Abstract:

A novel combination of agents for the treatment of HIV-associated adipose redistribution syndrome (HARS) comprising combination of agents, said combination of agents comprising a first agent which binds to and initiates signaling of the human growth hormone (hGH) receptor or a substance which stimulates release or potentiates the activity of endogenous hGH; and a second agent which increases insulin sensitivity in said patient.
NOVEL COMBINATION OF AGENTS FOR THE TREATMENT OF HIV-ASSOCIATED ADIPOSE REDISTRIBUTION SYNDROME (HARS)

Field of the Invention

The present invention is related to a novel combination of agents, comprising a first agent (a) comprising a substance which binds to and initiates signaling of the human growth hormone receptor or a substance which stimulates release or potentiates the activity of endogenous hGH and a second agent (b) which increases insulin sensitivity, for the treatment of HIV-associated adipose redistribution syndrome (HARS), and methods and uses of this novel combination in treatment of HIV-Associated Adipose Redistribution Syndrome (HARS).

Background of the Invention

Advances in antiviral treatment of HIV infection, along with developments in the prophylaxis and therapy of opportunistic infections, have greatly improved the long-term health of HIV-infected individuals. However, along with improved antiretroviral therapy a new syndrome has developed, which is identified herein as HIV-associated adipose redistribution syndrome (HARS).

HARS involves pathological accumulation of adipose tissue in specific regional depots. The pathologic adipose tissue accumulation of HARS may also be associated with abnormal adipose tissue depletion elsewhere (lipodystrophy or lipoatrophy), with or without associated metabolic abnormalities, premature atherosclerotic lesions, depletion of lean body mass, and/or other abnormal physiology.

This recently discovered clinical disorder, which has been referred to by a number of terms, including HIV lipodystrophy syndrome and other terminology, has important public health consequences, as described further below. The prevalence of HARS in the population of HIV-infected patients is widely estimated from 30 to >90% (Mauss, 2001). HARS remains a significant deterrent to commencing highly active antiretroviral therapy (HAART) and is a distressing disorder for those who have developed body composition changes on previous treatment regimens. HARS patients are also concerned about diagnostic disclosure as a consequence of their appearance and about associated cardiovascular morbidity and mortality.
HARS patients typically present with abnormal accumulation of adipose tissue in the abdomen, specifically in the visceral adipose tissue compartment (Miller et al., 1998, Kotler et al., 1999). HARS patients may also present with abnormal adipose tissue accumulation in the dorsocervical area ("buffalo hump"), the submandibular area ("horse collar"), the pectoral, mammary, and/or supraclavicular areas, and/or with subcutaneous lipomas (encapsulated benign fatty tumors, single or multiple).

Abnormal, involuntary, pathological, and often dysmorphic accumulation of adipose tissue is sufficient to diagnose a HIV-infected patient with HARS. However, in addition to developing abnormal adipose accumulation, some HARS patients develop abnormally depleted subcutaneous adipose tissue, termed "peripheral lipodystrophy" (or lipoatrophy) at other specific sites. This adipose depletion is typically observed in the face (buccal, parotid, and periauricular fat pads), and in the subcutaneous adipose tissue surrounding the limbs, trunk, and/or gluteal regions. Also, some HARS patients may present with metabolic abnormalities (Carr et al., 1998).

The metabolic abnormalities associated with HARS and lipodystrophy syndrome typically involve disordered lipid and glucose metabolism. Clinical manifestations may include fasting hypertriglyceridemia, hyperlipidemia, and abnormalities of the insulin/glucose axis (elevated fasting insulin, elevated C-peptide, insulin resistance or reduced insulin sensitivity), with or without overt diabetes (Carr et al., 1998).

In sum, HARS is a recently discovered multisystemic, gender dimorphic disorder associated with HIV infection that includes (1) regional changes in adipose distribution, frequently dysmorphic, that result from abnormal regional accumulation of adipose tissue, with or without lipodystrophy, (2) occasionally observed in conjunction with abnormalities of lipid and glucose metabolism, and (3) possibly associated with other physiologic abnormalities, including premature atherosclerotic lesions, depletion of lean body mass, and other abnormalities.

Since HARS and HIV-associated lipodystrophy syndrome have only recently been described, standardized nomenclature or consensus definition(s) for these syndromes are lacking. Kotler and Schambelan (1999) predict that we may eventually see an official case definition that has major and minor criteria, similar to what is already in place for rheumatic disorders such as systemic lupus erythematosus (Kotler and Schambelan, 1999).

In recent years, a plethora of terms and nomenclature have been used by scientists, clinicians, and patient advocates to describe the syndrome and its manifestations. Any of the manifestations may be observed in men, women, and children with HIV.
infection who develop the syndrome. The terms describing it may be found in the peer-reviewed scientific literature, posted in discussions and reviews on the internet. The terms provided below are non-inclusive. New terms are continually added.

Terms that have been used to describe the syndrome or subsets of it include: HIV-associated adipose redistribution syndrome (HARS); HIV-associated dysmorphism/dysmetabolic syndrome (HADDS); lipodystrophy syndrome and HIV-associated lipodystrophy syndrome (HALS); HIV-related peripheral lipodystrophy (HIPL); HIV-associated fat redistribution syndrome (HIVFRES) or fat redistribution syndrome (FRS); fat maldistribution syndrome (FHARS), HIV-associated dysmorphism/metabolic Syndrome (HADS), abnormal body fat (ABF) accumulation, and protease inhibitor-associated lipodystrophy (PI-AL).

Terms used to describe abnormal accumulation of abdominal adipose tissue in patients with the syndrome include: HIV-associated "Crix belly" and "protease paunch" (although the truncal adiposity may not be directly related to Crixivan or other protease inhibitors); "pouch belly"; truncal adiposity, truncal obesity, central obesity, abdominal adiposity, increased waist to hip ratio (WHR), increased waist-to-thigh circumference ratio (by anthropometry), increased truncal to limb fat ratio (by DEXA scan), increased abdominal visceral adipose tissue (VAT) with decreased subcutaneous adipose tissue (SAT) and increased VAT/SAT ratio by CT or MRI scan, and "Pseudo-Cushing's syndrome".

Terms used to describe other types of fat accumulation in patients with HARS include HIV-related buffalo hump, abnormal accumulation of dorsocervical fat, "dorsocervical lipodystrophy" (a misnomer because it implies depletion of the dorsocervical fat pad), increased neck fat, facial fat accumulation, double chin, moon face, submandibular fat accumulation ("horse collar"), supraclavicular fat pad accumulation, multiple symmetric lipomatosis, "lumps and bumps", Madelung's syndrome, HIV-related breast enlargement, mammary fat hyperplasia and gynecomastia. (Note that it is unclear if there is true gynecomastia, or whether there is hypertrophy of subcutaneous chest fat other than mammary tissue), "chest fat accumulation", and peripheral adiposity.

Terms used to describe abnormal depletion of adipose tissue found in some patients with HARS and lipodystrophy syndrome include: pseudocachexia, peripheral lipodystrophy, pure lipoatrophy, "Lipo", facial wasting, facial wrinkling, sunken cheeks, sunken eyes, temple hollowness, prominent zygomatic arch, "cadaveric fades", buccal, parotid, and periauricular fat pad wasting, limb wasting, skinny, stick
arms and stick legs with symmetrical, prominent non-varicose veins, muscularity and bones, butt wasting, saggy buttocks with loose skin folds, loss of buttock fat contour, and hollowing of the buttocks.

Treatment options for HARS remain limited. Beyond switching to a thymidine NRTI-sparing regimen (Martin AIDS 04; MITOX study), in which case reversal of body shape change is very slow and unpredictable, few interventions have shown reliable benefit. Since insulin resistance is a major component of the syndrome, some have advocated the use of glitazones which increase insulin sensitivity by acting as agonists for PPAR-γ, part of the intracellular signalling pathway for insulin activity. (Arioglu Ann Int Med 2000). Two small uncontrolled studies suggested that rosiglitazone can encourage fat accumulation in patients with lipoatrophy (Gelato M, JAIDS 02; Calmy A, AIDS 03). A double-blind placebo-controlled study of rosiglitazone (4mg/d) in HIV-infected patients selected for hyperinsulinaemia and lipoatrophy showed improved insulin resistance and increases in total body fat by BIA and leg fat by CT scanning (Hadigan Ann Int Med 2004). Not only does rosiglitazone ameliorate insulin resistance but it also induces increases in serum adiponectin concentration which may mediate the favourable insulin-sensitizing effects of rosiglitazone (Sutinen J AJPEM 04). In addition, Sutinen J 04 showed that twenty-four-week treatment with rosiglitazone (8 mg/day) compared with placebo and induced significant changes in gene expression in subcutaneous adipose tissue, specifically, increased expression of adiponectin, peroxisome proliferator-activated receptor-gamma (PPAR-γ), and PPAR-γ coactivator 1 and decreased expression of IL-6, but did not affect expression of other genes involved in lipogenesis, fatty acid metabolism, or glucose transport. However a longer study (48 weeks) using 8 mg daily failed to show any benefit in body composition by DEXA or CT (Carr Lancet 04).

More recent studies in HARS have shown that pioglitazone also improves insulin resistance in patients with elevated fasting insulin levels and dyslipidaemia (Gavrila CID 2005) but similarly, in this group of subjects, had no significant impact on body composition, although a trend towards increased fat, primarily in the visceral compartment was noted. Thus there is conflicting evidence regarding the impact of rosiglitazone on body composition in HARS, though it does increase insulin sensitivity.

Agents that bind to and initiate signaling of the human growth hormone (hGH) receptor or substances which stimulate release or potentiate the activity of endogenous hGH have been reported to be effective treatments for HARS. Reduction in visceral adipose tissue has been described in HARS patients treated
with recombinant human growth hormone (rhGH) treatment (US Patent 6,696,063) and in another study with growth hormone releasing hormone (GHRH) (Koutkia et al., 2004). In addition, an analogue of GHRH was shown to reduce truncal fat in HARS patients (Falutz et al. 2005).

Engelson (2003) reviewed the potential approaches for treatment of HARS, including switching antiretrovirals for improving metabolism, growth hormone administration for reduction in visceral fat and buffalo humps, and improvement in fat distribution and glucose metabolism by metformin and rosiglitazone. Engelson reported that rosiglitazone trials showed improved insulin sensitivity but inconsistent changes in fat distribution. Whilst a trial in 8 persons with lipodystrophy reduced visceral fat by 21% and increased subcutaneous fat by 25%, a trial in 30 persons showed no change in fat distribution.

While rhGH is an effective treatment for HARS, some patients may experience hyperglycemia, and preexisting diabetes mellitus may be exacerbated during treatment with rhGH. It may be advantageous to combine rhGH treatment with an agent that either reduces glucose levels or increases insulin sensitivity to balance the potential hyperglycemic effect of rhGH. Since glitazones have in some instances been shown to increase visceral fat and affect gene expression in subcutaneous adipose tissue (see supra), it is unpredictable whether the combination of an agent such as rhGH with a glitazone, e.g., rosiglitazone would be an effective treatment for HARS, or if the glitazone would antagonize the therapeutic effects of rhGH in HARS. Described herein is the surprising result that an agent such as rhGH in combination with a glitazone, such as rosiglitazone is an effective treatment for HARS. The present invention is thus drawn to the use of hGH, a functional derivative, fragment, variant, analog, or salt thereof which retains growth hormone biological activity, or a substance which binds to and initiates signaling of the human growth hormone receptor or a substance which stimulates release or potentiates the activity of endogenous growth hormone, in combination with an agent which increases insulin sensitivity, to treat HARS.

**Summary of the Invention**

The present invention is related to uses and methods relating to a novel combination of agents for the treatment of HIV-associated adipose redistribution syndrome (HARS).
The invention further relates to uses and methods relating to a novel combination of agents for the prevention or treatment of insulin resistance in HARS patients. In one embodiment of the present invention, the combination comprises a first agent (a) comprising a substance which binds to and initiates signaling of the human growth hormone receptor or a substance which stimulates release or potentiates the activity of endogenous hGH and a second agent (b) which increases insulin sensitivity.

In another embodiment of the present invention, said first agent (a) comprises human growth hormone or a functional derivative, fragment, variant or salt thereof which retains the biological activity of human growth hormone.

In another embodiment of the present invention, said first agent (a) comprises human growth hormone releasing hormone or a functional derivative, fragment, variant or salt thereof which retains the ability to stimulate the release of human growth hormone.

In one embodiment of the present invention, said second agent (b) comprises a glitazone or pharmaceutically acceptable salts thereof.

In another embodiment of the present invention, said glitazone is either pioglitazone or rosiglitazone or pharmaceutically acceptable salts thereof.

In a particularly preferred embodiment, said glitazone is rosiglitazone or pharmaceutically acceptable salts thereof.

**Detailed Description of the Invention**

This invention is related to a method of treating HARS comprising administering to a patient in need thereof, an effective amount of a combination of agents, said combination of agents comprising a first agent (a) comprising a substance which binds to and initiates signaling of the human growth hormone (hGH) receptor or a substance which stimulates release or potentiates the activity of endogenous growth hormone and a second agent (b) comprising an insulin sensitizing agent. Although said first agents (a) have been used for the treatment of HARS, and said second agents (b) have been used in the treatment of some forms of lipodystrophy (albeit with varied results), the combination of said agents (a) and (b) has not been previously used, taught or suggested for the treatment of HARS. The present invention is directed to the surprising discovery that combining said agents (a) and (b) is effective in treating a broad spectrum of HARS patients.
It has also surprisingly been found that there was no occurrence of insulin resistance in those patients receiving the combined treatment.

Therefore, the invention also relates to the use of a combination of agents, said combination of agents comprising a first agent (a) comprising a substance which binds to and initiates signaling of the human growth hormone (hGH) receptor or a substance which stimulates release or potentiates the activity of endogenous growth hormone and a second agent (b) comprising an insulin sensitizing agent, for prevention or treatment of insulin resistance in HARS patients. In an embodiment, HARS patients treated with the combination in accordance with the invention have no significant changes in insulin or glucose.

The invention also relates to the use of a combination of agents in the treatment of HARS, said combination of agents comprising a first agent (a) comprising a substance which binds to and initiates signaling of the human growth hormone (hGH) receptor or a substance which stimulates release or potentiates the activity of endogenous growth hormone and a second agent (b) comprising an insulin sensitizing agent.

In one embodiment of the present invention said first agent (a) is human growth hormone (hGH), preferably recombinant hGH (rhGH).

In another embodiment of the present invention, said first agent (a) comprises any art recognized functional derivative, fragment, variant of hGH, or salt thereof that retains the biological activity of hGH.

In another embodiment of the present invention, said first agent (a) comprises human growth hormone releasing hormone (hGHRH), preferably recombinant hGHRH (rhGHRH).

In another embodiment of the present invention, said first agent (a) comprises any art recognized functional derivative, fragment, variant of hGHRH, or salt thereof that retains the ability to stimulate the release of hGH.

In one embodiment of the present invention, said second agent (b) is selected from the group of glitazones consisting of pioglitazone or rosiglitazone or pharmaceutically acceptable salts thereof.

In a preferred embodiment of the present invention, said second agent (b) is rosiglitazone or pharmaceutically acceptable salts thereof.

In an embodiment of the invention, growth hormone is recombinant human growth hormone (rhGH) and is administered at 2 to 6, preferably at 2, 4 or 6 mg daily or
every other day, for at least 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 36 weeks. Preferably, rhGH is administered subcutaneously.

In another embodiment, rosiglitazone is administered at 2 to 8, preferably at 2, 4, 6 or 8 mg daily or every other day, for at least 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 36 weeks. Preferably, rosiglitazone is administered orally.

In a further embodiment of the present invention, agent (a) and agent (b) are formulated or adapted for simultaneous, sequential or separate administration or they are administered simultaneously, sequentially or separately.

In an embodiment of the present invention, the use or method according to the invention leads to a reduction in visceral adipose tissue (VAT) by at least 10 or 15 or 20 or 25% after the treatment period. VAT can e.g. be measured in a CT scan.

In a further embodiment, the use or method according to the invention leads to a reduction in trunk fat by at least 250 or 500 or 750 or 1000 g after the treatment period. Preferably, there is an increase in trunk lean tissue mass or limb lean tissue mass after the treatment period. A change in fat or lean tissue can e.g. be measured by DEXA scan.

In yet another embodiment, administration of the combination in accordance with the present invention leads to a reduction in plasma glucose.

Definitions

The term "treatment" within the context of this invention refers to any beneficial effect on progression of disease or of a symptom thereof, including attenuation, reduction, decrease or diminishing of the pathological development after onset of disease or any partial or substantial prevention, attenuation, reduction, decrease or diminishing of the beneficial effect before or at early onset of disease.

Symptoms of HARS include HIV-associated truncal adiposity, truncal obesity, central obesity, abdominal adiposity, increased waist to hip ratio (WHR), increased waist-to-thigh circumference ratio (by anthropometry), increased truncal to limb fat ratio (by DEXA scan), increased abdominal visceral adipose tissue (VAT) with decreased subcutaneous adipose tissue (SAT) and increased VAT/SAT ratio by CT or MRI scan, and "Pseudo-Cushing's syndrome".

Other types of fat accumulation in patients with HARS include HIV-related buffalo hump, abnormal accumulation of dorsocervical fat, increased neck fat, facial fat accumulation, double chin, moon face, submandibular fat accumulation ("horse collar"), supraclavicular fat pad accumulation, multiple symmetric lipomatosis, "lumps
and bumps", Madelung's syndrome, HIV-related breast enlargement, mammary fat hyperplasia and gynecomastia, "chest fat accumulation", and peripheral adiposity.

Further symptoms of HARS relating to abnormal depletion of adipose tissue found in some patients with HARS and lipodystrophy syndrome include: pseudocachexia, peripheral lipodystrophy, pure lipoatrophy, facial wasting, facial wrinkling, sunken cheeks, sunken eyes, temple hollowness, prominent zygomatic arch, "cadaveric fades", buccal, parotid, and periauricular fat pad wasting, limb wasting, skinny, stick arms and stick legs with symmetrical, prominent non-varicose veins, musculature and bones, butt wasting, saggy buttocks with loose skin folds, loss of buttock fat contour, and hollowing of the buttocks.

The term "human growth hormone", as used in the present invention, is intended to include the naturally-occurring derivatives, as noted above, including, without limitation, both the 20 kD and the 22 kD human growth hormone, GH-V, and other members of the growth hormone gene locus as described in Chen et al (1989). The term also includes functional derivatives, fragments, variants, analogs, or salts which retain the biological activity of growth hormone, i.e., which act as agonists to the growth hormone receptor. In other words, they are capable of binding to the growth hormone receptor to initiate the signaling activity of the receptor.

The term "human growth hormone releasing hormone", as used in the present invention, is intended to include the naturally-occurring derivatives. The term also included functional derivatives, fragments, variants, analogs, or salts thereof which are known in the art and retain the ability to stimulate the release of human growth hormone.

"Functional derivatives" of hGH as used herein covers derivatives which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e., they do not destroy the biological activity of hGH as described herein, i.e., the ability to bind the hGH receptor and initiate receptor signalling, and do not confer toxic properties on compositions containing it. Derivatives may have chemical moieties, such as carbohydrate or phosphate residues, provided such a derivative retains the biological activity of hGH and remains pharmaceutically acceptable.

For example, derivatives may include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives or free amino groups of the amino acid residues formed
with acyl moieties (e.g., alkanoyl or carbocyclic aryl groups) or O-acyl derivatives of
free hydroxyl group (e.g., that of seryl or threonyl residues) formed with acyl moieties. Such derivatives may also include for example, polyethylene glycol side-chains which may mask antigenic sites and extend the residence of the molecule in body fluids.

Of particular importance is a growth hormone that has been derivatized or combined with a complexing agent to be long lasting. For example, pegylated versions, or growth hormones genetically engineered to exhibit long lasting activity in the body, can be used to treat HARS according to the present invention.

hGH that is acetylated at the N-terminus has been isolated and identified (Lewis et al, 1979). It is not clear if acylation serves a regulatory role or is simply an artifact of the purification. However, it is expected that this molecule exhibits anti-HARS activity in a similar fashion to other hGH derivatives.

The term "derivatives" is intended to include only those derivatives that do not change one amino acid to another of the twenty commonly occurring natural amino acids.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the hGH molecule or analogs thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids, such as, for example, acetic acid or oxalic acid. Of course, any such salts must retain the biological activity of hGH relevant to the present invention, i.e., the ability to bind to the hGH receptor and initiate receptor signalling.

A "fragment" of the growth hormone according to the present invention refers to any subset of the molecule, that is, a shorter peptide which retains the desired biological activity. Fragments may readily be prepared by removing amino acids from either end of the hGH molecule and testing the resultant for its properties as an hGH receptor agonist. Proteases for removing one amino acid at a time from either the N-terminal or the C-terminal of a polypeptide are known, and so determining fragments retaining the desired biological activity involves only routine experimentation.

Additionally, the polypeptide which has such hGH receptor agonist activity, be it hGH, an analog or variant, salt, functional derivative or fragment thereof, can also contain
additional amino acid residues flanking the hGH polypeptide. As long as the resultant molecule retains the hGH receptor agonist ability of the core polypeptide, one can determine whether any such flanking residues affect the basic and novel characteristics of the core peptide, i.e., its receptor agonist characteristics, by routine experimentation. The term "consisting essentially of", when referring to a specified sequence, means that additional flanking residues can be present which do not affect the basic and novel characteristic of the specified sequence. This term does not comprehend substitutions, deletions or additions within the specified sequence.

A "variant" of the human growth hormone according to the present invention refers to a molecule, which is substantially similar to either the entire peptide or a fragment thereof. Variant peptides may be conveniently prepared by direct chemical synthesis of the variant peptide, using methods well known in the art. Of course, a variant human growth hormone would have similar similar hGH receptor binding and signal initiating activity as hGH and which would, therefore, be expected to have similar anti-HARS activity to hGH.

Amino acid sequence variants of the human growth hormone can be prepared by mutations in the DNAs, which encode the synthesized human growth hormone derivatives. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity. Obviously, the mutations that will be made in the DNA encoding the variant peptide must not alter the reading frame and preferably will not create complementary regions that could produce secondary mRNA structure.

At the genetic level, these variants ordinarily are prepared by site-directed autogenesis (as exemplified by Adelman et al, 1983) of nucleotides in the DNA encoding the peptide molecule, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. The variants typically exhibit the same qualitative biological activity as the non-variant peptide.

An "analog" of human growth hormone according to the present invention refers to a non-natural molecule, which is substantially similar to either the entire molecule or to an active fragment thereof. An analog of human growth hormone useful in the present invention would exhibit anti-HARS activity.

The types of substitutions, which may be made in the human growth hormone according to the present invention may be based on analysis of the frequencies of
amino acid changes between a homologous protein of different species. Based upon such analysis, conservative substitutions may be defined herein as exchanges within one of the following five groups:

i. Small, aliphatic, nonpolar or slightly polar residues:
   Ala, Ser, Thr, Pro, Gly

ii. Polar, negatively-charged residues and their amides:
   Asp, Asn, Glu, Gln

iii. Polar, positively-charged residues:
   His, Arg, Lys

IV. Large, aliphatic non-polar residues:
   Met, Leu, lle, Val, Cys

V. Large aromatic residues:
   Phe, Try, Trp

Within the foregoing groups, the following substitutions are considered to be "highly conservative":

Asp/Glu
His/Arg/Lys
Phe/Tyr/Trp
Met/Leu/ille/Val

Semi-conservative substitutions are defined to be exchanges between two of groups (I)-(IV) above which are limited to supergroup (A), comprising (I), (II), and (III) above, or to supergroup (B), comprising (IV) and (V) above. Substitutions are not limited to the genetically encoded or even the naturally occurring amino acids. When the epitope is prepared by peptide synthesis, the desired amino acid may be used directly. Alternatively, a genetically encoded amino acid may be modified by reacting it with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

Cysteinyl residues most commonly are reacted with alpha-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, alpha-bromo-beta-(5imidazoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl-2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.
Histidyl residues are derivatized by reaction with diethylprocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Parabromophenacyl bromide is also useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino acid-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisosurea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal; 2,3butanedione; and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine, as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues per se has been studied extensively, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidazole and tetranitromethane are used to form O-acetyl tyrosyl species and e-nitro derivatives, respectively.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'-N=C-N-R*), such as 1-cyclohexyl-3-[2-morpholinyl-(4-ethyl)]carbodiimide or 1 ethyl-3-(4-azonia-4,4-dimethylpentyl)carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Examples of production of amino acid substitutions in proteins which can be used for obtaining analogs of the hGH for use in the present invention include any known method steps, such as presented in U.S. patents RE 33,653; 4,959,314; 4,588,585 and 4,737,462, to Mark et al; 5,1 16,943 to Koths et al; 4,965,195 to Namen et al; and
5,017,691 to Lee, et al, and lysine substituted proteins presented in US patent 4,904,584 (Shaw et al).

Among the substances which bind to and initiate signalling of the human growth hormone receptor which may be used in accordance with the present invention are all of those growth hormone analogs and mimetics already known in the literature, such as, for example, are disclosed in U.S. patents 5,851,992; 5,849,704; 5,849,700; 5,849,535; 5,843,453; 5,834,598; 5,688,666; 5,654,010; 5,635,604; 5,633,352; 5,597,709; and 5,534,617.

Preferably, the hGH variant or analog will have a core sequence, which is the same as that of the native sequence or biologically active fragment thereof, which has an amino acid sequence having at least 70% identity to the native amino acid sequence and retains the biological activity thereof. More preferably, such a sequence has at least 80% identity, at least 90% identity, or most preferably at least 95% identity to the native sequence.

The term "sequence identity" as used herein means that the sequences are compared as follows. The sequences are aligned using Version 9 of the Genetic Computing Group's GAP (global alignment program), using the default (BLOSUM62) matrix (values -4 to +11) with a gap open penalty of -12 (for the first null of a gap) and a gap extension penalty of -4 (per each additional consecutive null in the gap).

After alignment, percentage identity is calculated by expressing the number of matches as a percentage of the number of amino acids in the claimed sequence.

Analogs or variants in accordance with the present invention may also be determined in accordance with the following procedure. The DNA of the native sequence is known to the prior art and is found in the literature (Martial et al, 1979). Polypeptides encoded by any nucleic acid, such as DNA or RNA, which hybridizes to the complement of the native DNA or RNA under highly stringent or moderately stringent conditions, as long as that polypeptide maintains the biological activity of the native sequence, are also considered to be within the scope of the present invention.

Stringency conditions are a function of the temperature used in the hybridization experiment, the molarity of the monovalent cations and the percentage of formamide in the hybridization solution. To determine the degree of stringency involved with any given set of conditions, one first uses the equation of Meinkoth et al. (1984) for determining the stability of hybrids of 100% identity expressed as melting temperature Tm of the DNA-DNA hybrid:

\[ T_m = 81.5^\circ C + 16.6 \times (\log M) + 0.41 \times (\% GC) - 0.61 \times (\% form) - 500/L \]
where M is the molarity of monovalent cations, %GC is the percentage of G and C nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. For each 1°C that the Tm is reduced from that calculated for a 100% identity hybrid, the amount of mismatch permitted is increased by about 1%. Thus, if the Tm used for any given hybridization experiment at the specified salt and formamide concentrations is 10°C below the Tm calculated for a 100% hybrid according to equation of Meinkoth, hybridization will occur even if there is up to 10% mismatch.

As used herein, highly stringent conditions are those which are tolerant of up to about 15% sequence divergence, while moderately stringent conditions are those which are tolerant of up to about 20% sequence divergence. Without limitation, examples of highly stringent (12-15°C below the calculated Tm of the hybrid) and moderately (15-20°C below the calculated Tm of the hybrid) conditions use a wash solution of 2 X SSC (standard saline citrate) and 0.5% SDS at the appropriate temperature below the calculated Tm of the hybrid. The ultimate stringency of the conditions is primarily due to the washing conditions, particularly if the hybridization conditions used are those which allow less stable hybrids to form along with stable hybrids. The wash conditions at higher stringency then remove the less stable hybrids. A common hybridization condition that can be used with the highly stringent to moderately stringent wash conditions described above is hybridization in a solution of 6 X SSC (or 6 X SSPE), 5 X Denhardt's reagent, 0.5% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA at a temperature approximately 20° to 25°C below the Tm. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC (Ausubel, 1987-1998).

While the present invention provides recombinant methods for making the human growth hormone derivatives, these derivatives may also be made by conventional protein synthesis methods which are well known to those skilled in the art.

The growth hormone treatment in accordance with the present invention may be accomplished either by administration of exogenous growth hormone or by administration of a substance which stimulates production of endogenous growth hormone either directly or indirectly by suppressing endogenous somatostatin secretion. It is known that human growth hormone releasing hormone (hGHRH) stimulates the release of hGH. GHRH has been isolated from human islet cell tumors and characterized by Guillemin and co-workers, Science, 218, 585-587 (Nov. 5, 1982) and Rivier and co-workers, Nature, 300, 276-278 (1982). The structure of GHRH 1-44 (SEQ ID NO: 1) and GHRH 1-40 (SEQ ID NO: 2) has been described
and both have been shown to cause the release of growth hormone. These two forms of GHRH are identical at their amino (NH2) termini but differ at the termination point at the carboxy termini (COOH). Id.

Rivier and Vale et al. have shown that the biological activity, i.e. the ability to stimulate the release of human growth hormone, resides in the NH2-terminal portion of the molecule and full intrinsic activity and potency was demonstrated with GHRH 1-29 (SEQ ID NO: 3). Thus, the biological activity of hGH can be obtained by administering GHRH comprising amino acids 1-44 (SEQ ID NO: 1), 1-40 (SEQ ID NO: 2) or 1-29 (SEQ ID NO: 3) of GHRH or a functional derivative, salt, variant, analog or fragment thereof which retains the biological activity of GHRH, i.e., the ability to stimulate the release of growth hormone. Thus, for example, besides GHRH there may be used functional derivatives thereof in accordance with the above Definitions, analogs or variants thereof, which have at least 70% sequence identity, more preferably 80% or 90% or, most preferably, 95% sequence identity therewith, yet retains the biological activity of GHRH, or a variant or analog which is a polypeptide encoded by a DNA which hybridizes to the native DNA encoding GHRH under moderately stringent conditions, or preferably under highly stringent conditions, all in accordance with the definitions given hereinabove. Any of the GHRH or GHRH analogs or agonists known in the literature and disclosed as simulating the release of growth hormone can be used in the present invention, including but not limited to those disclosed in U.S. patents 5,861,379; 5,817,627; 5,939,386; 6,020,311; 6,194,384; 6,458,764; 5,792,747; 5,776,901; 5,696,089; 5,137,872; 5,767,085; 5,612,470; 5,846,936; and 5,847,066, the contents of which are incorporated herein by reference. See also Thorner et al (1997), Felix et al (1995), Alba-Roth et al (1988), Friend et al (1997), Falutz et al. (2005). Of particular importance is a GHRH that has been derivatized or combined with a complexing agent to be long lasting or to increase biological activity. For example pegylated versions or GHRH genetically engineered to exhibit long lasting activity in the body, or versions that have been modified with, e.g., a hydrophobic moiety and said to have increased biological activity in the body, can be used to treat HARS according to the present invention.

Other substances capable of promoting the release of growth hormone in vivo which can be used in accordance with the present invention include those disclosed in U.S. patents 5,807,985; 5,804,578; 5,795,957; 5,777,112; 5,767,118; 5,731,317; 5,726,319; 5,726,307; 5,721,251; 5,721,250, etc.

There can also be used in accordance with the present invention any other molecule which binds to the hGH receptor and initiates signaling of that receptor. It is known,
for example, that small molecules, sometimes called secretagogues, have been developed which bind hGH receptors and cause them to aggregate and initiate signalling, which signal initiation is the same as one obtains with natural hGH binding to the receptor. Such molecules are known, for example, from U.S. patents 5,773,441; 5,798,337; 5,830,433; 5,767,124; and 5,723,616. See also Bowers et al (1991), Thorner et al (1997), Camanni et al (1998), Ankersen et al (1998), Smith et al (1993) and Ghigo et al (1998). Thus, the present invention is intended to include any substance which binds to hGH receptor and initiates signalling thereof so as to obtain the same ultimate qualitative effect as the administration of natural hGH, insofar as the treatment of HARS is concerned.

As used herein, "effective amount" when used in the context of a dosing regimen of rhGH in combination with an insulin sensitizing agent, refers to that amount of either rhGH or an insulin sensitizing agent, which if given in the absence of the other would result in the maximal treatment of HARS that each respective agent could effect if given as a single agent.

As used herein, "efficacy" when used in the context of a dosing regimen refers to the effectiveness of a particular treatment regimen. Efficacy can be measured based on changes in the course of disease in response to a dosing regimen of this invention. For example, treatment of HARS efficacy can be measured by reemergence of abnormal fat deposits.

As used herein, "in combination with" where used to describe administration of rhGH and an insulin sensitizing agent means that the rhGH may be administered prior to, together with, or after the insulin sensitizing agent.

As used herein, "treatment" and "treating" and the like generally mean obtaining a desired pharmacological and physiological effect. The effect may be prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof and/or may be therapeutic in terms of a partial or complete cure of a disease, condition, symptom or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or relieving the disease, i.e., causing regression of the disease and/or its symptoms or conditions.
Combination

According to the present invention, rhGH or an agent which binds to and initiates signaling of the human growth hormone receptor or a substance which stimulates release or potentiates the activity of endogenous hGH, is administered in combination with an insulin sensitizing agent.

The two agents may be administered simultaneously or sequentially with respect to each other. When administered simultaneously, the agents may be formulated in the same composition or in different compositions. When the two agents are not administered as part of the same formulation, the two agents may be administered by the same or different routes of administration. The relative administration of rhGH and an insulin sensitizing agent may be dependent on the specific route of administration and/or formulation of each component. The relative administration of rhGH and an insulin sensitizing agent may be different depending on whether the patient is experiencing remission or exacerbation of symptoms.

Insulin Sensitizing Agents

Insulin sensitizing agents such as the glitazones such as rosiglitazone and pioglitazone, have been shown to reduce glucose levels and are effective treatments for diabetes.

Rosiglitazone has been described in US patents 5,002,953; 5,194,443, 5232,925; 5,260,445; 5,521,201; 5,646,169; 5,756,525; and 6,288,095, the contents of which are herein incorporated by reference.

Rosiglitazone has the following structure:

![Rosiglitazone structure]

and is marketed by GlaxoSmithKline under the trade name Avandia for the treatment of non-insulin dependent diabetes. It is a peroxisome proliferator-activated receptor (PPAR)-gamma receptor agonist and acts to increase insulin sensitivity in the body. Also included in the present invention are pharmaceutically acceptable salts of rosiglitazone.
Pioglitazone has been described in US patent 4,687,777, the contents of which are incorporated herein by reference.

Pioglitazone has the following structure:

and is co-marketed by Takeda and Eli Lilly under the trade name Actos for the treatment of non-insulin dependent diabetes.

Also included in the present invention are pharmaceutically acceptable salts of pioglitazone.

rhGH

Human growth hormone, also known as somatotropin, is a protein hormone produced and secreted by the somatotropic cells of the anterior pituitary. Secretion is regulated by a releasing factor, i.e., the growth hormone-releasing hormone (GHRH), and by an inhibitory factor, somatostatin. Human growth hormone plays a key role in somatic growth through its effects on the metabolism of proteins, carbohydrates and lipids.

Human growth hormone is a single polypeptide chain of 191 amino acids (Bewley et al, 1972) having two disulfide bonds, one between Cys-53 and Cys-165, forming a large loop in the molecule, and the other between Cys-182 and Cys-189, forming a small loop near the C-terminus. The DNA sequence that confirmed the amino acid sequence was reported by Martial et al (1979). Purified hGH is a white amorphous powder in its lyophilized form. It is readily soluble (concentrations >10 mg/L) in dilute aqueous buffers at pH greater than 7.2.

In solution, hGH exists predominantly as a monomer, with a small fraction as dinners and higher molecular weight oligomers. Under certain conditions, hGH can be induced to form larger amounts of dinners, trimers and higher oligomers.

Several derivatives of hGH are known, including naturally-occurring derivatives, variants and metabolic products, degradation products primarily of biosynthetic hGH and engineered derivatives of hGH produced by genetic methods. One example of a
naturally-occurring derivative of hGH is GH-V, a variant of growth hormone found in the placenta. Other members of the gene locus are described in Chen et al (1989). Any derivative of hGH, including derivatives designed to be long-lasting in the body, can be used for the purpose of the present invention as long as it retains the biological activity of hGH.

Methionyl hGH was the first form of hGH to be produced through recombinant DNA technology. This compound is actually a derivative of hGH having one additional methionine residue at its N-terminus (Goeddel et al, 1979).

A naturally-occurring variant of hGH called 20-K-hGH has been reported to occur in the pituitary as well as in the bloodstream (Lewis et al, 1978; Lewis et al, 1980). This compound, which lacks the 15 amino acid residues from Glu-32 to Gln-46, arises from an alternative splicing of the messenger ribonucleic acid (DeNoto et al, 1981). This compound shares many, but not all of the biological properties of hGH.

20-K-hGH is made in the pituitary and secreted into the blood. It makes up about 5% of growth hormone output of adults, and about 20% of growth hormone output of children. It has the same growth promoting activity as 22 kD growth hormone, and has been reported to have equal to or greater the amount of lipolytic activity as the 22 kD form. It binds to growth hormone receptors with equal affinity as the 22 kD growth hormone, and has one tenth the lactogenic (prolactin-like) bioactivity as the 22 kD hormone. Unlike 22 kD, the 20-k-hGH has weak anti-insulin activity.

A number of derivatives of hGH arise from proteolytic modifications of the molecule. The primary pathway for the metabolism of hGH involves proteolysis. The region of hGH around residues 130-150 is extremely susceptible to proteolysis, and several derivatives of hGH having nicks or deletions in this region have been described (Thorlacius-Ussing, 1987). This region is in the large loop of hGH, and cleavage of a peptide bond there results in the generation of two chains that are connected through the disulfide bond at Cys-53 and Cys-165. Many of these two-chain forms are reported to have increased biological activity (Singh et al, 1974). Many derivatives of human growth hormone have been generated artificially through the use of enzymes. The enzymes trypsin and subtilisin, as well as others, have been used to modify hGH at various points throughout the molecule (Lewis et al, 1977; Graff et al, 1982). One such derivative, called two-chain anabolic protein (2-CAP), was formed through the controlled proteolysis of hGH using trypsin (Becker et al, 1989). 2-CAP was found to have biological properties very distinct from those of the intact hGH molecule, in that
the growth-promoting activity of hGH was largely retained and most of the effects on carbohydrate metabolism were abolished.

Asparagine and glutamine residues in proteins are susceptible to deamidation reactions under appropriate conditions. Pituitary hGH has been shown to undergo this type of reaction, resulting in conversion of Asn-152 to aspartic acid and also, to a lesser extent, conversion of Gln-137 to glutamic acid (Lewis et al, 1981). Deamidated hGH has been shown to have an altered susceptibility to proteolysis with the enzyme subtilisin, suggesting that deamidation may have physiological significance in directing proteolytic cleavage of hGH. Biosynthetic hGH is known to degrade under certain storage conditions, resulting in deamidation at a different asparagine (Asn-149). This is the primary site of deamidation, but deamidation at Asn-152 is also seen (Becker et al, 1988). Deamidation at Gln-137 has not been reported in biosynthetic hGH.

Methionine residues in proteins are susceptible to oxidation, primarily to the sulfoxide. Both pituitary-derived and biosynthetic hGH undergo sulfoxidations at Met-14 and Met-125 (Becker et al, 1988). Oxidation at Met-170 has also been reported in pituitary but not biosynthetic hGH. Both desamide hGH and Met14 sulfoxide hGH have been found to exhibit full biological activity (Becker et al, 1988).

Truncated forms of hGH have been produced, either through the actions of enzymes or by genetic methods. 2-CAP, generated by the controlled actions of trypsin, has the first eight residues at the N-terminus of hGH removed. Other truncated versions of hGH have been produced by modifying the gene prior to expression in a suitable host. The first 13 residues have been removed to yield a derivative having distinctive biological properties (Gertler et al, 1986) in which the polypeptide chain is not cleaved.

Although human growth hormone was originally obtained from pituitary glands of cadavers, these preparations were not electrophoretically homogeneous, and antibodies appeared in the serum of patients treated with preparations of the order of 50% purity, the immunogenicity being attributed to inactive components.

Recombinant DNA technology permitted production of an unlimited supply of hGH in a number of different systems. Purification of hGH from the culture medium is facilitated by the presence of only low amounts of contaminating proteins. In fact, it has been shown that hGH can be purified on a laboratory scale by a single purification step on a reversed-phase HPLC column (Hsiung et al, 1989).
Recombinant human growth hormone, rhGH, is produced by Serono Laboratories, Inc., as SEROSTIM®, which product has been given accelerated FDA approval for treating weight loss and wasting in AIDS patients. PROTROPIN®, produced by Genentech, Inc. (South San Francisco, CA), differs slightly in structure from natural sequence hGH, having an additional methionine residue at the N-terminus.

Recombinant hGH is generally marketed as vials containing hGH plus additional excipients, e.g., glycine and mannitol, in a lyophilized form. A companion diluent vial is provided, allowing the patient to reconstitute the product to the desired concentration prior to administration of the dose. Recombinant hGH can also be marketed in other well-known manners, such as prefilled syringes, etc.

**Compositions**

Compositions of this invention may further comprise one or more pharmaceutically acceptable additional ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like.

Compositions of this invention may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including, but not limited to, binding agents, fillers, lubricants, disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers include, but are not limited to, lactose, sugar, microcrystalline cellulose, maize starch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycinate. Wetting agents include, but are not limited to, sodium lauryl sulfate). Tablets may be coated according to methods well known in the art.

Compositions of this invention may also be liquid formulations including, but not limited to, aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The compositions may also be formulated as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including, but not limited to, suspending agents, emulsifying agents, nonaqueous vehicles and preservatives. Suspending agent include, but are not limited to, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate, and acacia. Nonaqueous vehicles include, but are not limited to, edible oils, almond oil,
fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid.

Compositions of this invention may also be formulated as suppositories, which may contain suppository bases including, but not limited to, cocoa butter or glycerides. Compositions of this invention may also be formulated for inhalation, which may be in a form including, but not limited to, a solution, suspension, or emulsion that may be administered as a dry powder or in the form of an aerosol using a propellant, such as dichlorodifluoromethane or trichlorofluoromethane. Compositions of this invention may also be formulated transdermal formulations comprising aqueous or nonaqueous vehicles including, but not limited to, creams, ointments, lotions, pastes, medicated plaster, patch, or membrane.

Compositions of this invention may also be formulated for parenteral administration including, but not limited to, by injection or continuous infusion. Formulations for injection may be in the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulation agents including, but not limited to, suspending, stabilizing, and dispersing agents. The composition may also be provided in a powder form for reconstitution with a suitable vehicle including, but not limited to, sterile, pyrogen-free water.

Compositions of this invention may also be formulated as a depot preparation, which may be administered by implantation or by intramuscular injection. The compositions may be formulated with suitable polymeric or hydrophobic materials (as an emulsion in an acceptable oil, for example), ion exchange resins, or as sparingly soluble derivatives (as a sparingly soluble salt, for example).

Compositions of this invention may also be formulated as a liposome preparation. The liposome preparation can comprise liposomes which penetrate the cells of interest or the stratum corneum, and fuse with the cell membrane, resulting in delivery of the contents of the liposome into the cell. For example, liposomes such as those described in U.S. Patent No. 5,077,211 of Yarosh, U.S. Patent No. 4,621,023 of Redziniak et al. or U.S. Patent No. 4,508,703 of Redziniak et al. can be used. Other suitable formulations can employ niosomes. Niosomes are lipid vesicles similar to liposomes, with membranes consisting largely of non-ionic lipids, some forms of which are effective for transporting compounds across the stratum corneum.
Administration

Compositions of this invention may be administered in any manner including, but not limited to, orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, or combinations thereof. Parenteral administration includes, but is not limited to, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intrathecal, and intraarticular. The compositions of this invention may also be administered in the form of an implant, which allows slow release of the compositions as well as a slow controlled i.v. infusion.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent application, issued U.S. or foreign patents or any other references, are entirely incorporated by reference herein, including all data, tables, figures and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various application such specific embodiments, without undue experimentation, without
departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning an range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

Example

Subjects

HIV-infected men and women were recruited from those receiving HAART at two London clinics. Subjects were selected on the basis of self-reported, physician-confirmed symptoms and signs of fat redistribution, either lipoatrophy or fat accumulation or combinations thereof.

Inclusion/Exclusion Criteria

Inclusion criteria: Subjects were included on the basis of self reported and physician confirmed HIV associated lipodystrophy (HARS) defined as 2 or more of the following features: loss of subcutaneous fat at the naso-labial fold, on the arms, on the legs, or on the trunk or accumulation of abdominal fat or the acquisition of a dorso-cervical fat pad. All were taking approved antiretroviral medication and were at least 18 years of age. Subjects were excluded if they had any history of liver disease, diabetes mellitus, angina pectoris, coronary artery disease or heart failure, pancreatitis, allergy or hypersensitivity to thiazolidinediones, HMG-CoA reductase inhibitors or rhGH, severe renal insufficiency or an active medical condition. Subjects were also excluded if they had a history of recent treatment with HMG-CoA reductase inhibitors, anabolic steroids, megestrol acetate, testosterone, glucocorticoids, human recombinant growth hormone, or thiazolidinediones.

All gave written informed consent under protocols approved by local ethics committees and studies were performed in accordance with the principles of the Declaration of Helsinki.

Interventions

This study was designed as a randomised, prospective, open label study of rosiglitazone, alone or in combination with pravastatin or recombinant human growth
hormone in the treatment of HIV associated lipodystrophy syndrome (HARS). 60 HIV-infected men with HARS were randomized into 5 arms, as illustrated, to receive daily therapy with: (1) rosiglitazone, 4 mg, (2) pravastatin, 40mg, or (3) rosiglitazone and pravastatin, or (4) recombinant human growth hormone (rhGH, Serostim®, 2mg) or (5) rhGH (2 mg) and rosiglitazone (4 mg) for 12 weeks. The primary endpoint for therapy with rosiglitazone, pravastatin, both or standard care was at 24 weeks but therapy was continued in the active treatment groups to ensure that late changes, beyond 24 weeks, were not missed. Subjects in the two standard care arms did not receive any pharmacologic intervention for the first 24 weeks in order to be able to describe the natural history of HALS in this setting but went on to receive rhGH from 24 to 36 weeks followed by a monitoring phase to establish the longevity of rhGH effects (from 36 to 48 weeks).

Assessments

Assessments every 12 weeks included dual energy X-ray absorptiometry (DEXA), computed tomography (CT), biochemistry and clinical anthropometries.

DEXA assessment consisted of a single whole body scan from which segmental analysis was used to derive trunk and limb fat and lean tissue mass. For CT assessment, single computed tomography slices were taken at the L4/5 level and mid thigh and assessed for fat composition as previously described (ref) to derive fat and lean areas in visceral or muscle and subcutaneous compartments. Self assessment of severity of lipodystrophy consisted of a seven-element linear 10cm visual analogue scale questionnaire relating to changes in fat loss on the face, arms, legs, and buttocks, increased abdominal fat, enlarged breasts or a "buffalo hump". Scores were reported as "change since last assessment" with a score of zero representing no change.

Results

Subjects

In total, 64 subjects entered into the study. Baseline demographics are shown in Table 1. More men than women were included in this study, reflecting the demographics of the clinic populations affected by lipodystrophy. All subjects were taking combination antiretroviral therapy at study entry. At this time, median CD4 count was 421 cells/ul. Most subjects had a fully suppressed viral load; only four of those subjects with a documented contemporaneous viral load reading had detectable viremia. When subjects were randomized, there were no differences in baseline parameters between groups.
Of the 64 subjects who entered the study, 46 completed all stages and assessments up to 48 weeks.

**Body composition changes**

Changes in body composition were assessed clinically, by single slice CT scanning of abdomen and thigh, by whole-body DEXA scanning, and by a visual analogue scale self-assessment as described. Clinically, there were no changes in the first 24 weeks in terms of body mass index (BMI) or waist-hip ratio (WHR). With rhGH therapy however, there was a significant increase in BMI and a fall in WHR.

**CT scan changes**

**Abdominal body composition changes**

No changes were seen in visceral adipose tissue (VAT) in any group in the first 24 weeks. Neither Rosiglitazone nor Pravastatin appeared to reduce VAT. However, VAT was significantly reduced in the group receiving rhGH treatment by about 30cm$^2$ or 26% after 12 weeks of therapy (from week 24 to 36 of the overall study, and by a similar amount in the group receiving rhGH and rosiglitazone in combination.

**DEXA scan changes**

When DEXA scan data were reviewed a similar pattern was observed with the greatest changes occurring in the rhGH treatment groups.

In both rhGH alone and rhGH in combination with rosiglitazone there was a marked reduction in Trunk Fat by a mean of over 1kg in both rhGH-receiving groups (P<0.05). There was a corresponding increase in trunk lean tissue mass after 12 weeks of treatment. rhGH also resulted in a large increase in limb lean tissue, although limb fat remained largely unaffected.

The results indicate that rhGH both alone and in combination with rosiglitazone were effective treatments for HARS.

**Changes in insulin secretion/ sensitivity and blood lipids.**

When fasting glucose and insulin concentrations in plasma were reviewed, it was noted that with rosiglitazone treatment there was a fall in plasma glucose. The major change seen, however, was in the rhGH-alone group where subjects developed significant insulin resistance, which was completely abrogated by the co-administration of Rosiglitazone. There were no significant changes in insulin or glucose in those subjects receiving rhGH and Rosiglitazone together, indicating that
the combination of rhGH and rosiglitazone effectively treats HARS without causing an increase in glucose levels that may occur with rhGH alone.
References


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Martin et al., AIDS. 2004 Apr 30;18(7):1029-36
Claims

1. Use of a combination of
   a. an agent which binds to and initiates signaling of the human growth hormone (hGH) receptor or a substance which stimulates release or potentiates the activity of endogenous hGH; and
   b. an agent which increases insulin sensitivity in said patient for the treatment of HARS.

2. The use according to claim 1, for prevention or treatment of insulin resistance.

3. The use according to claim 1 or 2, wherein said agent (a) is hGH or a functional derivative, fragment, variant, or salt thereof which retains the biological activity of human growth hormone.

4. The use according to any one of the preceding claims, wherein said agent (a) is an isolated human growth hormone.

5. The use of any one of the preceding claims, wherein said agent (a) is recombinant human growth hormone.

6. The use according to claims 1 or 2, wherein said agent (a) is
   i. Human growth hormone releasing hormone (hGHRH); or
   ii. A fragment of (i) that retains the ability to stimulate the release of growth hormone; or
   iii. A variant or analog of (i) or (ii) that retains the ability to stimulate the release of growth hormone; or
   iv. A functional derivative or salt or (i), (ii) or (iii) that retains the ability to stimulate the release of growth hormone.
7. The use according to claim 6, wherein said agent (a) comprises human growth hormone releasing hormone (hGHRH).

8. The use according to any one of the preceding claims, wherein said agent (b) is a glitazone selected from the group consisting of pioglitazone and rosiglitazone, or a pharmaceutically acceptable salt thereof.

9. The use according to any one of the preceding claims, wherein said agent (b) is rosiglitazone, or a pharmaceutically acceptable salt thereof.

10. The use according to any one of the preceding claims, wherein agent (a) and agent (b) are formulated for simultaneous, sequential or separate administration.
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/EP2007/059288

### A. CLASSIFICATION OF SUBJECT MATTER

**INV.** A61K31/4439  A61K38/27  A61P3/00

According to International Patent Classification (IPC) into both national classification and IPC.

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

Electronic database consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, EMBASE, BIOSIS

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication where appropriate, of the relevant passages</th>
<th>Relevant to claim</th>
</tr>
</thead>
</table>
| Y        | ANDERSEN O ET AL: "Low-dose growth hormone and human immunodeficiency virus-associated lipodystrophy syndrome: a pilot study"
EUROPEAN JOURNAL OF CLINICAL INVESTIGATION, BLACKWELL SCIENTIFIC PUBLICATIONS, XX, vol. 34, no. 8, August 2004 (2004-08), pages 561-568, XP009095112
ISSN: 0014-2972 *cf. abstract and introduction part, page 567, left-sided col., 2nd para.* | 1-10 |

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* Special categories of cited documents
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Stolter, Anton
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