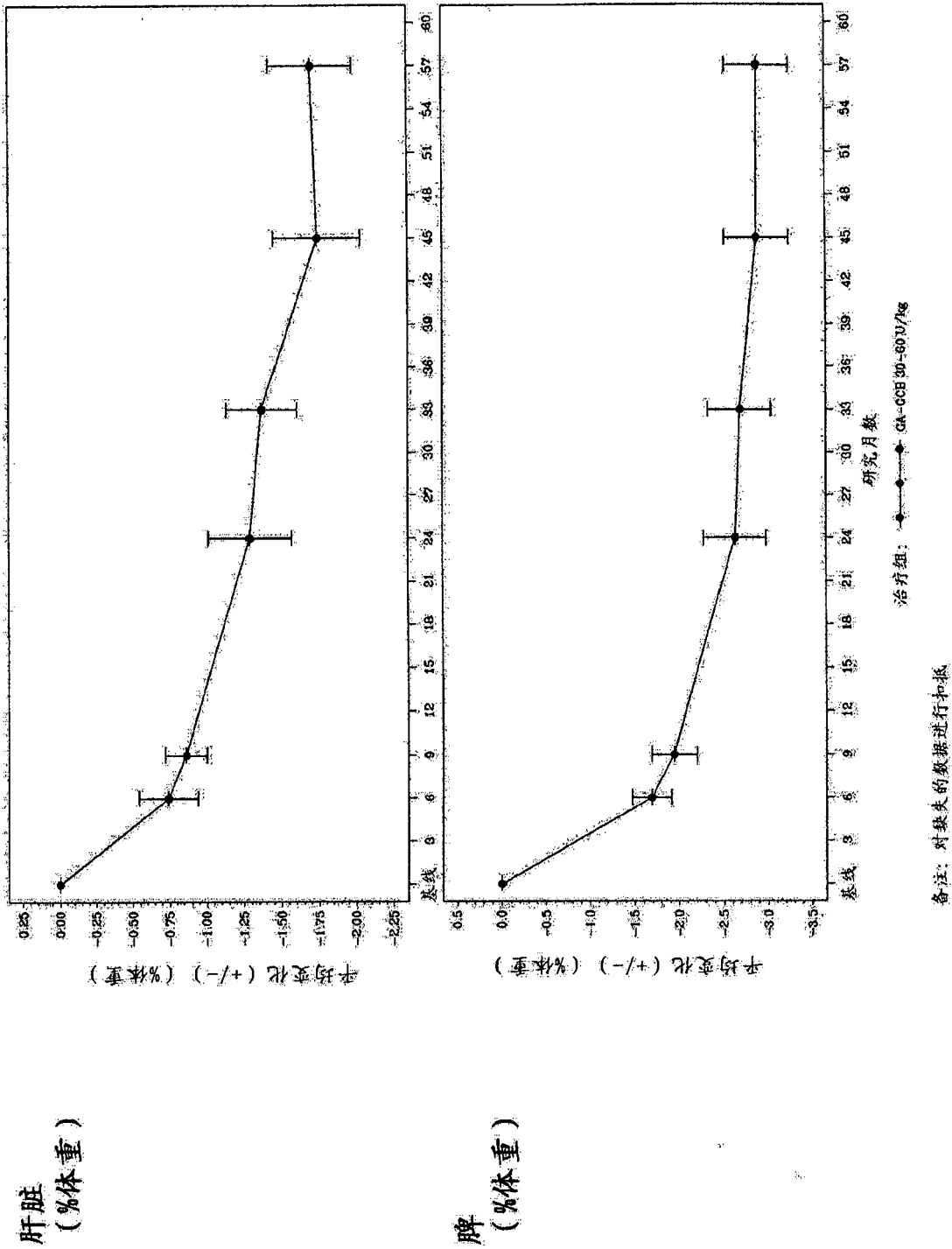


图 4

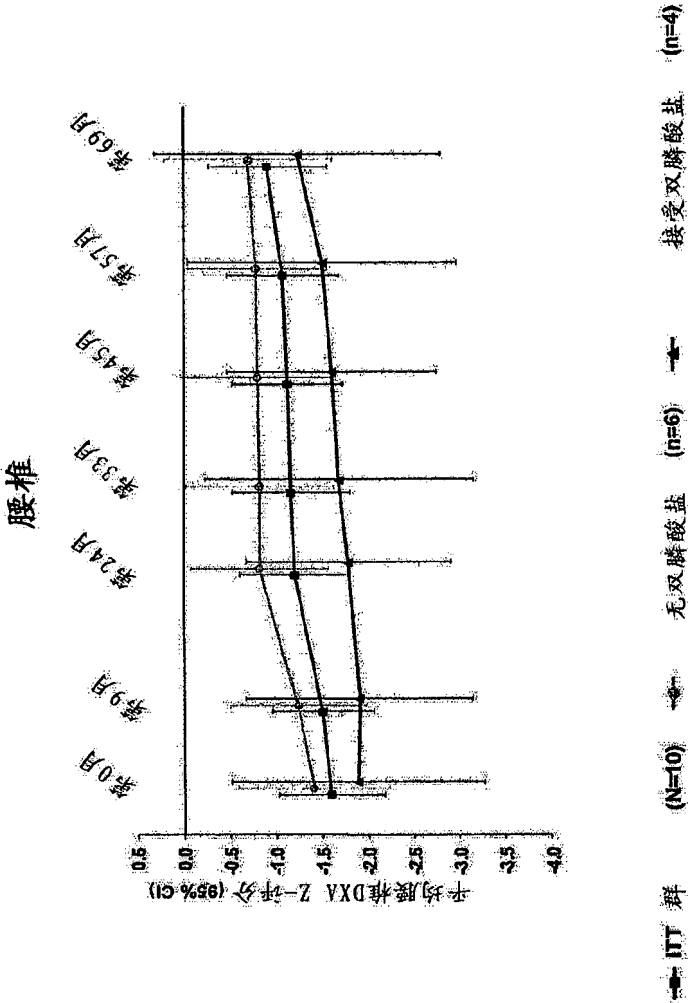


图 5

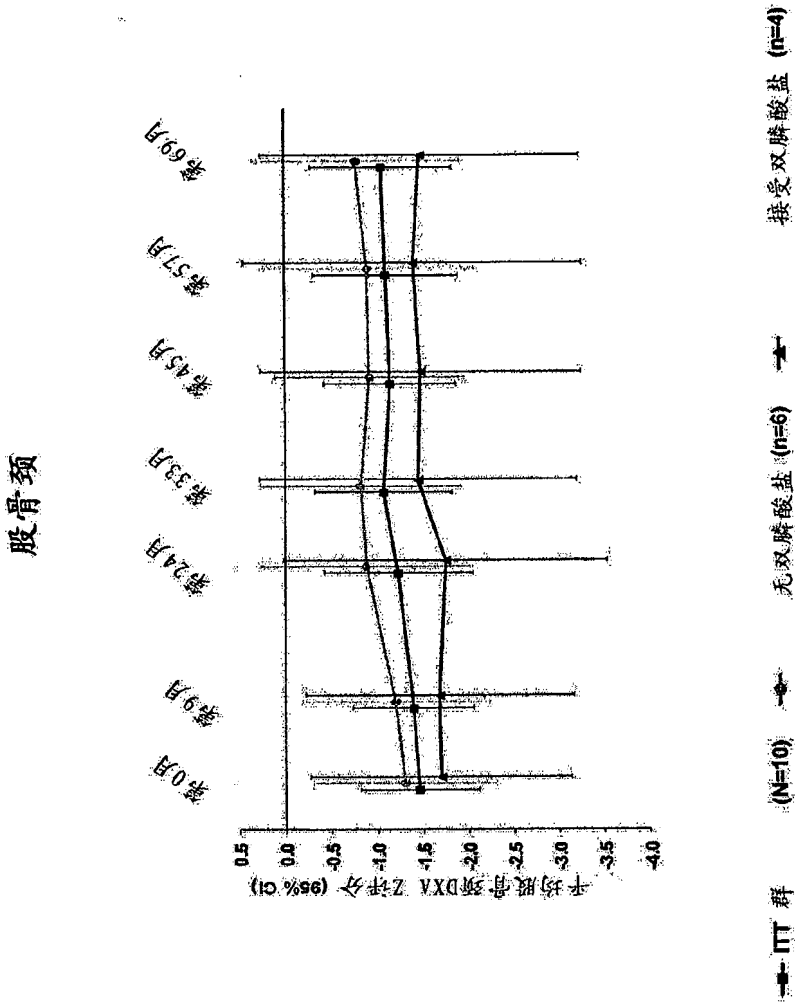


图 6

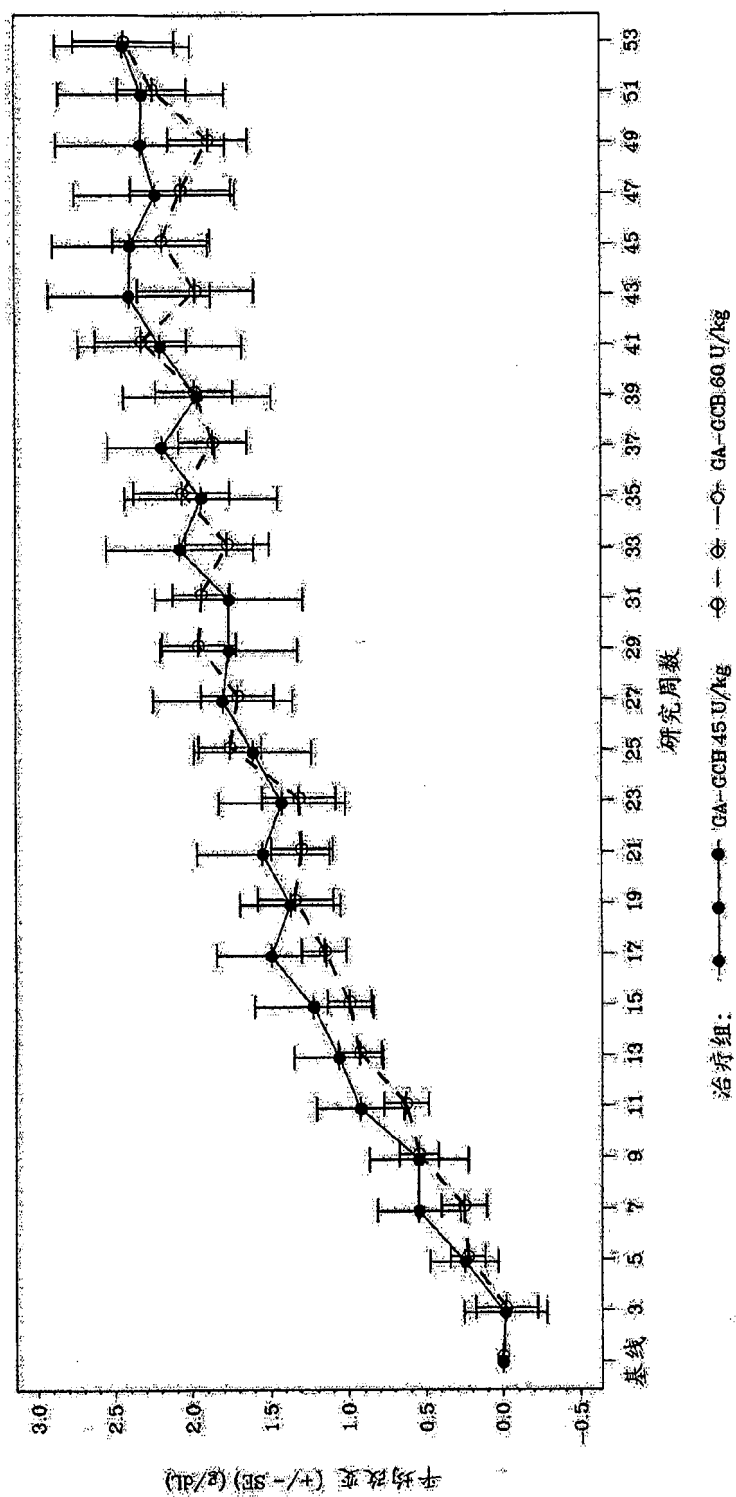


图 7

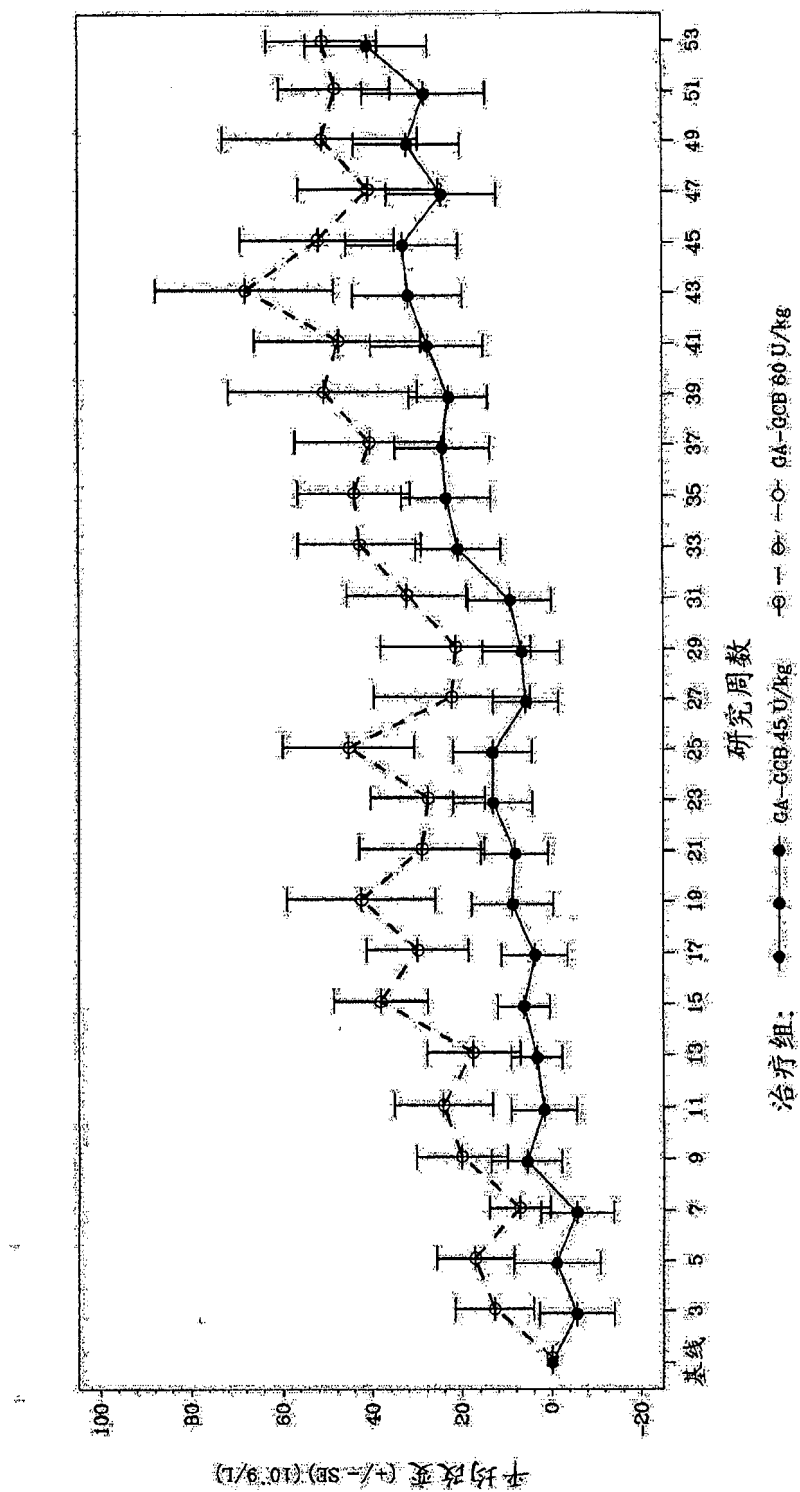


图 8

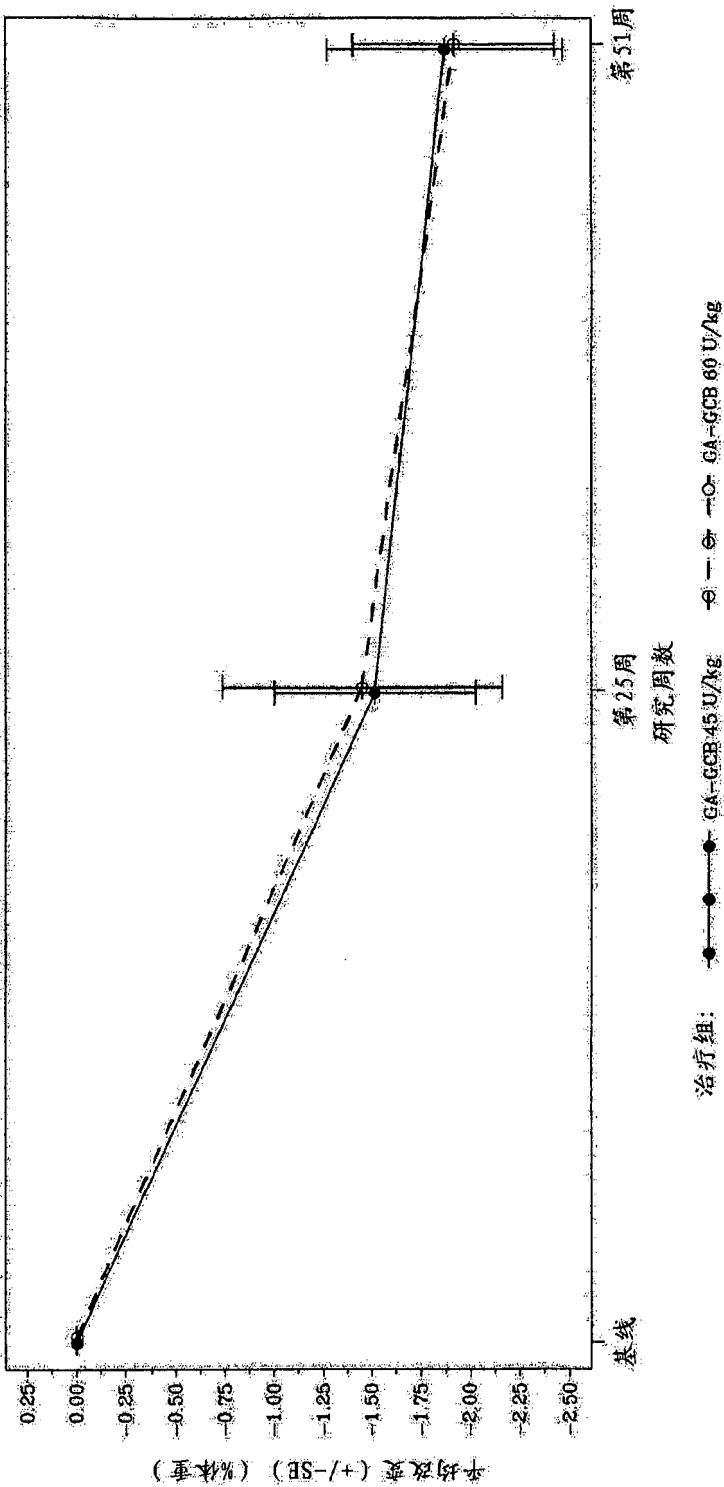


图 9

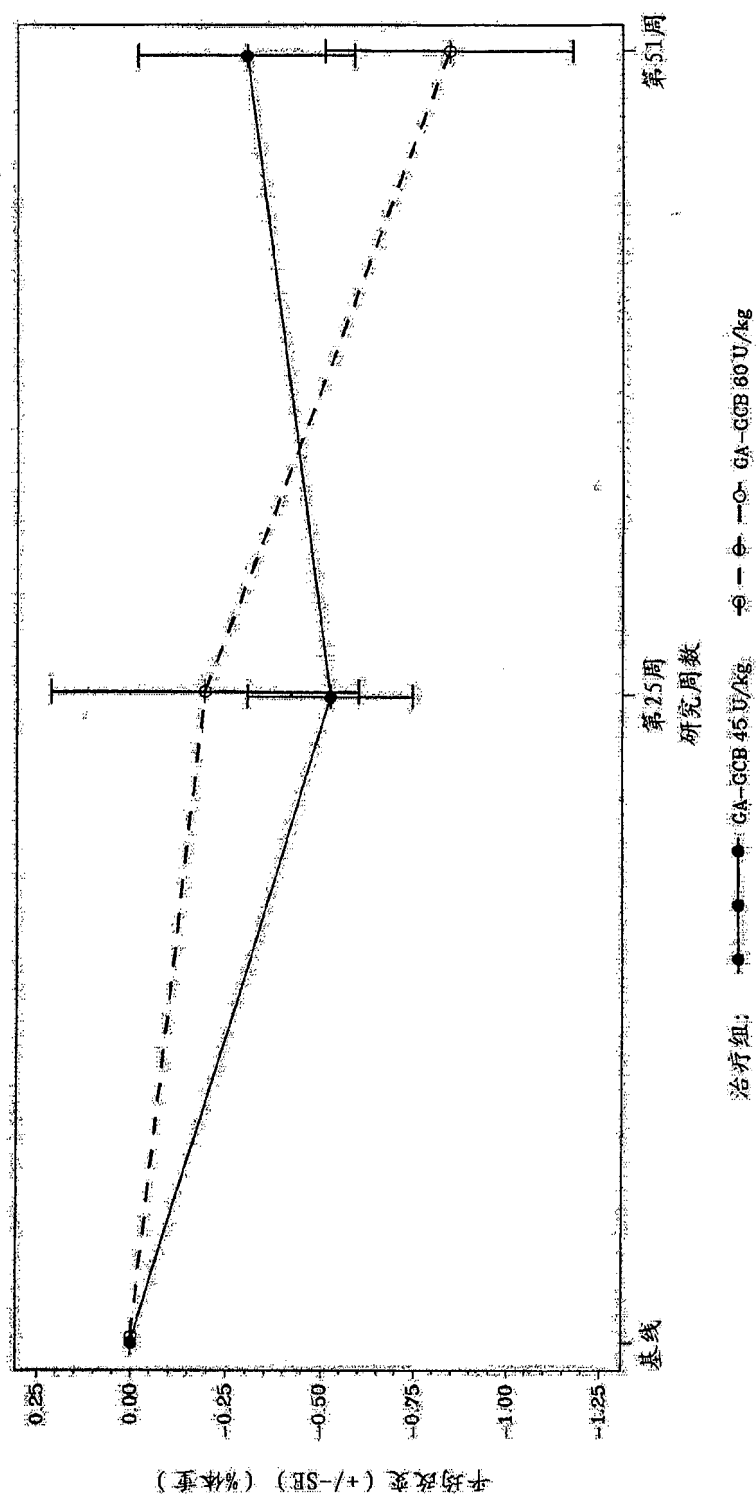


图 10

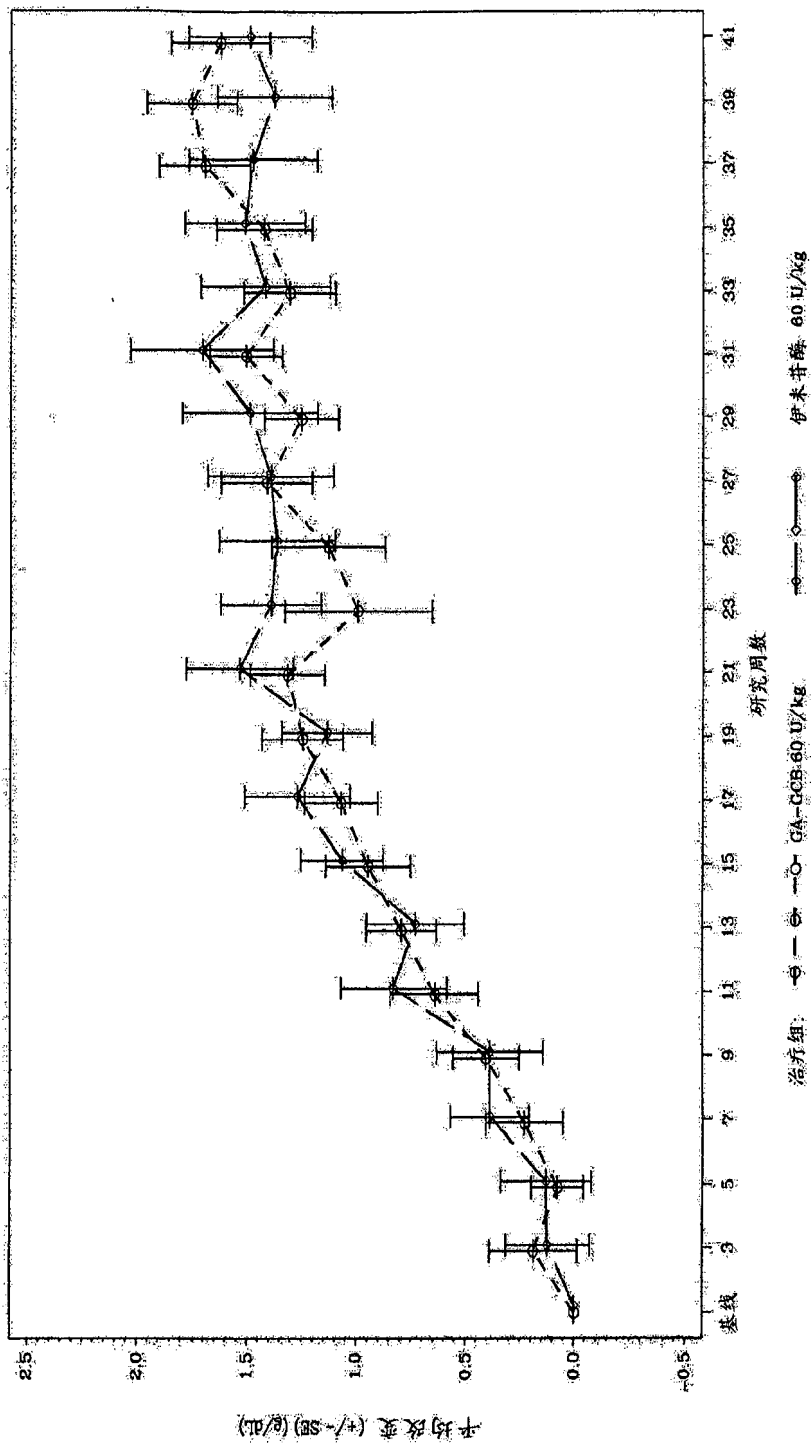


图 11

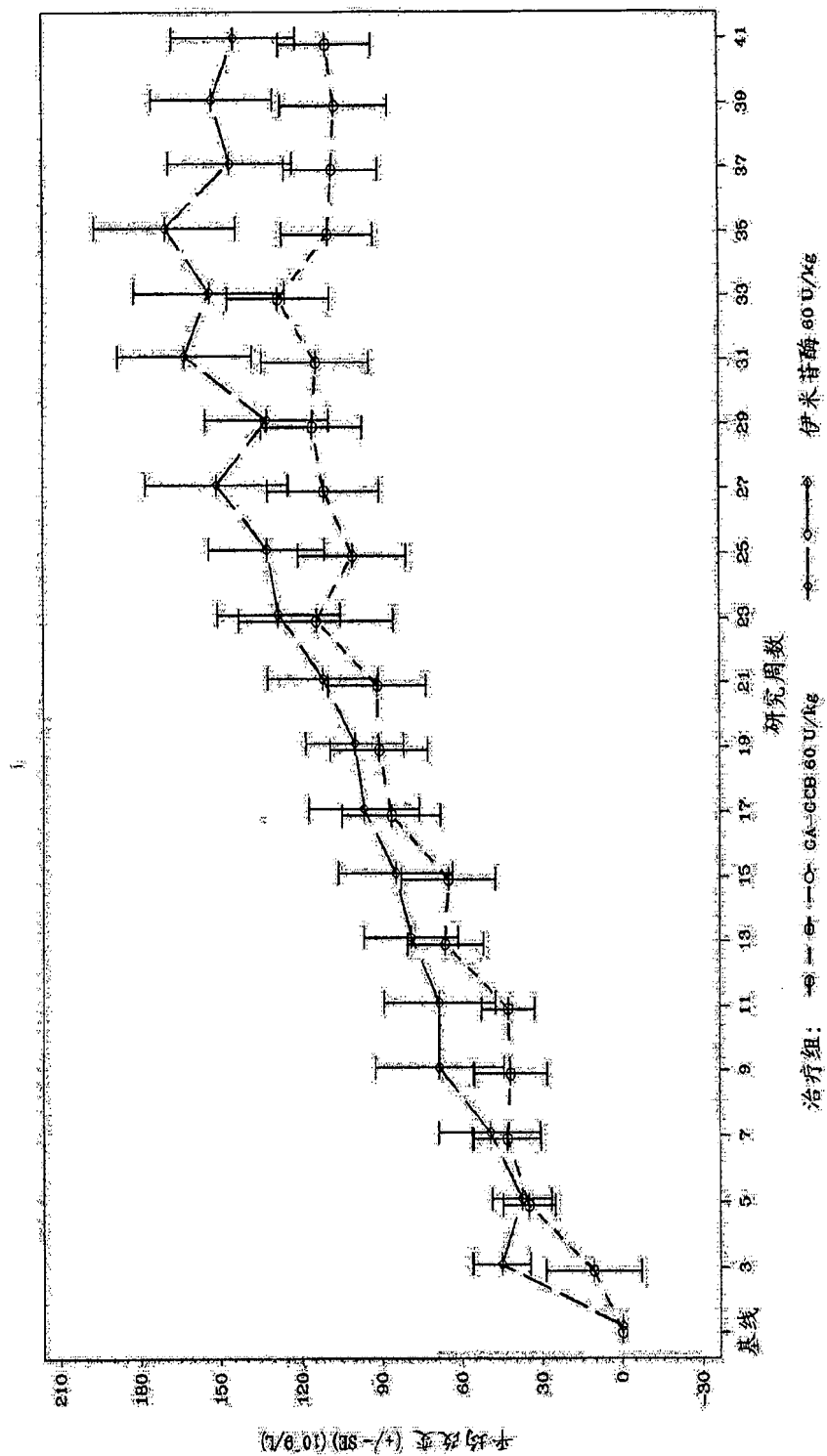


图 12

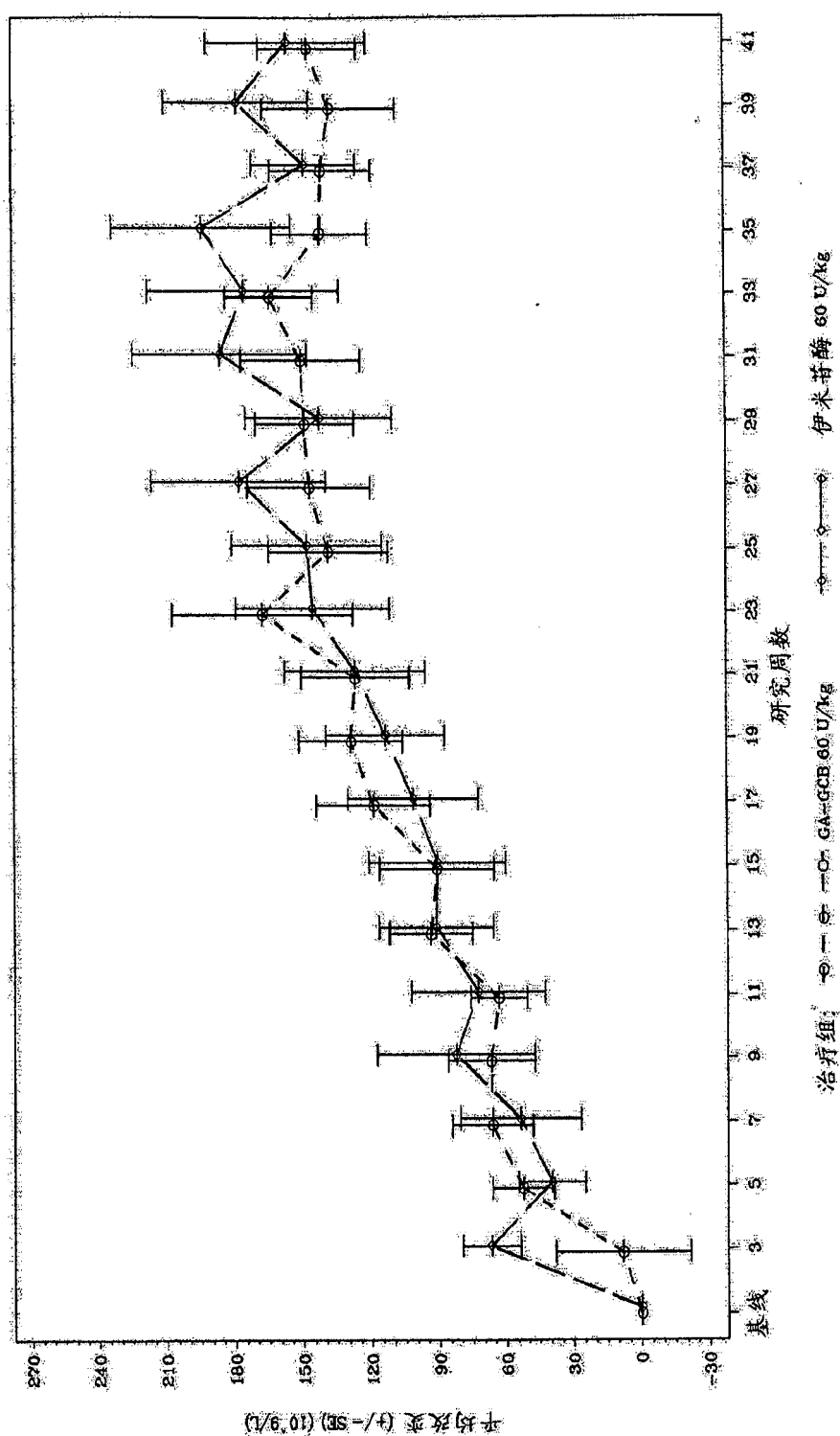


图 13

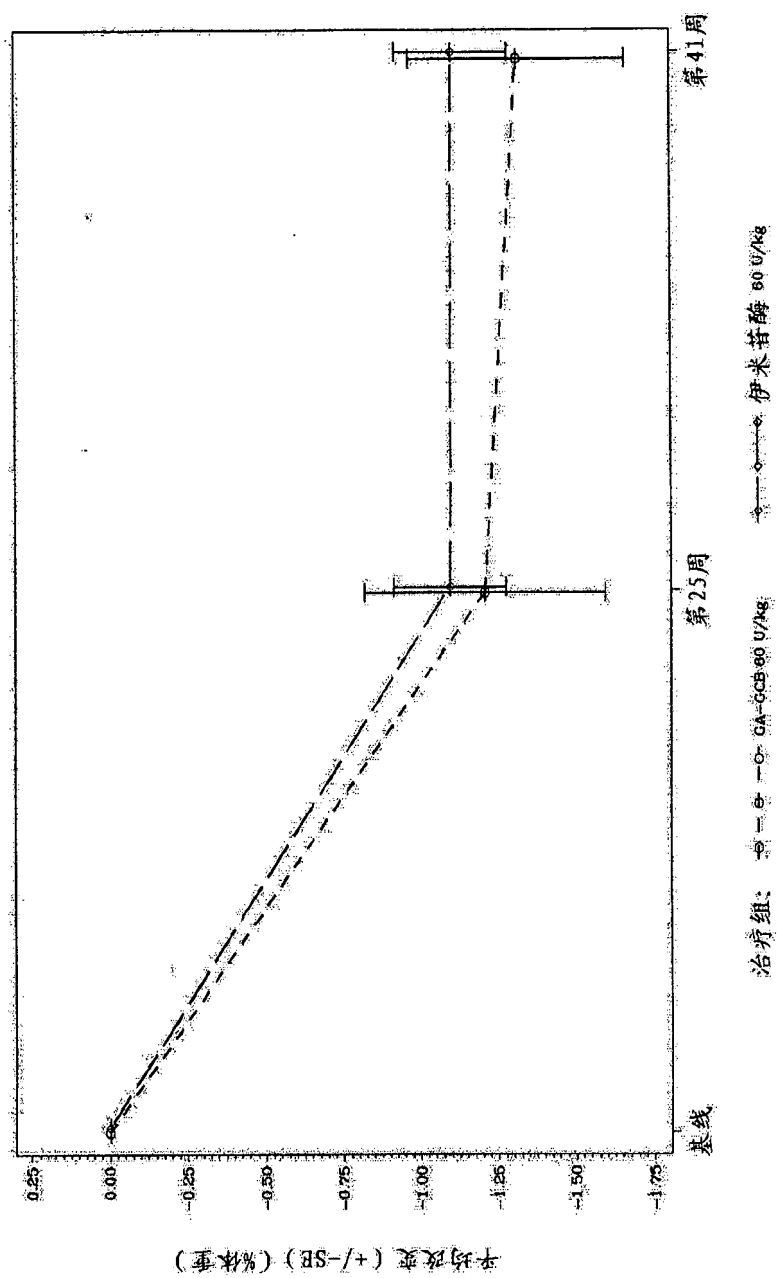


图 14

血红蛋白浓度相对于基线的平均改变

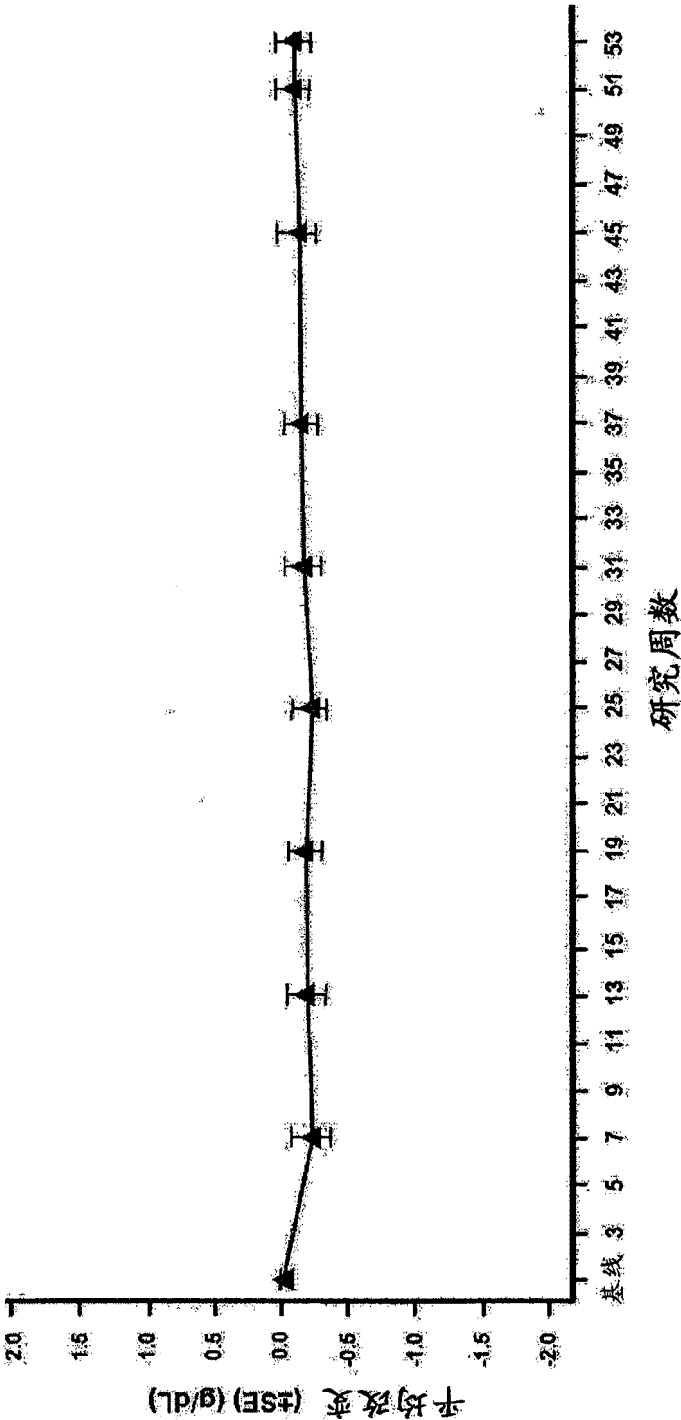


图 15

血小板计数相对于基线的平均改变百分比

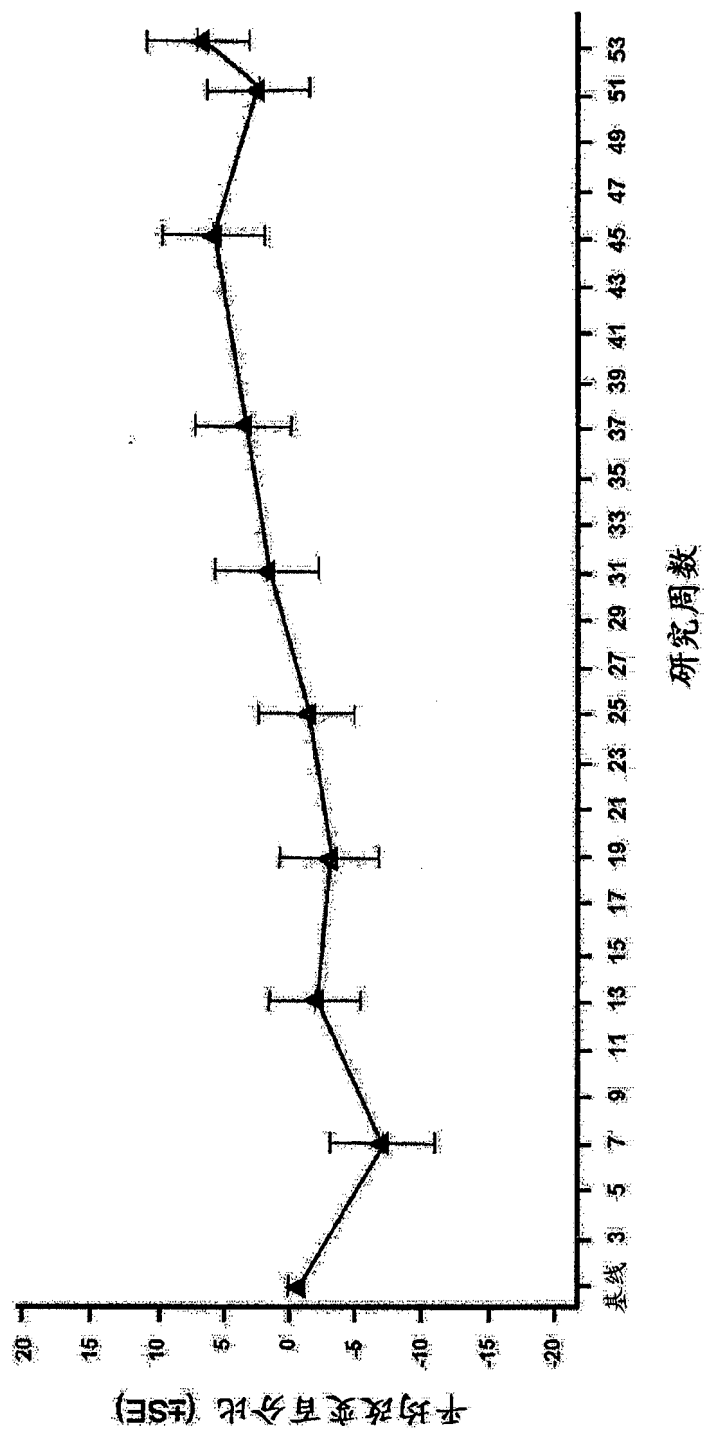


图 16

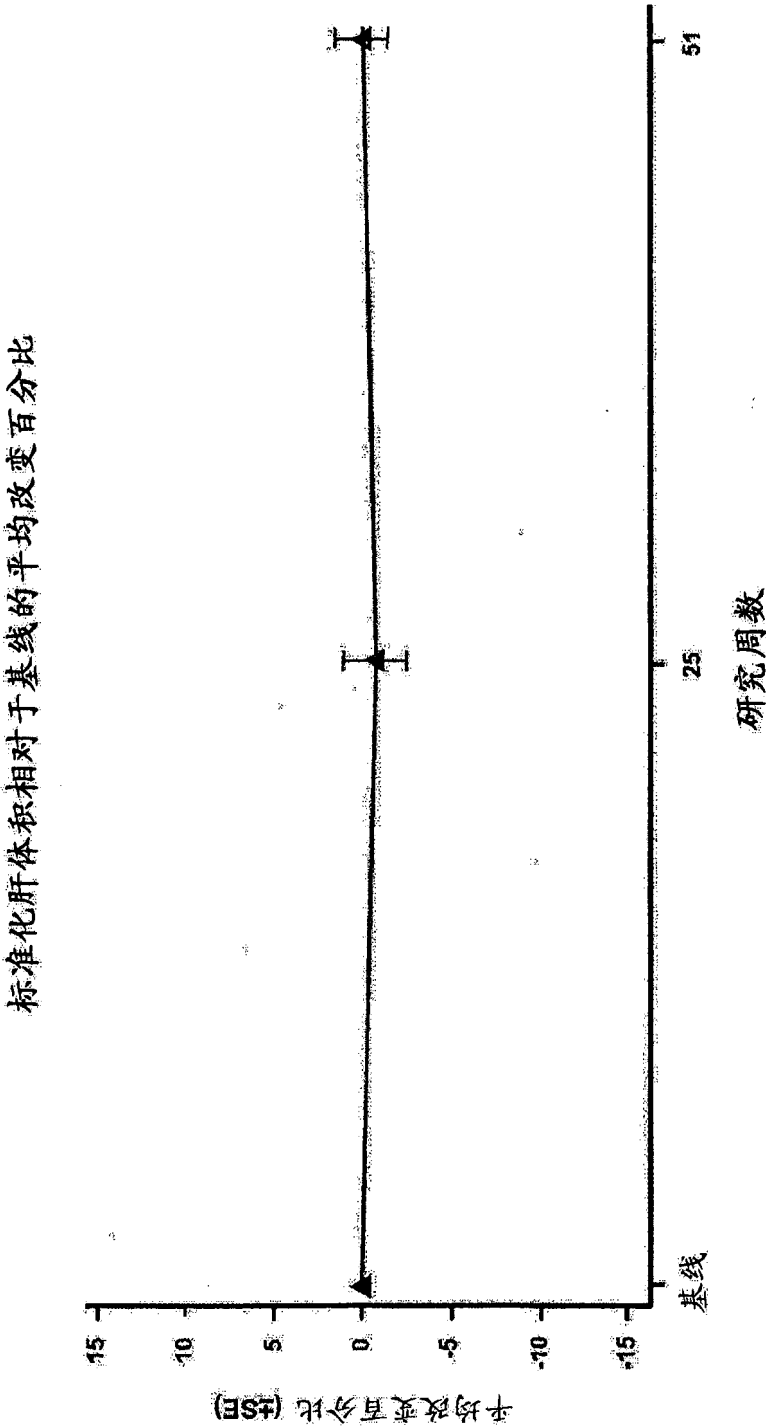


图 17

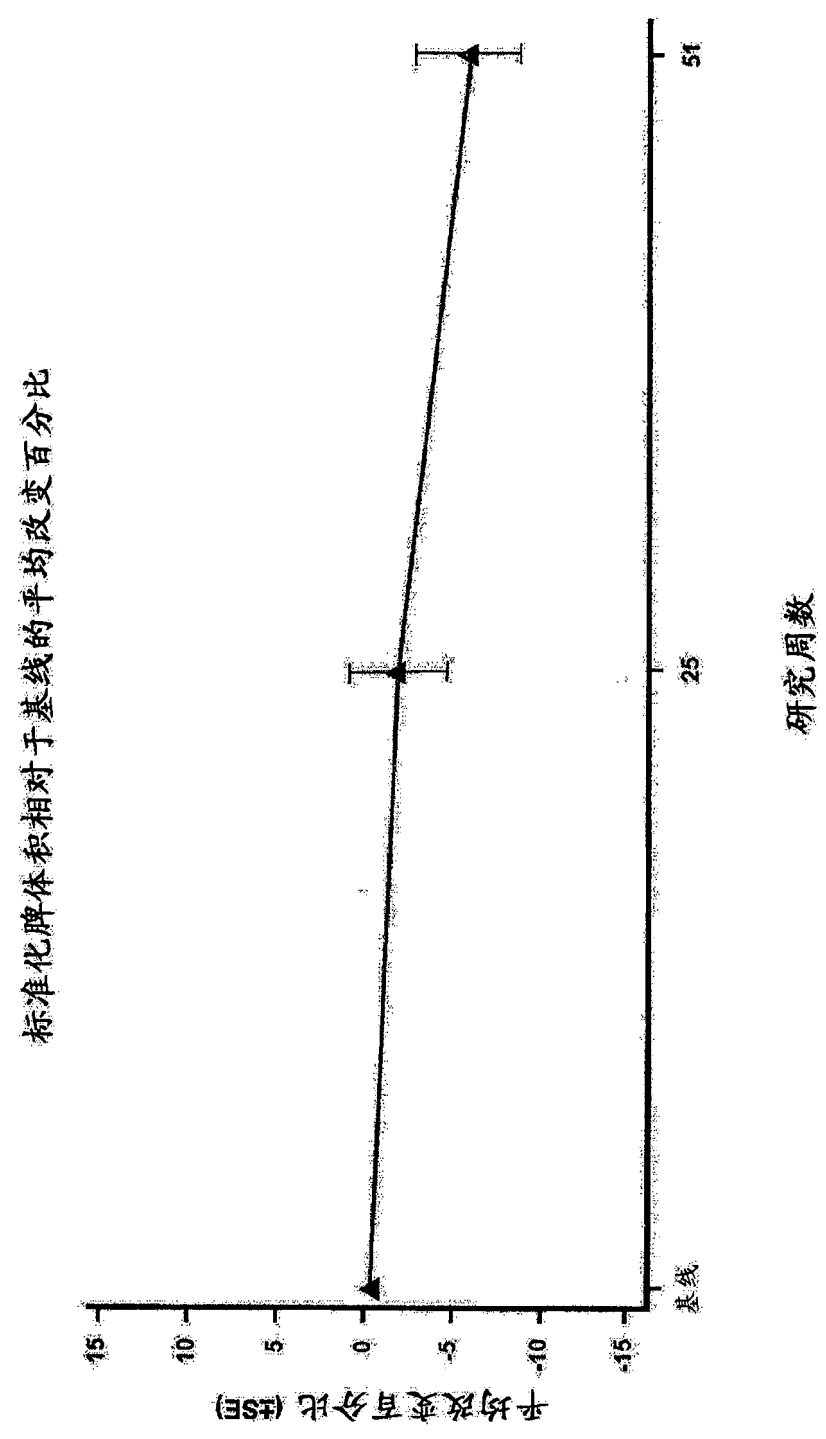


图 18

血浆壳三糖苷酶相对于基线的平均改变百分比

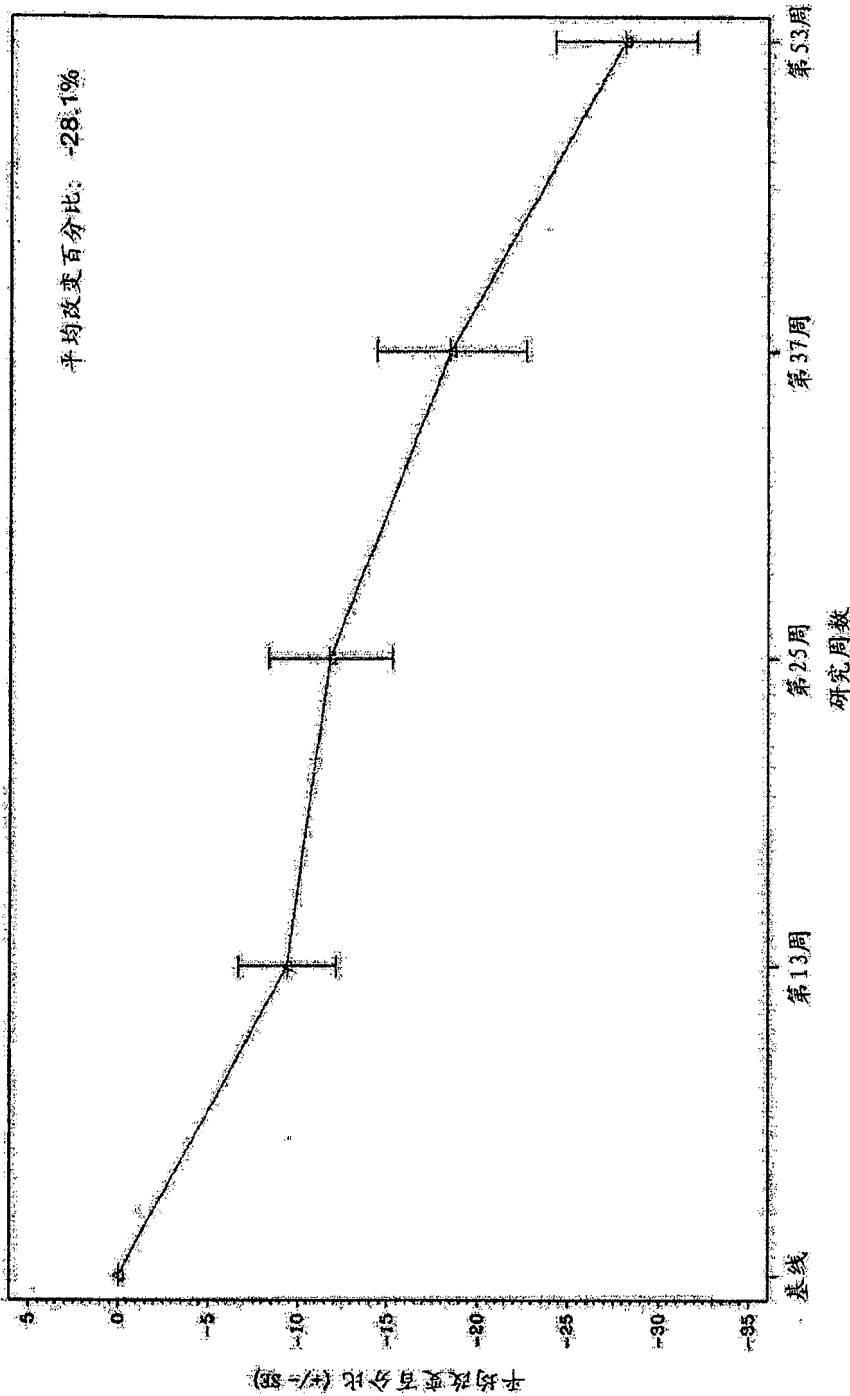


图 19

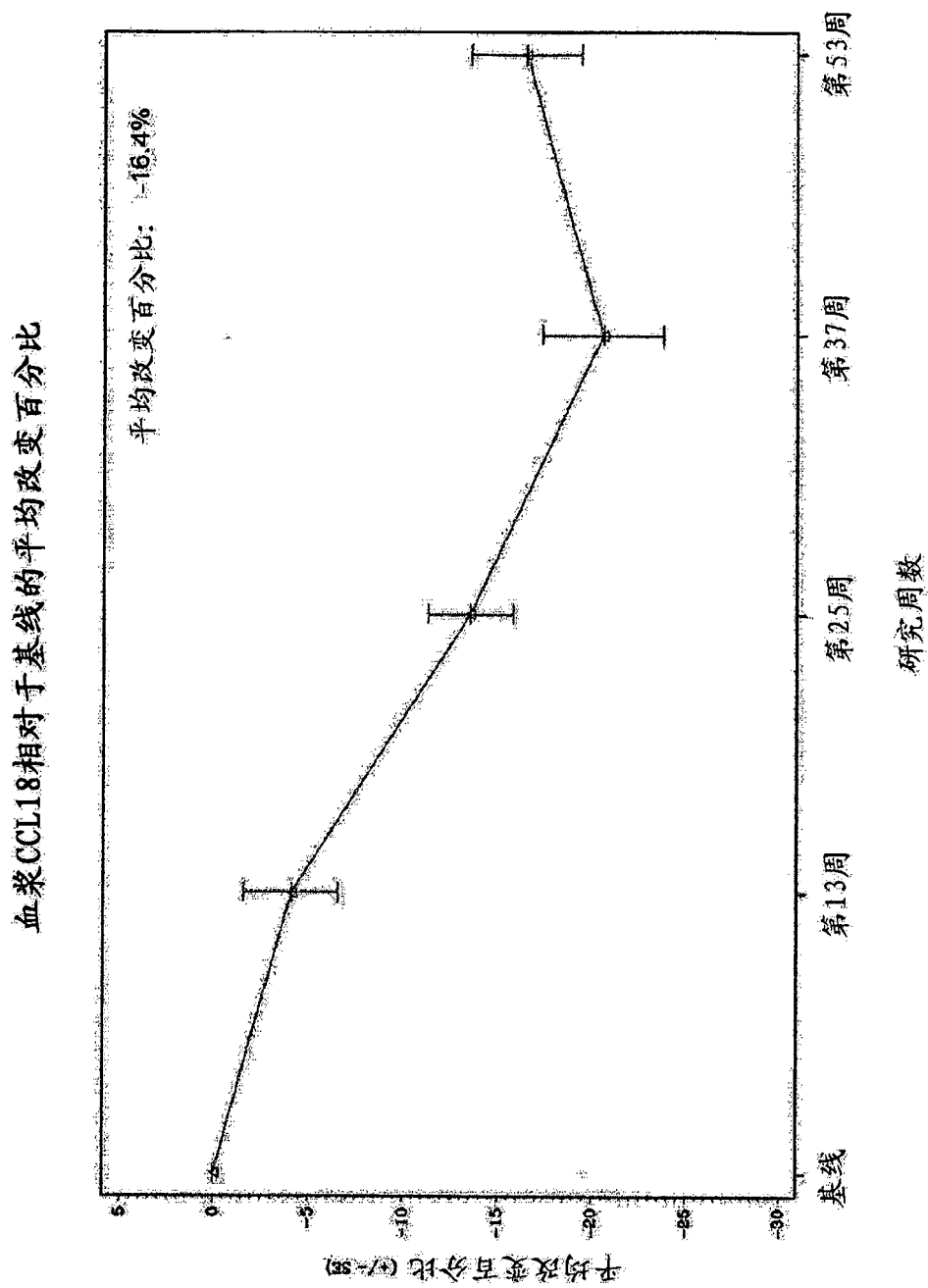
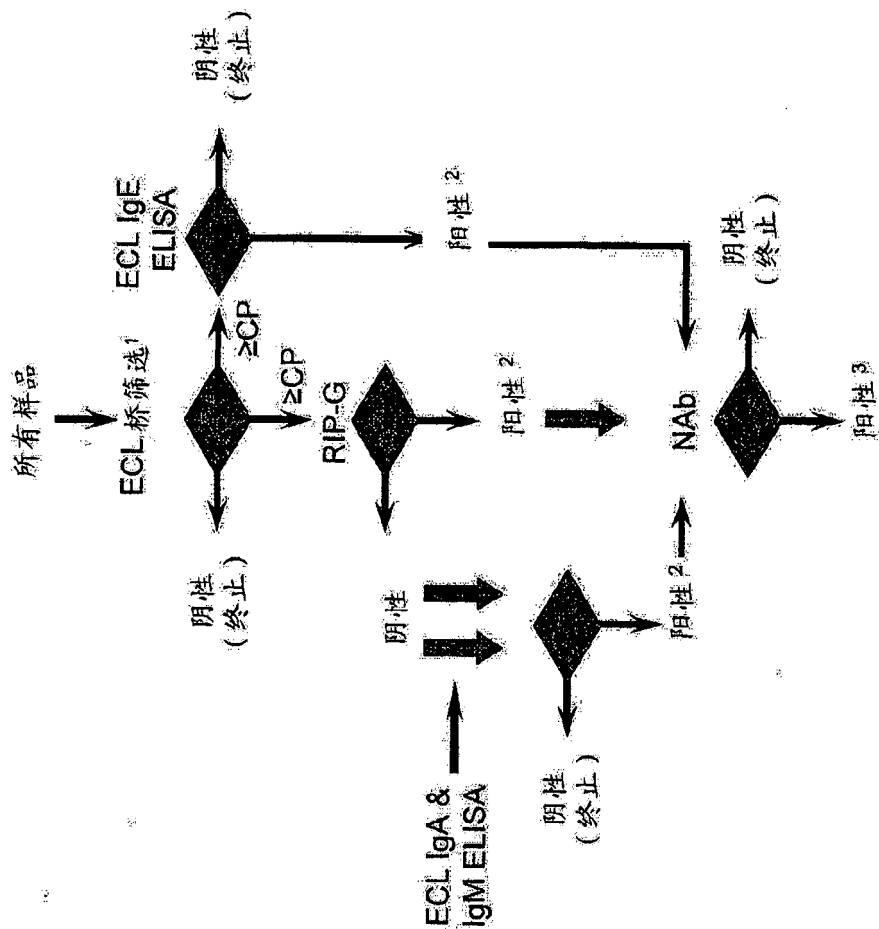


图 20

在维拉昔酶α临床研究中患者的免疫原性评估



¹ 以ng/ml表示的阳性截止点 (CP) ; ² 报道滴度; ³ 报道%NAb

图 21

通过电化学发光 (ECL) 免疫测定进行抗药物抗体筛选

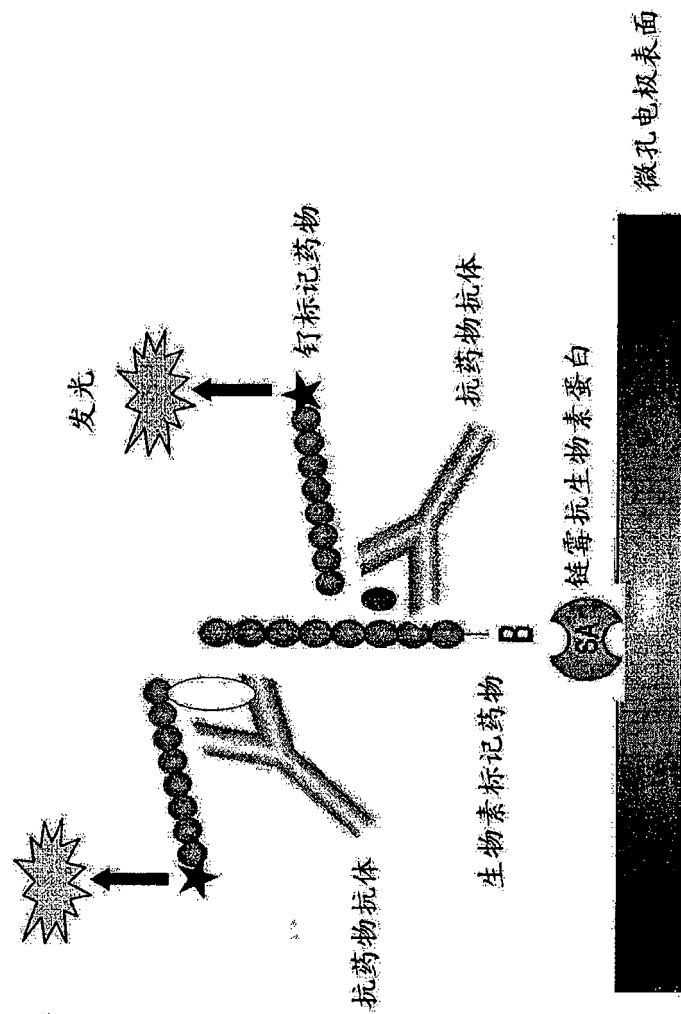


图 22

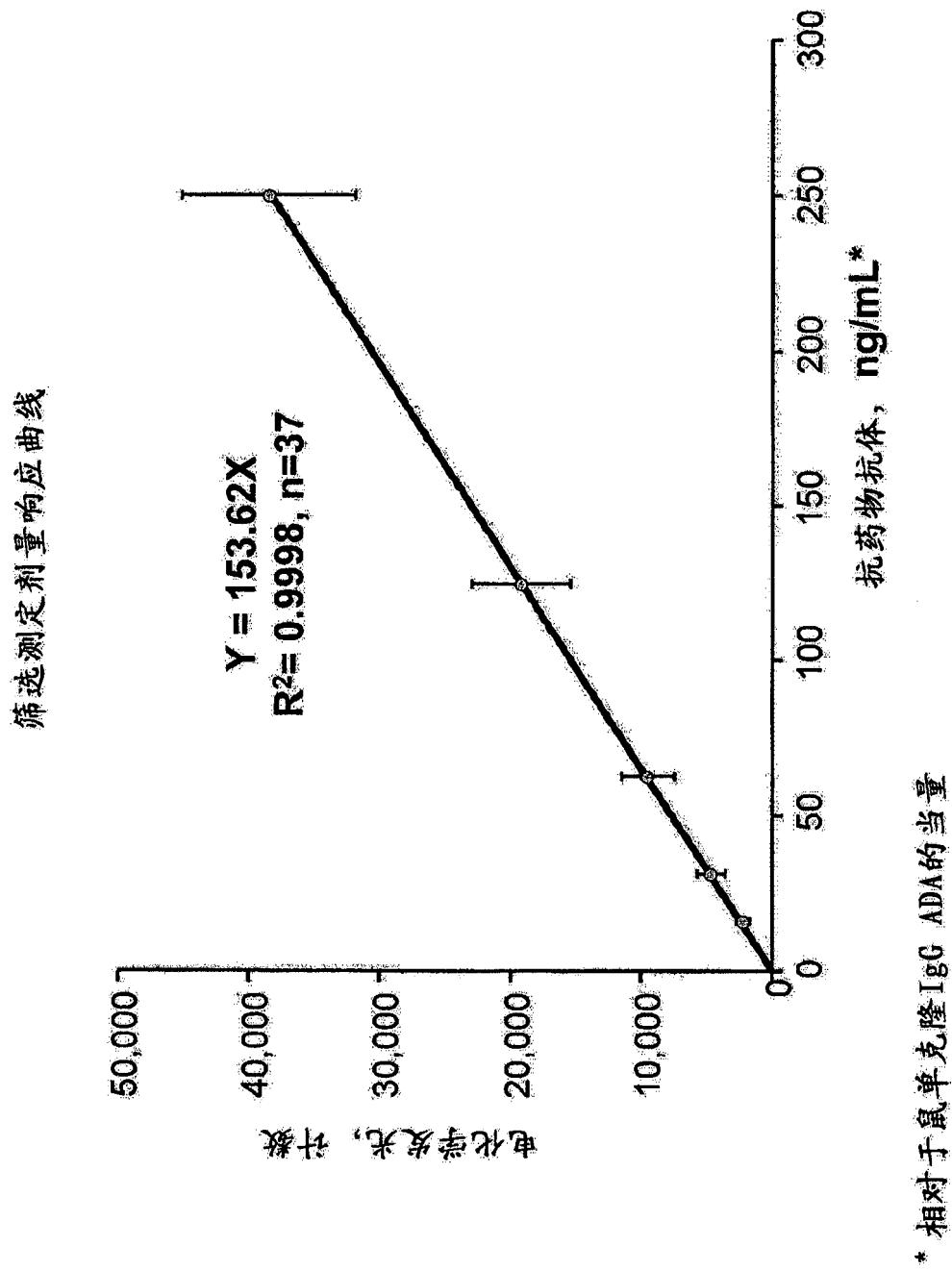


图 23

IgG ADA 验证性测定

放射免疫沉淀 (RIP) 测定



图 24

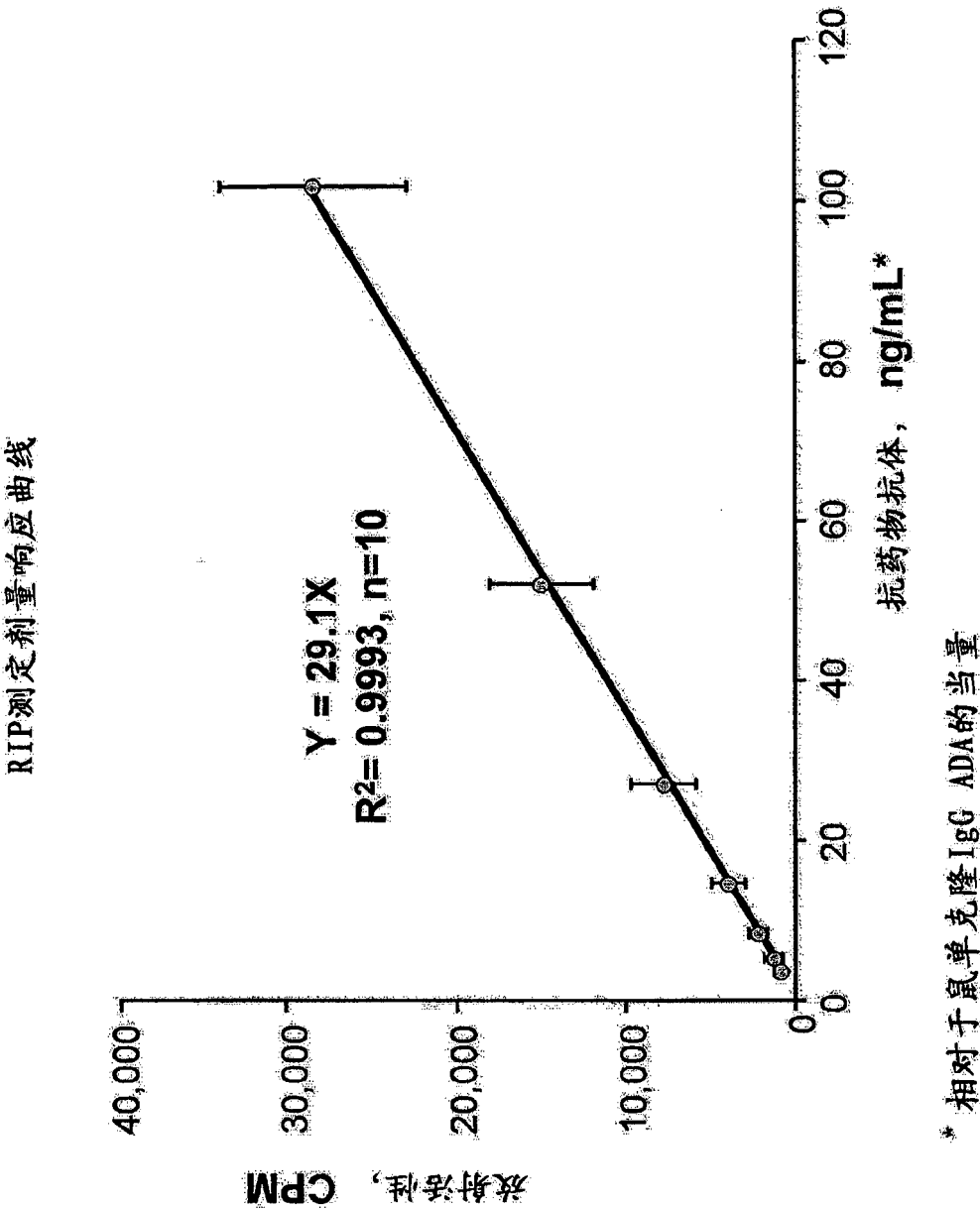


图 25

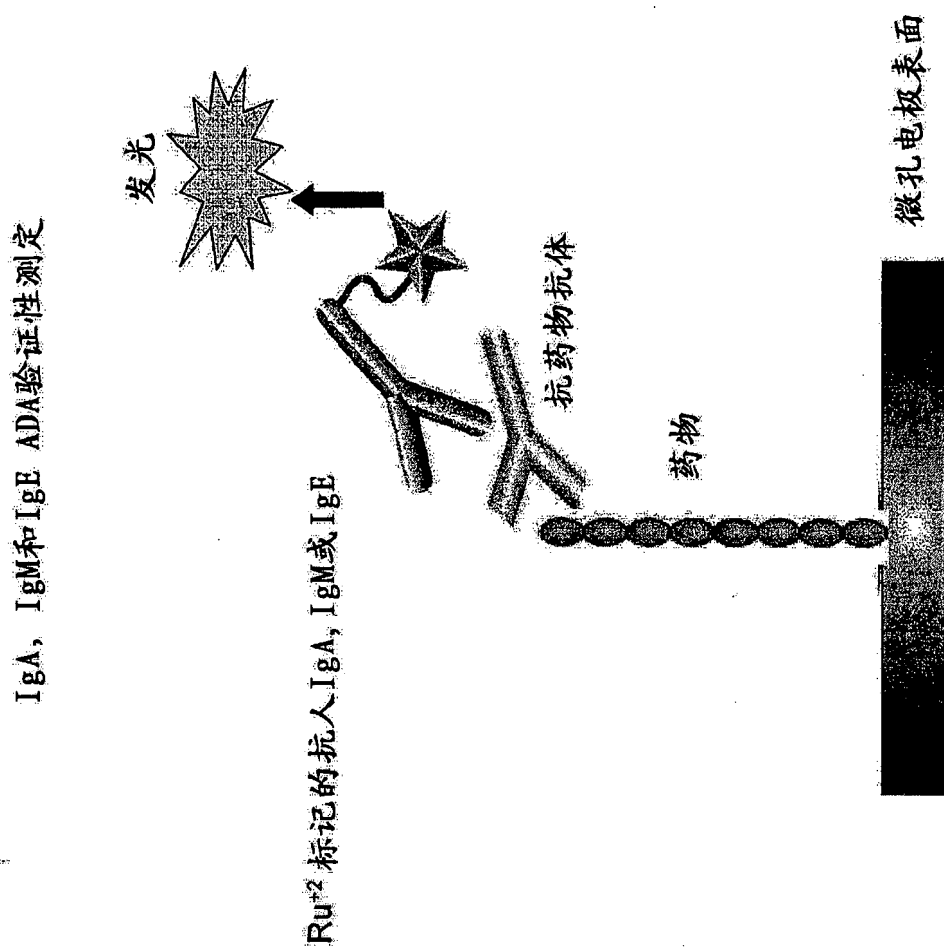


图 26