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(54) **Title**: MYOSTATIN INHIBITORS FOR TREATMENT OF DIABETES

(57) **Abstract**: Disclosed are methods of treating or modulating diabetes in an ESRD patient by administration of a Pinta (745) myostatin antagonist.

TITLE

[0001] Myostatin inhibitors for treatment of diabetes.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Patent Application No. 62/006,780, filed June 2, 2014, the disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0003] The invention relates to methods of treatment and/or ameliorating a condition of diabetes in a human subject with End Stage Renal Disease.

SEQUENCE LISTING

[0004] The instant application contains a Sequence Listing in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy is named 29654PCT_CRF_SequenceListing.txt.

BACKGROUND OF THE INVENTION

[0005] End Stage Renal Disease (ESRD) has become an increasingly important global public health concern with the current estimated hemodialysis population of more than 2 million people predicted to increase by 7% annually (Jha et al. 2012). The major risk factors for chronic kidney disease (CKD) and subsequent ESRD, such as diabetes, hypertension, obesity, cardiovascular disease and smoking, are becoming common throughout the world.

[0006] ESRD patients typically undergo Maintenance Hemodialysis (MHD). Patients on MHD have high rates of morbidity and mortality; in the United States (US), approximately one in five will die ≤ 1 year after commencing dialysis (US Renal Data System 2012). In addition, quality of life for patients on hemodialysis is impaired by a low tolerance for physical activity, low endurance and impaired muscle strength (Storer et al. 2005; Lewis et al. 2012). The risk for hospitalization and death are increased in those with low muscle mass and low serum albumin levels (Kalantar-Zadeh et al. 2001; Kalantar-Zadeh et al. 2004; Huang et al. 2010; Lopes et al. 2010; Noori et al. 2010).

[0007] As described in more detail below, this syndrome of muscle wasting and poor nutritional state in ESRD patients is termed Protein Energy Wasting (PEW) (Fouque et al. 2008). Although nutrition supplements are often administered according to consensus guidelines (Ikizler et al. 2013), the metabolic and catabolic alterations of dialysis and underlying uremia render increased food intake alone unable to correct the pathophysiology

of PEW (Carrero et al. 2013). PEW increases the risk for infections, cardiovascular disease complications, frailty, depression and mortality.

[0008] Therapies with the potential ability to increase sensitivity to insulin and improve therapeutic outcome, including survival, and are urgently needed for ESRD and diabetes patients including those on maintenance hemodialysis.

[0009] Myostatin is a transforming growth factor–β (TGF-β) family member that is expressed mainly in skeletal muscle and acts as a negative regulator of muscle growth (Roth et al. 2004; McPherron 2010). Guo et al (Diabetes 61:2414-2423, 2012) described myostatin inhibition in a mouse model of lipodystrophy. Bonala et al (JBC 289:7654-7670, 2014) described myostatin induced insulin resistance in response to high calorie diet intake.

SUMMARY OF THE INVENTION

[0010] Disclosed herein are methods of treatment of diabetes by administration of a myostatin inhibitor, e.g., the dimeric peptibody Pinta 745. In an embodiment, the method provides improved glycemic control to the patient. In an embodiment, the myostatin inhibitor is used to treat a patient having protein energy wasting. In an embodiment, the myostatin inhibitor is used to treat a patient having end stage renal disease. In an embodiment, the myostatin inhibitor is used to treat a patient undergoing maintenance hemodialysis.

[0011] In an embodiment of the invention, provided herein is a method of treating diabetes in a human subject with End Stage Renal Disease (ESRD) and/or ameliorating a condition of diabetes in the subject comprising administering to the subject a therapeutically effective amount of a myostatin inhibitor, wherein the myostatin inhibitor comprises Pinta 745, thereby treating diabetes in the subject.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0012] The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

[0013] Figure 1 shows data for screening and week 12 insulin sensitivity and beta cell function.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0014] Effective amount. The term "effective amount" refers to an amount of a composition an amount that is effective to ameliorate a symptom of a disease, e.g., muscle wasting. An effective amount can be a therapeutically effective amount or a prophylactically effective amount. An effective amount of a pharmaceutical composition will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient.

[0015] Inhibition. The term "inhibition of a protein" refers to a reduction in the activity of a protein, e.g., myostatin,. The inhibition can be direct or indirect. The inhibition can be, e.g., via a reduction in the expression level, stability, or activity of the protein, or of the mRNA encoding the protein.

[0016] Mammal. The term "mammal" as used herein includes both humans and non-humans and includes but is not limited to humans, non-human primates, non-primate mammals, canines, felines, murines, bovines, equines, and porcines.

[0017] Muscle wasting. The term "muscle wasting" refers to any amount of muscle atrophy and/or muscle loss in a subject. As used herein the term "cachexia" refers to the condition of accelerated muscle wasting and loss of lean body mass resulting from a number of diseases such as those described herein.

[0018] End stage renal disease (ESRD). As described in more detail herein, end-stage kidney disease is a condition when the kidneys are no longer able to work at a level needed for day-to-day life. The most common causes of ESRD in the U.S. are diabetes and high blood pressure. ESRD almost always comes after chronic kidney disease.

[0019] Protein energy wasting (PEW). As described in more detail below, PEW is a syndrome of muscle wasting and poor nutritional status in ESRD patients.

[0020] Homeostatic Model Assessment–Insulin Resistance (HOMA-IR). As described in more detail herein, HOMA-IR is a method used to quantify insulin resistance derived by use of the insulin-glucose product divided by a constant.

[0021] Hemoglobin A1c (HbA1c). The hemoglobin A1c test is a measure of the amount of sugar in blood cells by measuring the percentage of hemoglobin that has become glycated.

This test can serve as a marker for average blood sugar levels during the life of the blood cells.

[0022] Myostatin. Myostatin, a growth factor also known as GDF-8, is a member of the TGF-ß family. Myostatin known to be a negative regulator of skeletal muscle tissue. Myostatin is synthesized as an inactive preproprotein which is activated by proteolyic cleavage (Zimmers et al., supra (2002)). The precurser protein is cleaved to produce an NH2-terminal inactive prodomain and an approximately 109 amino acid COOH-terminal protein in the form of a homodimer of about 25 kDa, which is the mature, active form (Zimmers et al, supra (2002)). It is now believed that the mature dimer circulates in the blood as an inactive latent complex bound to the propeptide (Zimmers et al, supra (2002)). Full-length myostatin refers to the full-length human preproprotein sequence described in McPherron et al. PNAS USA 94, 12457 (1997), as well as related full-length polypeptides including allelic variants and interspecies homologs (McPherron et al. supra (1997)). As used herein the term "myostatin" or "mature myostatin" refers to the mature, biologically active COOH-terminal polypeptide, in monomer, dimer, multimeric form or other form. "Myostatin" or "mature myostatin" also refers to fragments of the biologically active mature myostatin, as well as related polypeptides including allelic variants, splice variants, and fusion peptides and polypeptides. Myostatin may or may not include additional terminal residues such as targeting sequences, or methionine and lysine residues and /or tag or fusion protein sequences, depending on how it is prepared.

[0023] Subject. The term "subject" or "individual" is a mammal. In one embodiment, a subject is a human.

[0024] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition/Explanation
6MWT	6-minute walk test
ALM	Appendicular Lean Mass
BMI	Body mass index
BUN	Blood urea nitrogen
CI	Confidence interval
CKD	Chronic kidney disease
CL	Clearance
CRP	C-reactive protein

Abbreviation	Definition/Explanation
DXA	Dual-energy X-ray absorptiometry
eCRF	Electronic case report form
ESRD	End stage renal disease
FAACT	Functional Assessment of Anorexia/Cachexia Treatment
FACIT	Functional Assessment of Chronic Illness Therapy
GDF	Growth differentiation factor
HbA1c	Hemoglobin A1c / glycated hemoglobin
HOMA	Homeostatic model assessment
IL-6	Interleukin-6
IR	Insulin resistance
IV	Intravenous(ly)
KDQOL™	Kidney Dialysis Quality of Life™
LBM	Lean body mass
mCCI	Modified Charlson comorbidity index
MHD	Maintenance hemodialysis
nPCR	Normalized protein catabolic rate
PDW	Post-dialysis weight
PEW	Protein energy wasting
PK	Pharmacokinetic(s)
PRO	Patient-reported outcomes
SCPT	Stair climbing power test
TEAE	Treatment-emergent adverse event
TGF-β	Transforming growth factor -β
TNF-α	Tumor necrosis factor-α

Protein energy wasting in ESRD patients

[0025] Disclosed herein are methods for treating protein energy wasting in ESRD patients. Protein energy wasting, or PEW, is a syndrome of muscle wasting and poor nutritional status in ESRD patients leading to low muscle mass, low serum albumin levels, impaired quality of life, low tolerance for physical activity, low endurance, impaired muscle strength, increased risk for hospitalization, prolonged hospitalization/poor outcomes, increased risk for infections, cardiovascular disease complications, frailty, depression and mortality. PEW is not only associated with muscle breakdown and loss of physical function but also high degrees of inflammation and uremia (high blood levels nitrogen) uncorrected by dialysis which may play a role in a decrease in platelet blotting.

[0026] The nature of PEW is different from other muscle wasting syndromes such as cachexia resulting from cancer, as treatment with androgenic hormones have not worked for PEW. Additional differences include that cancer cachexia is typically a result of inadequate nutritional intake (anorexia), whereas PEW can result even in the face of protein intake that would be adequate for an unaffected person. In PEW this may be because abnormal proteolysis of the muscle is a key factor (Proteolytic mechanisms, not malnutrition, cause loss of muscle mass in kidney failure. Mitch WE J Ren Nutr. 2006 Jul; 16(3):208-11.) that can outweigh nutrition. In addition, PEW manifests a large component of inflammation, whereas cachexia is not always associated with inflammation

Serum albumin levels

[0027] In some embodiments, the ESRD patient having PEW is selected for treatment by measurement of blood serum albumin levels. Serum albumin levels can be determined by any method well known to one of skill in the art and/or any method typically performed by a clinical laboratory. Examples include but are not limited to bromocresol green methods and bromocresol purple methods. In some embodiments, the method of treatment includes determining the serum albumin level of the human subject.

[0028] In some embodiments, the human subject selected for treatment has a blood serum albumin level of less than or equal to 3.8 g/dL. In other embodiments, the human subject selected for treatment has a blood serum albumin level of less than or equal to 3.7 or 3.6 or 3.5 g/dL. In some embodiments, the human subject has a serum albumin level $\leq$ 3.8 g/dL at any time within 60 days prior to start of treatment.

Body mass index

[0029] In some embodiments, the human subject undergoing the method of treatment has a low body mass index (BMI). BMI is determined by dividing weight (in kg) by the square of height (in meters). In one embodiment, the human subject has a BMI of $\leq$ 28 kg/m^2, based on a post-dialysis weight and a height of the human subject. In other embodiments, the human subject has a BMI of <=25 or a BMI of <=23. In some embodiments, the method of treatment includes determining the BMI of the human subject.

Hemodialysis

[0030] In some embodiments, the ESRD patient treated with Compound 745 is undergoing hemodialysis. In some embodiments, the ESRD patient is undergoing

maintenance hemodialysis (MHD), e.g., MHD greater than or equal to 3 times per week. In other embodiments, the ESRD patient is undergoing MHD 2, 3, 4, 5, or 6 times per week. In other embodiments, the ESRD patient is undergoing daily hemodialysis, e.g., 2 hours 6 days a week. In other embodiments, the ESRD patient is undergoing nocturnal hemodialysis, e.g., three to six nights per week and between 6 and 10 hours per session while the patient sleeps. In other embodiments, the ESRD patient is undergoing peritoneal dialysis.

[0031] In some embodiments, the ESRD patient treated with Compound 745 is undergoing hemodialysis and is taking a nutritional supplement having at least 10 grams of protein per day. Examples of nutritional supplements include but are not limited to nutritional bars, yoghurt, and the like.

Clinical endpoints

[0032] The methods of treatment disclosed herein results in an improvement in at least one clinical endpoint in the ESRD patient, e.g., at least one improvement in a measurement of a parameter related to PEW. In one embodiment, the method results in a decrease in insulin resistance in the human subject. In one embodiment, the method results in an increase in lean body mass (LBM) in the human subject. Other clinical endpoints include but are not limited to an increase in LBM as determined by Dual-energy X-ray absorptiometry (DXA), an increase in muscle cross sectional area as determined by CT, an increase in appendicular lean mass (ALM), an increase in physical function by the Stair climbing power test (SCPT), an increase in distance on the and 6-minute walk test (6MWT), an increase in quality of life using at least one of three Patient Reported Outcome (PRO tools) (the Kidney Dialysis Quality of Life™ (KDQOL™)-36 short form questionnaire, the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) scale, and the Functional Assessment of Anorexia/Cachexia Treatment (FAACT) anorexia/cachexia subscale). Other clinical endpoints include a reduction in hospitalizations, fractures, and/or cardiovascular events. Additional clinical endpoints that can result from the methods of treatment are described below in the examples.

[0033] In other embodiments, the methods of treatment results in a reduction in Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), a reduction in fasting glucose level, a reduction in fasting insulin level, a decrease in insulin resistance, an increase in beta/cell function, and/or a reduction of effective dose of long acting insulin dose in the human subject. In some embodiments, the methods of treatment result in a reduction of HOMA-IR by at least 0.5%, 1.0%, 1.5%, or 2.0%. In some embodiments, the methods of

treatment result in a reduction of fasting glucose level by at least 10%, 20%, 30%, 40%, or 50%. In some embodiments, the methods of treatment result in a final fasting plasma glucose of between 70 and 130 mg/dL (3.9-7.2 mmol). In some embodiments, the methods of treatment result in a reduction of peak post-prandial glucose levels by at least 20%, 30%, 40%, or 50%. In some embodiments, the methods of treatment result in a peak post-prandial glucose level of less than 180 mg/dL (10 mmol). In some embodiments, the methods of treatment result in a reduction of fasting insulin level by at least 2-fold, 3-fold, 4-fold, or 5-fold. In some embodiments, the methods of treatment result in a fasting insulin level of 1, 2, 3, 4, or 5 mIU/mL. In some embodiments, the methods of treatment result in an increase in insulin sensitivity by at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold. In some embodiments, the methods of treatment result in an increase in beta cell function by at least 12.5%, 25%, 50%, or 100%. In some embodiments, the methods of treatment result in a decrease in an effective dose of long acting insulin by at least 20%, 30%, 40%, 50%, or greater than 50%. In some embodiments, the methods of treatment result in an increase in lean body mass by at least 1%, 2%, 3%, 4%, 5%, or more than 5%.

[0034] In some embodiments, the methods of treatment result in reduction of insulin dose intensity; discontinuation of insulin/no longer meeting criteria for diabetes (i.e. diabetes remission); reduction in fasting blood glucose; reduction in postprandial blood glucose; reduction in fasting insulin; reduction in HOMA-IR corrected for adiponectin; modulation of adipose hormones (adiponectin, resistin, leptin); prevention of progression to diabetes (in prediabetic patients with impaired fasting glucose, gestational diabetes or impaired glucose tolerance); reduction in incidence rate or severity of microvascular complications (e.g. renal failure, retinopathy); or reduction in incidence rate or severity of macrovascular complications (e.g., myocardial infarction, cerebrovascular accident, peripheral vascular disease).

[0035] In other embodiments, the methods of treatment results in a change in at least one biomarker selected from the group consisting of a serum myostatin, C-reactive protein, interleukin-6 (IL-6), tumor necrosis factor- α (TNF-α), adiponectin, leptin, resistin, serum lipid, hemoglobin, and serum albumin. Additional biomarkers are described below in the examples.

[0036] Assays and methods for measuring clinical endpoints can be any that are well known to one of skill in the art and/or are used in a clinical laboratory setting. In some

embodiments, the assays and methods for measuring clinical endpoints are those described in the examples below.

[0037] The clinical endpoints may be measured in absolute values or as a percent change over baseline. The changes will typically be statistically significant, e.g., a two-sided one-sample t-test at the alpha = 0.05 level of significance. In most cases, analysis of variance (ANOVA) or analysis of covariance (ANCOVA) models are used.

Pharmaceutical compositions of the invention

[0038] The methods of the invention include administering an effective amount of a myostatin inhibitor, e.g., Pinta 745. Pinta 745 is an anti-myostatin peptibody disclosed in International application no. PCT/US2003/040781, filed on December 19, 2003 and published as WO/2004/058988. The amino acid sequence of Pinta 745 is found below SEQ ID NO:1.

MDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV 60
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA 120
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD 180
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGGAQLADHG 240
QCIRWPWMCP PEGWE (**SEQ ID NO:1**)

[0039] The compositions of the invention can be formulated in pharmaceutical compositions. These compositions can comprise, in addition to one or more of the inhibitors, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material can depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

[0040] Pharmaceutical compositions for oral administration can be in tablet, capsule, powder or liquid form. A tablet can include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol can be included.

[0041] In the methods of the invention, the pharmaceutical composition may be administered in a solution. A pharmaceutical composition may be administered in an unbuffered solution, e.g., in saline or in water. Alternatively, the pharmaceutical composition may also be administered in a suitable buffer solution. The buffer solution may comprise

acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In a preferred embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution can be adjusted such that it is suitable for administering to a subject. Other components of a liquid pharmaceutical composition include detergents and compounds with detergent properties.

[0042] In some embodiments, the buffer solution further comprises an agent for controlling the osmolarity of the solution, such that the osmolarity is kept at a desired value, *e.g.*, at the physiologic values of the human plasma. Solutes which can be added to the buffer solution to control the osmolarity include, but are not limited to, proteins, peptides, amino acids, non-metabolized polymers, vitamins, ions, sugars, metabolites, organic acids, lipids, or salts. In some embodiments, the agent for controlling the osmolarity of the solution is a salt. In certain embodiments, the agent for controlling the osmolarity of the solution is sodium chloride or potassium chloride or sodium acetate. For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives can be included, as required.

[0043] The administration of the compositions is in an "effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

[0044] A composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Kits

[0045] Also described herein are kits for treatment of diabetes in a human subject with End-Stage Renal Disease (ESRD) comprising a vial comprising Pinta 745 and instructions

for use. The myostatin inhibitor can be in any suitable pharmaceutical composition as described herein, e.g., a liquid suspension suitable for IV infusion. Alternatively, the myostatin inhibitor can be in a lyophilized state suitable for re-suspension before use. The instructions for use can include storage instructions, patient selection, dosages, administration methods, time periods for use, clinical endpoints, and the like.

Polypeptides and polynucleotides

[0046] In some embodiments, the compositions of the invention include a polypeptide, e.g., an antibody or peptibody, e.g., Pinta 745. Exemplary sequences are disclosed herein.

[0047] In some embodiments, the compositions of the invention include a polypeptide or polynucleotide that is less than 100% identical to a amino acid or nucleic acid sequence disclosed herein. In some embodiments, the polypeptide or polynucleotide is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or between 99 and 100% identical to a sequence disclosed herein.

[0048] The term "percent identical" in the context of two or more amino acid or nucleic acid sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent identity can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

[0049] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0050] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and

TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., infra).

[0051] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).

Variants

[0052] The compositions described herein also encompass variants of the polypeptides described herein. As used herein, the term "variants" refers to polypeptides having one or more amino acid residues inserted, deleted or substituted into the original amino acid sequence and which retain at least a portion of the function of the polypeptide described herein. As used herein, fragments of the polypeptides are included within the definition of "variants". It is understood that any given peptide or peptibody may contain one or two or all three types of variants. Insertional and substitutional variants may contain natural amino acids, as well as non-naturally occurring amino acids or both. Variants can include, e.g., polypeptides that include a leader or signal sequence; polypeptides with additional amino terminal residues, e.g., Met1 or Lys 2; polypeptides with expression tags, e.g., histidine tags; and polypeptides expressed as fusion proteins.

[0053] Variants of the polypeptides described herein can include amino acid substitutions. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional (naturally occurring) amino acids, non-naturally occurring amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of non-naturally occurring amino acids include, for example: aminoadipic acid, beta-alanine, beta-aminopropionic acid, aminobutyric acid, piperidinic acid, aminocaprioic acid, aminoheptanoic acid, aminoisobutyric acid, aminopimelic acid, diaminobutyric acid, desmosine, diaminopimelic acid, diaminopropionic acid, N-ethylglycine, N-ethylaspargine, hyroxylysine, all0-hydroxylysine, hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, sarcosine, N-methylisoleucine, N-methylvaline, norvaline, norleucine, orithine, 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-

acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and amino acids (*e.g.*, 4-hydroxyproline).

[0054] Naturally occurring residues may be divided into (overlapping) classes based on common side chain properties:

1) neutral hydrophobic: Met, Ala, Val, Leu, Ile, Pro, Trp, Met, Phe;
2) neutral polar: Cys, Ser, Thr, Asn, Gln, Tyr, Gly;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

[0055] Substitution with naturally occurring amino acids can be conservative or non-conservative. Conservative amino acid substitutions involve exchanging a member of one of the above classes for another member of the same class. Conservative changes may encompass unconventional amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties.

EXAMPLES

[0056] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[0057] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Example 1: Composition Pinta 745.

[0058] Pinta 745 is an anti-myostatin peptibody. A peptibody represents the component peptide (the "pepti-") and the Fc portion of an immunoglobulin in an overall structure that resembles an antibody (the "-body"). In this format, the peptide "warhead" interacts with myostatin and inhibits signaling through its receptor. The second domain, the Fc component, stabilizes the complex in the body, allows for endothelial cell trancytosis and recycling through FcRn1 and extends residence time into a therapeutically useful range.

[0059] Pinta 745 consists of 2 identical polypeptide chains, which are covalently linked through disulfide bonds. The N-terminal portion of each chain consists of the human IgG1 Fc sequence which is fused at the C-terminus via a glycine (five glycines plus AQ) linker to an anti-myostatin peptide. Each polypeptide chain consists of 255 amino acids beginning with the amino acid methionine and ending with glutamic acid. There are 3 intrachain disulfide links between residues Cys^{42}-Cys^{102}, Cys^{148}-Cys^{206}, and Cys^{242}-Cys^{249} on each polypeptide chain and 2 interchain disulfide links between Cys^{7}_{chain1}-Cys^{7}_{chain2}, and Cys^{10}_{chain1}-Cys^{10}_{chain2}. The 510 amino acids that constitute the Pinta 745 molecule yield a theoretical molecular mass of 57,099 daltons. As a microbially expressed protein, Pinta 745 is not glycosylated.

[0060] The 255 residue amino acid sequence of each polypeptide chain (SEQ ID NO:1) of Pinta 745 is shown below. The plain font portion of the sequence indicates the IgG1 Fc sequence. The bold font portion indicates the five glycine plus AQ linker sequence (GGGGGAQ; SEQ ID NO:2). The bold and italic portion of the sequence indicates the anti-myostatin peptide (LADHG QCIRWPWMCP PEGWE; SEQ ID NO:3).

Pinta 745 Sequence (amino acid)
MDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK**GG GGGAQ*LADHG QCIRWPWMCP PEGWE* (SEQ ID NO:1)**

Expression and Preparation

[0061] Pinta 745 was expressed as insoluble inclusion bodies by fermentation of E coli (e.g., pAMG -Fc fusion constructs in E. coli strain 2596) as described in International patent application nos. PCT/US2003/040781 filed on December 19, 2003 (WO/2004/058988) and PCT/US2006/046546 filed on December 6, 2006 (WO/ 2007/067616).

[0062] Typical fermentation proceeds for 12 to16 hours post induction, followed by cell harvest with a disk-stack centrifuge. Lysing the cells with high-pressure homogenization isolated the inclusion bodies. After wash and centrifugation, the resulting double-washed inclusion body slurry (DWIBs) was stored at -30° ± 10°C until purification.

[0063] Following solubilization of the DWIBs, Pinta 745 was refolded in a solution containing urea, glycerol, arginine, and the redox pair cysteine/cystamine. After refolding, the product was concentrated and the refold reagents removed by means of an ultrafiltration and diafiltration (UF/DF) process. The diafiltered product was acidified, followed by clarification. The product was subsequently purified through 3 different chromatography steps: 2 anion-exchange (Q Sepharose Fast Flow) columns, one operated in flow-through mode and one in bind and elute mode, and a HIC (Butyl Sepharose Fast Flow) column. The product was then further concentrated and diafiltered into formulation buffer with a UF/DF process. The formulated product was then filtered through a 0.2 μm filter into bulk containers and frozen at -30° ± 10°C.

[0064] An additional chromatography step was added to the purification process for the clinical drug substance process to remove host cell-related impurities.

Formulation

[0065] The final dosage formulation for Pinta 745 at 30 mg/mL was 10 mM sodium acetate, 9% (w/v) sucrose, 0.004% (w/v) polysorbate 20, pH 4.75.

Drug Supply and Storage

[0066] Compound 745 was stored in a non-frost-free freezer set between −20° and −70°C. A range of −20 (+5)°C to −70 (−10)°C is acceptable to accommodate fluctuations in the temperature of the freezer. Exposure to higher temperatures and vigorous shaking of the vial should be avoided because these conditions may lead to loss of Compound 745 potency and structural integrity.

Example 2: Treatment of Protein Energy Wasting in End-Stage Renal Disease Patients.

[0067] A study was conducted with Pinta 745 in patients with end stage renal disease (ESRD) who require maintenance hemodialysis (MHD) and have evidence of protein energy wasting (PEW). The goals of the study included evaluation of the safety, dose-limiting toxicities, maximum tolerated dose, and pharmacokinetics of Pinta 745, to evaluate

percentage change in lean body mass, and to evaluate changes in muscle function as measured by stair climbing power, 6-minute walk test, or grip strength.

[0068] This was a pilot, multicenter, double-blind, randomized, dose-escalation study of Pinta 745 in adults with ESRD who require MHD and have evidence of PEW.

Pinta 745

[0069] For the purposes of this application, Pinta 745, AMG 745, and PINTA 745 refer to the same composition. Pinta 745 is expressed and prepared using the same methods as in Example 1. Pinta 745 is provided as a liquid for intravenous (IV) administration, in a sterile vial containing approximately 3 mL of 30 mg/mL Pinta 745. The final dosage formulation for Pinta 745 at 30 mg/mL is 10 mM sodium acetate, 9% (w/v) sucrose, 0.004% (w/v) polysorbate 20, pH 4.75. The placebo comprises the excipients used in the formulation of Pinta 745, and has a final dosage formulation of 10 mM sodium acetate, 9% (w/v) sucrose, 0.004% (w/v) polysorbate 20, pH 4.75.

[0070] Pinta 745 or a placebo is administered once weekly for 12 weeks at a dose of 3 mg/kg or 10 mg/kg, or at a different dose between 3 mg/kg and 10 mg/kg. Pinta 745 is administered by intravenous (IV) push over 1-2 minutes at the end of dialysis on any one of the dialysis days. Up to 40 subjects are enrolled in the study, randomized in a 3:1 allocation ratio to Pinta 745 or placebo. Dose escalation decisions are based on a blinded safety review of treatment-emergent adverse events (TEAEs), vital signs and other laboratory data. All subjects are to take one oral nutritional supplement containing $\geq$ 10 g of protein daily while undergoing study treatment from Day 1 through Day 78.

Study Subjects

[0071] Eligible subjects are men and women between 18-85 years old who have ESRD; received outpatient maintenance hemodialysis for at least 6 months prior to enrollment; received adequate dialysis with Kt/V $\geq$ 1.2 on two occasions within 12 weeks prior to enrollment; undergone dialysis $\geq$ 3 times per week, on average; hypoalbuminemia and low BMI defined as serum albumin level $\leq$ 3.8 g/dL and body mass index (BMI) $\leq$ 28 kg/m^2; and a life expectancy of $\geq$ 6 months.

Study Procedures

[0072] The following procedures are to be performed pre-study and periodically during the study for all cohorts: physical examination, vital signs, electrocardiogram, demographics and

medical and dialysis histories, height and most recent post-dialysis weight (PDW), eCRF calculation of body mass index (BMI) based on height and most recent PDW, concomitant medications, anti-Pinta 745 antibody screen, screen for CRP, IL-6, TNF-α, myostatin, adiponectin, resistin, and leptin, eCRF calculation of normalized protein catabolic rate from most recent blood urea nitrogen level and PDW, vitamin D levels, muscle and body composition assessments, muscle function tests, 6-minute walk test (6MWT), and pharmacokinetic sampling. A full listing of the study procedures and schedule of events is found in Table 6.

Efficacy

[0073] Pharmacokinetics: Pinta 745 levels in pharmacokinetic blood samples are determined by a central laboratory. The following parameters are estimated from blood levels using standard noncompartmental pharmacokinetic methods: terminal half-life, clearance, mean residence time, volume of distribution, and volume of steady state.

[0074] Historically-based Assessments: Historical information includes general medical history, diabetes history, dialysis history, dialysis vintage, history of decrease in PDW, and type of vascular access for dialysis (arteriovenous fistula versus arteriovenous graft) and baseline score on the mCCI.

[0075] Normalized Protein Catabolic Rate (nPCR): The protein catabolic rate (PCR) quantifies protein catabolism and can be derived from the interdialytic increase in the rate of urea generation. The nPCR, calculated by dividing the PCR by the subject's weight, is correlated to dietary protein intake and studies have shown that moderate protein intake results in positive nitrogen balance without causing a need for increase in dialysis dose (Grupe et al. 1983). In this study, PCR is estimated according to the method of Randerson (Keshaviah et al. 1991) using the BUN and nPCR is calculated based on the PCR and the most recent PDW (Uribarri et al. 1997), using the following equations: PCR (g/day) = 5.02 x (BUN in mg/min + 3.12), and nPCR = PCR/PDW. The nPCR is calculated automatically on the eCRF based on the reported values for BUN and PDW.

[0076] Muscle Function Tests: Two muscle function tests, the SCPT and the 6MWT, are used in this study. Both the SCPT and 6MWT are performed on non-dialysis days due to expected subject fatigue on dialysis days (Majchrzak et al. 2005).

[0077] Myostatin, Cytokine, and Adipokine Biomarkers: As Pinta 745 inhibits myostatin, serum myostatin levels are monitored to determine whether serum myostatin levels are

differentially affected in subjects treated with Pinta 745 compared with subjects treated with placebo. Also, levels of cytokines such as C-reactive protein, interleukin-6, and tumor necrosis factor alpha, as well as levels of adipokines such as adiponectin, leptin, and resistin are monitored to determine whether cytokine and adipokine levels are differentially affected in subjects treated with Pinta 745 compared with subjects treated with placebo.

[0078] Serum Lipids: Levels of serum lipids are monitored in patients to determine if serum lipid levels are differentially affected in subjects treated with Pinta 745 compared with subjects treated with placebo.

[0079] Serum Albumin: Serum albumin levels below 4.0 g/dL are correlated with increased mortality in patients with ESRD. Thus, serum albumin levels are monitored to determine if serum albumin levels are differentially affected in subjects treated with Pinta 745 compared with subjects treated with placebo.

[0080] Lean Body Mass (LBM): LBM, appendicular lean mass (ALM), and fat body mass is determined by using dual energy X-ray absorptiometry.

Efficacy Analysis

[0081] The primary analysis tests the null hypothesis that the mean percentage change in LBM from baseline to 12 weeks in the Pinta 745 group is equal to zero. This hypothesis is tested using a two-sided paired t test at the alpha = 0.05 level of significance.

[0082] At each time point, the paired t test is used to test the null hypothesis that the mean percentage change from baseline in LBM is equal to zero. These tests are carried out separately for each Pinta 745 treatment group and for placebo subjects. Exploratory comparisons between treatment groups are made. In most cases, analysis of variance (ANOVA) or analysis of covariance (ANCOVA) models are used. For stair climb power, comparisons between groups are made using the Wilcoxon-Mann-Whitney test. All additional efficacy analyses are completed using two-sided tests at the alpha = 0.05 level of significance.

[0083] The pharmacokinetic parameters of Pinta 745 in the hemodialysis population is estimated using standard noncompartmental pharmacokinetic methods and summarized using descriptive statistics for means, standard deviations, medians, minima and maxima.

Example 3: Treatment of diabetes with Pinta 745

[0084] The Patient is a 55 year old Native American woman who has had end stage renal disease requiring dialysis since 2008 (6 years), because of kidney damage due to

hypertension and Type 2 Diabetes. She has a history of hypoalbuminemia despite the use of 20g oral protein supplements with each dialysis run, and a past medical history of pancreatitis. The patient initiated linagliptin (a DPP4 inhibitor) in 12/2013.

[0085] The patient was treated with Pinta 745 for 12 weeks as described in Example 2.

Results

[0086] Screening and 4 week fasting glucose data was obtained and is shown in Table 2 and Table 3:

Table 2: Fasting blood glucose level at screening

Screening	
Fasting?	Blood glucose level
Yes	101
Yes	164
Yes	169
Yes	145
Yes	88
Yes	149
Yes	100
Average	130.8571429

Table 3: Fasting blood glucose level after 4 weeks of treatment with Pinta 745

Week 4	
Fasting?	Blood glucose level
Yes	127
Yes	125
Yes	127
Yes	82
Yes	91
Yes	116
Yes	64
Average	104.5714286

[0087] Data for screening and week 12 glucose and insulin levels and the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) are shown in Table 4:

Table 4: Glucose, Insulin, and HOMA-IR levels at screening and after 12 weeks of treatment with Pinta 745

	GLUCOSE (mg/dL)	INSULIN (mIU/mL)	HOMA-IR

SCREENIN G	249	10	6.14
WEEK 12	102	2	0.50

[0088] Data showing correlation of glucose and insulin levels from screening and week 12 to insulin sensitivity and beta cell function are shown in Figure 1.

[0089] The patient's insulin requirements decreased during the trial. The patient's hgbA1c was 7.8% at screen, 6.9% at Week 1, 6.5% at Week 5, 6.1% at Week 9, and 6.0% at Week 12. The patient's post-dialysis weight decreased from 60.9 kg at screen to 57.1 kg at Week 12. The patient has more endurance and is able to complete housework in a shorter period of time, but she has not started an exercise program.

[0090] The following was also observed in the patient:

- Insulin sensitivity increased 8 fold from 25% to 200%
- Beta Cell function doubled from 12.5% to 25%
- Lantus (long acting) insulin dose halved from 8.0 to 4.0 units on Day 8 of dosing
- Novolog insulin sliding scale with meals discontinued during week 9
- Glucose tablets prescribed for hypoglycemia Grade 1 on Day 8 of dosing

[0091] The results show that Pinta 745 can be used for treatment of diabetes and glycemic management in dialysis patients suffering from end stage renal failure.

[0092] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[0093] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

CLAIMS

1. A method of treating diabetes in a human subject with End Stage Renal Disease (ESRD) and/or ameliorating a condition of diabetes in the subject comprising administering to the subject a therapeutically effective amount of a myostatin inhibitor, wherein the myostatin inhibitor comprises Pinta 745, thereby treating diabetes in the subject.

2. The method of claim 1, wherein the myostatin inhibitor comprises a polypeptide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

3. The method of any one of the above claims, wherein the myostatin inhibitor comprises a polypeptide sequence consisting of the group selected from: SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

4. The method of any one of the above claims, wherein the subject is a human subject.

5. The method of any one of the above claims, wherein treating diabetes comprises reducing Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), reducing fasting glucose level, reducing fasting insulin level, increasing insulin sensitivity, increasing beta cell function, and/or reducing effective dose of long acting insulin dose in the subject.

6. The method of any one of the above claims, wherein the condition of diabetes is type 2 diabetes.

7. The method of any one of the above claims, wherein the subject is undergoing maintenance hemodialysis (MHD).

8. The method of claim 7, wherein the subject is undergoing MHD $\geq$ 3 times per week.

9. The method of any one of the above claims, wherein the subject is receiving insulin treatment.

10. The method of any one of the above claims, wherein the subject has protein-energy wasting (PEW).

11. The method of any one of the above claims, wherein the subject has a serum albumin level of $\leq$ 3.8 g/dL.

12. The method of any one of the above claims, wherein the subject has a body mass index (BMI) of ≤ 28 kg/m2, based on a post-dialysis weight and a height of the subject.

13. The method of any one of the above claims, wherein the subject has a Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) before treatment of greater than 1.0, 1.5, 2.0, 2.5, or 3.0.

14. The method of any one of the above claims, wherein HOMA-IR is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80% in the subject.

15. The method of any one of the above claims, wherein fasting glucose level is reduced by at least 10%, 20%, 30%, 40%, 50% and/or fasting plasma glucose is 70-130 mg/dL (3.9-7.2 mmol) in the subject.

16. The method of any one of the above claims, wherein peak post-prandial glucose level is reduced by at least 20%, 30%, 40%, 50% and/or is at a level of < 180 mg/dL (10 mmol) in the subject.

17. The method of any one of the above claims, wherein fasting insulin level is reduced by at least 2-fold, 3 fold, 4 fold, or 5 fold in said subject.

18. The method of any one of the above claims, wherein fasting insulin level is reduced to less than 1, 2, 3, 4, or 5 mIU/mL in said subject.

19. The method of any one of the above claims, wherein insulin resistance is decreased in said subject.

20. The method of any one of the above claims, wherein insulin sensitivity is reduced by at least 2, 3, 4, 5, 6, 7, 9 or 10 fold in said subject.

21. The method of any one of the above claims, wherein the subject has a hemoglobin A1c value of greater than 5%, 6%, 7%, 8%, 9% or 10%.

22. The method of any one of the above claims, wherein hemoglobin A1c is reduced by more than 0.5%, 1.0%, or 1.5% in said subject.

23. The method of any one of the above claims, wherein beta cell function is increased by at least 12.5%, 25%, 50%, or 100% in said subject.

24. The method of any one of the above claims, wherein effective dose of long acting insulin for said subject is decreased by at least 20%, 30%, 40%, 50%, or more.

25. The method of any one of the above claims, wherein said treatment results in an increase in lean body mass by at least 1%, 2%, 3%, 4%, 5%, or more in said subject.

26. The method of any one of the above claims, wherein treating diabetes comprises reducing Homeostasis Model Assessment-Insulin Resistance (HOMA-IR), reducing fasting glucose level, reducing fasting insulin level, decreasing insulin resistance, increasing beta cell function, and/or reducing effective dose of long acting insulin dose in the subject.

27. The method of any one of the above claims, wherein the subject is undergoing dialysis, has end stage renal disease, has protein energy wasting, has prediabetes (impaired glucose tolerance or impaired fasting glucose ie abnormal results that are not yet high enough to qualify as diabetic), has type 2 diabetics, has type 2 diabetics with chronic kidney disease, has type 2 diabetics with retinopathy, has type 2 diabetics with nephropathy, and/or has gestational diabetes.

28. The method of any one of the above claims, wherein the method results in at least one of the following: reduction of insulin dose intensity; discontinuation of insulin/no longer meeting criteria for diabetes ie diabetes remission; reduction in fasting blood glucose; reduction in postprandial blood glucose; reduction in fasting insulin; reduction in HbA1c; reduction in HOMA-IR; reduction in HOMA-IR corrected for adiponectin; modulation of adipose hormones (adiponectin, resistin, leptin); prevention of progression to diabetes (in prediabetic patients with impaired fasting glucose, gestational diabetes or impaired glucose tolerance); reduction in incidence rate or severity of microvascular complications (eg renal failure, retinopathy); reduction in incidence rate or severity of macrovascular complications (myocardial infarction, cerebrovascular accident, peripheral vascular disease).

29. The method of any one of the above claims, wherein the myostatin inhibitor is in a pharmaceutical composition.

30. The method of any one of the above claims, wherein the myostatin inhibitor is administered orally or by intravenous (IV) infusion.

31. A kit for treatment of diabetes in a human subject with End-Stage Renal Disease (ESRD) comprising a container comprising a myostatin inhibitor comprising Pinta 745, and instructions for use.

32. Use of a myostatin inhibitor for the treatment of diabetes in a patient with ESRD undergoing MHD, wherein said myostatin inhibitor comprises Pinta 745.

33. Use of a myostatin inhibitor comprising Pinta 745 for the manufacture of a medicament for treatment of diabetes in a patient with ESRD undergoing MHD.

34. Method for manufacturing a medicament intended for treatment of diabetes in a patient with ESRD undergoing MHD, characterized in that a myostatin inhibitor comprising Pinta 745 is used.

35. The method of claim 34, wherein the patient has a serum albumin level $\leq$ 3.8 g/dL.

36. The method of claim 34 or 35, wherein the myostatin inhibitor is administered intravenously once weekly, optionally once weekly at a loading dose for a first time period followed by a maintenance dose for a second time period, the loading dose greater than the maintenance dose.

37. The method of any one of claims 34-36, wherein treatment results in a reduction of HOMA-IR by at least 0.5%, 1.0%, 1.5%, or 2.0%.

38. A method of treating diabetes in a human subject with End Stage Renal Disease (ESRD) and/or ameliorating a condition of diabetes in said subject comprising administering to the subject a therapeutically effective amount of a compound comprising Pinta 745, thereby treating diabetes in the subject.

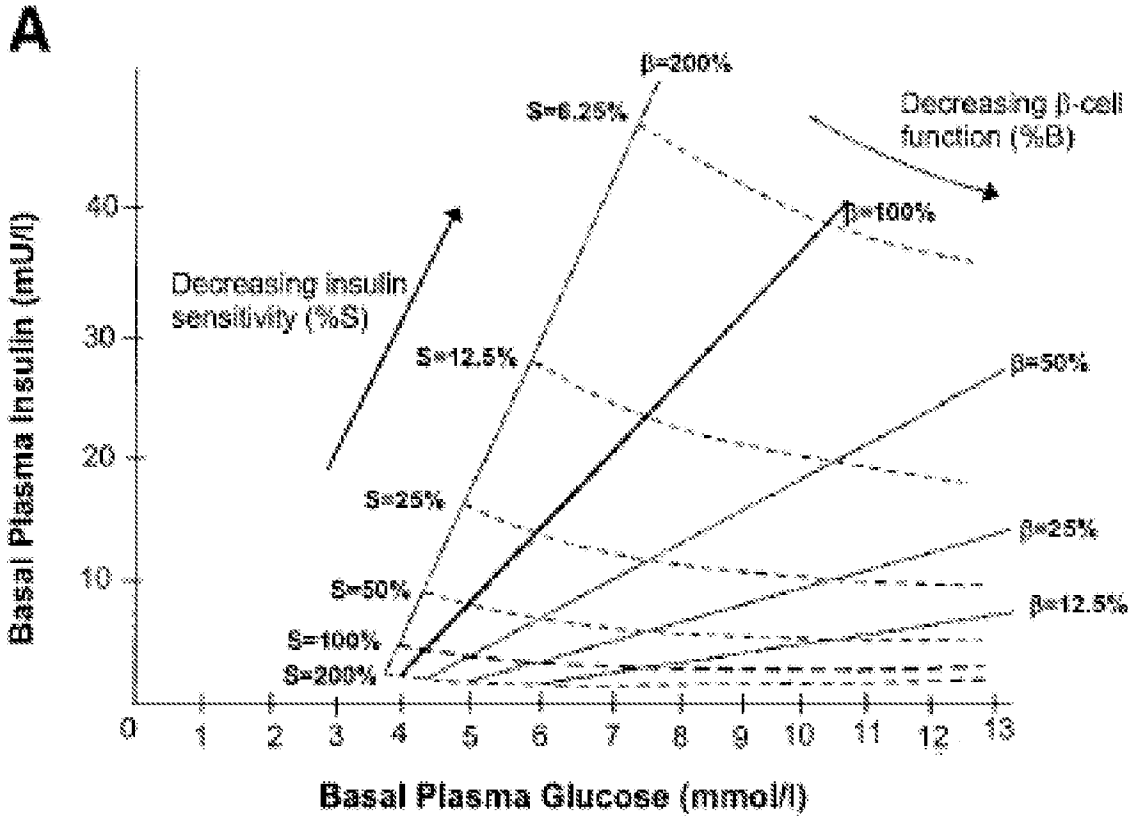
A
β=200%
S=6.25%
Decreasing β-cell function (%B)
β=100%
Decreasing insulin sensitivity (%S)
S=12.5%
β=50%
S=25%
β=25%
S=50%
β=12.5%
S=100%
S=200%
Basal Plasma Insulin (mU/l)
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Basal Plasma Glucose (mmol/l)

Figure 1