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(54) Title: MODULATION OF ADIPOSE TRIGLYCERIDE LIPASE FOR PREVENTION AND TREATMENT OF CACHEXIA, LOSS OF WEIGHT AND MUSCLE ATROPHY AND METHODS OF SCREENING THEREFOR

(57) Abstract: The present invention relates to agents, and methods for identifying compounds, which agents and compounds are effective in the treatment, and more particularly, that inhibit cachexia, and its associated or related disorders and conditions. In addition, the invention relates to compositions and methods for the use thereof in treating conditions that are characterized by cachexia, and its associated or related disorders and conditions and/or cachexia, and its associated or related disorders and conditions, such as for example, cancer cachexia and cachexia associated with AIDS, autoimmune disorders, drug addiction, alcoholism, chronic inflammatory disorders, cirrhosis of the liver, anorexia and neurodegenerative disease.



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**MODULATION OF ADIPOSE TRIGLYCERIDE LIPASE FOR PREVENTION AND  
TREATMENT OF CACHEXIA, LOSS OF WEIGHT AND MUSCLE ATROPHY  
AND METHODS OF SCREENING THEREFOR**

**FIELD OF THE INVENTION**

[0001] The present invention relates to methods of prevention, treatment or alleviation of cachexia and other wasting syndromes or conditions of loss of weight and/or muscle atrophy by modulation, including inhibition, of lipase, particularly adipose triglyceride lipase (ATGL). The invention relates to agents, and methods for identifying compounds, which agents and compounds result in the inhibition of lipase, particularly ATGL, and the alleviation of cachexia or related conditions or disorders.

**BACKGROUND OF THE INVENTION**

[0002] Animals, seed plants, and fungi commonly store excessive amounts of energy substrates in the form of intracellular triglyceride (TG) deposits. In mammals, TG are stored in adipose tissue providing the primary source of energy during periods of food deprivation. Whole body energy homeostasis depends on the precisely regulated balance of lipid storage and mobilization. Mobilization of fatty acids from triglyceride stores in adipose tissue critically depends on the activation of lipolytic enzymes, which degrade adipose TG and release non-esterified fatty acids (FA) into the circulation. Dysfunctional lipolysis affects energy homeostasis and may contribute to the pathogenesis of obesity and insulin resistance. Dysregulation of TG-lipolysis in man has been linked to variation in the concentration of circulating FA, an established risk factor for the development of insulin resistance (Bergman, R.N. et al (2001) *J Investig Med* 49: 119-26; Blaak, E.E. (2003) *Proc Nutr Soc* 62: 753-60; Boden, G. and G.I. Shulman (2002) *Eur J Clin Invest* 32(Suppl 3):14-23; Arner, P. (2002) *Diabetes Metab Res Rev* 18(Suppl 2): S5-9).

[0003] During periods of increased energy demand, lipolysis in adipocytes is activated by hormones, such as catecholamines. Hormone interaction with G-protein coupled receptors is followed by increased adenylate cyclase activity, increased cAMP levels, and the activation of cAMP-dependent protein kinase (protein kinase A, PKA) (Collins, S. and R.S. Surwit (2001) *Recent Prog Horm Res* 56:309-28). PKA then phosphorylates targets with established function in lipolysis including hormone-sensitive lipase (HSL), resulting in the translocation of HSL from the cytoplasm to the lipid droplet where efficient TG hydrolysis occurs (Sztalryd, C. et al (2003) *J Cell Biol* 161:1093-103).

[0004] The mobilization of free fatty acids from adipose triacylglycerol (TG) stores requires the activities of triacylglycerol lipolysis. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase

(HSL) are the major enzymes contributing to TG breakdown. ATGL (also named PNPLA 2 (patatin-like phospholipase domain containing protein-2, desnutrin, phospholipase A2 $\delta$ , and transport-secretion protein)) is highly expressed in adipose tissue and specifically removes the first fatty acid from the TG molecule, generating FFA and DG (Zimmerman, R. et al (2006) Science 306:1383-1386; Wang, SP et al (2001) Obes Res 9:119-128; Villena, JA et al (2004) J Biol Chem 279:47066-47075; Jenkins, CM et al (2004) J Biol Chem 279:48968-48975). An essential role of ATGL in lipolysis has been demonstrated in studies of ATGL-deficient (ATGL-ko) mice (Haemmerle, G. et al (2006) Science 312:734-737). ATGL-deficient mice accumulated large amounts of lipid in the heart, causing cardiac dysfunction and premature death. The relative contribution of these hydrolases to the lipolytic catabolism of fat has been determined, including in mutant mouse models lacking ATGL or HSL (Schweiger, M. et al (2006) J Biol Chem 281(52):40236-40241). Both HSL and ATGL enzymes contribute to hydrolysis of TG, however, ATGL deficient mice studies indicate that ATGL is rate limiting in the catabolism of cellular fat deposits and plays an important role in energy homeostasis (Haemmerle, G. et al (2006) Science 312(5774):734-737).

**[0005]** Cachexia is loss of weight, muscle atrophy, fatigue, weakness and significant loss of appetite in someone who is not actively trying to lose weight. It can be a sign of various underlying disorders; when a patient presents with cachexia, a doctor will generally consider the possibility of cancer, certain infectious diseases (e.g. tuberculosis, AIDS), and some autoimmune disorders, or addiction to drugs such as amphetamines or cocaine, chronic alcoholism and cirrhosis of the liver. Cachexia physically weakens patients to a state of immobility stemming from loss of appetite, asthenia, and anemia, and response to standard treatment is usually poor (Lainscak M, et al (2007) Curr Opin Support Palliat Care 1(4): 299–305; Bossola M et al (2007) Expert Opin Investig Drugs 16 (8): 1241–53).

**[0006]** Cachexia is often seen in end-stage cancer, and in that context is called "cancer cachexia". It was also prevalent in HIV patients before the advent of highly active anti-retroviral therapy (HAART) for that condition; now it is seen less frequently in those countries where such treatment is available. In those patients who have Congestive Heart Failure, there is also a cachectic syndrome. Also, a cachexia co-morbidity is seen in patients that have any of the range of illnesses classified as "COPD" (chronic obstructive pulmonary disease), particularly emphysema. Some severe cases of schizophrenia can present this condition where it is named *vesanic cachexia* (from *vesania*, a Latin term for insanity).

**[0007]** The exact mechanism by which these diseases cause cachexia is poorly understood, but there is postulated a role for inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukins 1 and 6 (IL-1 and IL-6), interferon gamma (IFN- $\gamma$ ), leukemia-inhibitory factor (LAF), as well as ZAG, a 43 kDa soluble glycoprotein as mediators of the cachectic process. However, the results of a number of clinical and laboratory studies suggest that the action of the cytokines alone is

unable to explain the complex mechanism of wasting in cancer cachexia. In addition, cachexia has been observed in some xenograft models even with concomitant administration of anti-TNF- $\alpha$  antibody without a cytokine involvement, suggesting that TNF- $\alpha$  may not be responsible for the cachexia and that other factors may be involved (Costelli P et al (1993) J Clin Invest 92:2783-2789; Sherry BA et al (1989) FASEB J 3:1956-1962).

[0008] About half of all cancer patients show a syndrome of cachexia, characterized by loss of adipose tissue and skeletal muscle mass. Such patients have a decreased survival time, compared with the survival time among patients without weight loss, and loss of total body protein leads to substantial impairment of respiratory muscle function. The definitive treatment of cancer cachexia is removal of the causative tumor. Short of achieving this goal, which is often compromised by the patients' inability to tolerate cancer treatments due to their cachexia, various measures have been undertaken to ameliorate cachexia, however with limited success. Various agents have been administered in attempts to retard or halt progressive cachexia in cancer patients. These agents include orexigenic agents (appetite stimulants), corticosteroids, cannabinoids, serotonin antagonists, prokinetic agents, androgens and anabolic agents, anticytokine agents, NSAIDs, and regulators of circadian rhythm.

[0009] Despite an increased understanding of ATGL and its role in TG hydrolysis and lipolysis, there is still a need for a fuller and specific understanding of its physiological role and its potential application and/or role in pathological conditions. Cachexia and other relevant wasting syndromes provide a significant and largely unaddressed condition. Improved and specific therapies for these conditions and for the particular modulation of the fat and muscle wasting associated therewith are needed.

[0010] The citation of references herein shall not be construed as an admission that such is prior art to the present invention.

### **SUMMARY OF THE INVENTION**

[0011] In its broadest aspect, the present invention extends to the recognition that inhibition of lipase activity provides a novel approach to prevention, treatment and/or alleviation of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, and reduction of white adipose tissue (WAT). Thus inhibition or reduction of the activity of lipases, particularly inhibition of ATGL alone or in combination with inhibition of HSL, is provided for the prevention, treatment and/or alleviation of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, and reduction of WAT.

[0012] The present invention is based on the discovery that inhibition of the lipase ATGL by knockout or elimination of its expression in animal models results in prevention of cachexia in a lung cancer tumor-induced cachexia model system. ATGL inhibition maintains white adipose tissue, and muscle mass significantly in tumor-bearing animals versus either wild type or HSL inhibited animals.

The present invention therefore provides ATGL as a TARGET which is involved in the pathway involved in cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting. The invention includes methods of screening for agents capable of prevention, treatment or alleviation of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, and uses of these agents in the prevention and /or treatment of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting.

**[0013]** The present invention relates to a method for identifying compounds that inhibit ATGL expression and/or activity, comprising contacting the compound with the identified TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below), or cells expressing the TARGET, or animals expressing the TARGET under conditions that allow said TARGET or active fragments or lipase domains thereof to bind to the compound, and measuring a property related to ATGL expression or activity, including but not limited to lipase activity, TG hydrolysis, fatty acid release, ATGL mRNA expression, ATGL protein levels, or physiological markers such as cachexia, body weight, WAT, and muscle mass.

**[0014]** Aspects of the present method include the *in vitro* assay of compounds using identified TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below), and cellular assays wherein identified TARGET inhibition is followed by observing indicators of efficacy, including alteration of the release of free fatty acid, TG hydrolysis, etc. Another aspect of the invention is a method of treatment or prevention of a condition involving cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, in a subject suffering or susceptible thereto, by administering a pharmaceutical composition comprising an agent which is able to inhibit ATGL. Also contemplated herein, are compositions comprising one or more ATGL-inhibiting agents of the invention, alone, or in combination with each other or in combinations with one or more inhibitors of inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukins 1 and 6 (IL-1 and IL-6), interferon gamma (IFN- $\gamma$ ), and leukemia-inhibitory factor (LAF).

**[0015]** The present invention relates to a method for identifying compounds that inhibit the TARGET(s) (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below), comprising contacting the compound with the identified TARGET or active fragments or lipase domains thereof under conditions wherein the compounds may interact with or influence the TARGET(s), measuring the expression of ATGL, levels of TG, release of fatty acid, lipase activity, etc. and selecting compounds which alter, particularly inhibit or reduce the expression of ATGL, release of fatty acid, lipase activity, etc. or stabilize the levels of TG in the presence of other TG lipases such as HSL. In one such method the release of free fatty acid from adipose tissue is measured.

[0016] Aspects of the present method include the *in vitro* assay of compounds using polypeptide of a TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below), or fragments thereof, including the amino acid sequences described in Figures 7 (human ATGL; SEQ ID NO: 1 shows the amino acid sequence and SEQ ID NO: 2 shows the cDNA including the coding sequence) and 8 (mouse ATGL; SEQ ID NO: 3 shows the amino acid sequence and SEQ ID NO: 4 shows the cDNA including the coding sequence) hereof and the publicly known sequences of ATGL, and cellular assays wherein TARGET inhibition is followed by observing indicators of efficacy including, for example, TARGET expression levels, TARGET enzymatic activity, release of FA, TG hydrolysis, lipase activity, and/or other assessments of fat, white adipose tissue, muscle mass, weight loss, and /or cachexia.

[0017] The present invention also relates to

- (1) expression inhibitory agents comprising a polynucleotide selected from the group of an antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said polynucleotide comprises a nucleic acid sequence complementary to, or engineered from, a naturally occurring polynucleotide sequence encoding a TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below) polypeptide and
- (2) pharmaceutical compositions comprising said agent(s), useful in the treatment, or prevention, of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting.

[0018] Another aspect of the invention is a method of treatment or prevention of a disease or condition characterized by fat loss, weight loss, muscle atrophy, wasting, anorexia, or cachexia, and in particular those diseases/conditions characterized by involuntary or unintended loss of weight, fat and/or muscle, in a subject suffering from or susceptible thereto, by administering a pharmaceutical composition comprising an effective TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below)-expression inhibiting amount of a expression-inhibitory agent or an effective TARGET activity inhibiting amount of an activity-inhibitory agent.

[0019] Another aspect of this invention relates to the use of agents which inhibit a TARGET as disclosed herein in a therapeutic method, a pharmaceutical composition, and the manufacture of such composition, useful for the treatment of a disease or condition involving cachexia, and its associated or related disorders and conditions. In particular, the present method relates to the use of the agents which inhibit a TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below) in the treatment of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, fat loss, and /or reduced WAT.

[0020] Another aspect of this invention relates to the use of agents which inhibit a TARGET as disclosed herein in a therapeutic method, a pharmaceutical composition, and the manufacture of such composition, useful for the treatment of a disease involving cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, fat loss, and /or reduced WAT.

[0021] Other objects and advantages will become apparent from a consideration of the ensuing description taken in conjunction with the following illustrative drawings.

#### **BRIEF DESCRIPTION OF THE FIGURES**

[0022] **Figure 1A and 1B.** **A:** depicts average weight change for WT, Lipase A (ATGL) ko mice and Lipase B (HSL) ko mice. Weight changes compared to the initial weight is depicted in g for tumor bearing animals and normal mock injected animals; tumor tissue weight was subtracted from the body weight. **B:** depicts average food weight per day for WT, Lipase A (ATGL) ko mice and Lipase B (HSL) ko mice. Food intake was recorded daily. Average daily food consumption is given in g.

[0023] **Figure 2A, 2B and 2C** depict visual inspections of white adipose tissue (WAT) in tumor bearing mice. **A** shows no visible fat in tumor bearing wildtype mice. **B** shows that WAT is preserved in tumor bearing ATGL ko mice. WAT is clearly evident at the arrow indicated. **C** depicts a closer view of preserved perirenal WAT in ATGL ko mice at arrows where indicated.

[0024] **Figure 3A and 3B.** **A:** WAT weight normalized to body weight (mg/g) is given for mock injected and tumor bearing wildtype, Lipase A (ATGL) ko mice and Lipase B (HSL) ko mice. Almost identical results were obtained when WAT was normalized to the length of the tibia bone. **B:** Average fat change by NMR for mock injected and tumor bearing wildtype, Lipase A (ATGL) ko mice and Lipase B (HSL) ko mice. NMR analysis for the assessment of total body fat content was performed before injection and immediately before sacrificing the animals respectively. Using NMR analysis, fat content is recorded as percentage and total fat weight is calculated from the animals' weight. Change (in g) is reported compared to the initial values before injection.

[0025] **Figure 4** depicts results of lipase assay of mock injected and tumor bearing mice including wildtype, Lipase A (ATGL) ko mice, and Lipase B (HSL) ko mice, in the presence or absence of a lipase B inhibitor. Animals were sacrificed 14 days after injection of tumor cells or mock injection, a time point where adipose tissue still can be dissected from wild type tumor bearing mice. Lipase activity was measured in homogenates of adipose tissue without (total lipase activity) or with inhibition of HSL (Lipase B) using an HSL inhibitor.

[0026] **Figure 5A and 5B.** **A:** depicts average normalized gastrocnemius muscle weight and **B:** depicts average normalized soleus muscle weight in mock injected and tumor bearing mice including wildtype, Lipase A (ATGL) ko mice, and Lipase B (HSL) ko mice. 21 days after injection of tumor cells or mock injections, mice were sacrificed. *M. gastrocnemius* and *M. soleus* were dissected from

both hind legs and the weight recorded. Muscle weight normalized to body weight is compared to the mock injected animals.

[0027] **Figure 6A and 6B.** **A** depicts proteasome activity in mock injected and tumor bearing mice including wildtype, Lipase A (ATGL) ko mice, and Lipase B (HSL) ko mice. **B** depicts caspase activity in mock injected and tumor bearing mice including wildtype and Lipase A (ATGL) ko mice. Wildtype mice were measured at 8, 14 and 21 days, and Lipase A mice were measured at 14 and 21 days. Proteasome as well as caspase activities were measured in muscle homogenates. Values (arbitrary fluorescence or luminescence respectively) were normalized to protein concentration and compared to mock injected animals.

[0028] **Figure 7** as well as SEQ ID NOs: 1 and 2 depict the amino acid and cDNA including the nucleic acid coding sequence, respectively, of human ATGL. These sequences correspond to Genbank NM\_020376 (nucleotide) and NP\_065109 (protein).

[0029] **Figure 8** as well as SEQ ID NOs: 3 and 4 depict the amino acid and cDNA including the nucleic acid coding sequence, respectively, of mouse ATGL. These sequences correspond to Genbank NM\_025802 (nucleotide) and NP\_080078 (protein).

#### **DETAILED DESCRIPTION OF THE INVENTION**

[0030] The following terms are intended to have the meanings presented therewith below and are useful in understanding the description and intended scope of the present invention.

[0031] The term 'agent' means any molecule, including polypeptides, antibodies, polynucleotides, chemical compounds and small molecules. In particular the term agent includes compounds such as test compounds or drug candidate compounds.

[0032] The term 'agonist' refers to a ligand that stimulates the receptor the ligand binds to in the broadest sense.

[0033] As used herein, the term 'antagonist' is used to describe a compound that does not provoke a biological response itself upon binding to a receptor, but blocks or dampens agonist-mediated responses, or prevents or reduces agonist binding and, thereby, agonist-mediated responses.

[0034] The term 'assay' means any process used to measure a specific property of an agent. A 'screening assay' means a process used to characterize or select agents based upon their activity from a collection of agents.

[0035] The term 'binding affinity' is a property that describes how strongly two or more compounds associate with each other in a non-covalent relationship. Binding affinities can be characterized qualitatively (such as 'strong', 'weak', 'high', or 'low') or quantitatively (such as measuring the  $K_D$ ).

[0036] The term 'carrier' means a non-toxic material used in the formulation of pharmaceutical compositions to provide a medium, bulk and/or useable form to a pharmaceutical composition. A



carrier may comprise one or more of such materials such as an excipient, stabilizer, or an aqueous pH buffered solution. Examples of physiologically acceptable carriers include aqueous or solid buffer ingredients including phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS®.

[0037] The term 'complex' means the entity created when two or more compounds bind to, contact, or associate with each other.

[0038] The term 'compound' is used herein in the context of a 'test compound' or a 'drug candidate compound' described in connection with the assays of the present invention. As such, these compounds comprise organic or inorganic compounds, derived synthetically, recombinantly, or from natural sources.

[0039] The compounds include inorganic or organic compounds such as polynucleotides, lipids or hormone analogs. Other biopolymeric organic test compounds include peptides comprising from about 2 to about 40 amino acids and larger polypeptides comprising from about 40 to about 500 amino acids, including polypeptide ligands, enzymes, receptors, channels, antibodies or antibody conjugates.

[0040] The term 'condition' or 'disease' means the overt presentation of symptoms (i.e., illness) or the manifestation of abnormal clinical indicators (for example, biochemical indicators or diagnostic indicators). Alternatively, the term 'disease' refers to a genetic or environmental risk of or propensity for developing such symptoms or abnormal clinical indicators.

[0041] The term 'contact' or 'contacting' means bringing at least two moieties together, whether in an *in vitro* system or an *in vivo* system.

[0042] The term 'derivatives of a polypeptide' relates to those peptides, oligopeptides, polypeptides, proteins and enzymes that comprise a stretch of contiguous amino acid residues of the polypeptide and that retain a biological activity of the protein, for example, polypeptides that have amino acid mutations compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may further comprise additional naturally occurring, altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally occurring form of the polypeptide. It may also contain one or more non-amino acid substituents, or heterologous amino acid substituents, compared to the amino acid sequence of a naturally occurring form of the polypeptide, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence.

[0043] The term 'derivatives of a polynucleotide' relates to DNA-molecules, RNA-molecules, and oligonucleotides that comprise a stretch of nucleic acid residues of the polynucleotide, for example, polynucleotides that may have nucleic acid mutations as compared to the nucleic acid sequence of a naturally occurring form of the polynucleotide. A derivative may further comprise nucleic acids with modified backbones such as PNA, polysiloxane, and 2'-O-(2-methoxy) ethyl-phosphorothioate, non-naturally occurring nucleic acid residues, or one or more nucleic acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection.

[0044] The term 'endogenous' shall mean a material that a mammal naturally produces. Endogenous in reference to the term 'protease', 'kinase', 'factor', or 'receptor' shall mean that which is naturally produced by a mammal (for example, and not limitation, a human). In contrast, the term non-endogenous in this context shall mean that which is not naturally produced by a mammal (for example, and not limitation, a human). Both terms can be utilized to describe both *in vivo* and *in vitro* systems. For example, and without limitation, in a screening approach, the endogenous or non-endogenous TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below) may be in reference to an *in vitro* screening system. As a further example and not limitation, where the genome of a mammal has been manipulated to include a non-endogenous TARGET, screening of a candidate compound by means of an *in vivo* system is viable.

[0045] The term 'expressible nucleic acid' means a nucleic acid coding for a proteinaceous molecule, an RNA molecule, or a DNA molecule.

[0046] The term 'expression' comprises both endogenous expression and overexpression by transduction.

[0047] The term 'expression inhibitory agent' means a polynucleotide designed to interfere selectively with the transcription, translation and/or expression of a specific polypeptide or protein normally expressed within a cell. More particularly, 'expression inhibitory agent' comprises a DNA or RNA molecule that contains a nucleotide sequence identical to or complementary to at least about 15-30, particularly at least 17, sequential nucleotides within the polyribonucleotide sequence coding for a specific polypeptide or protein. Exemplary expression inhibitory molecules include ribozymes, double stranded siRNA molecules, self-complementary single-stranded siRNA molecules (shRNA), genetic antisense constructs, and synthetic RNA antisense molecules with modified stabilized backbones.

[0048] The term 'fragment of a polynucleotide' relates to oligonucleotides that comprise a stretch of contiguous nucleic acid residues that exhibit substantially a similar, but not necessarily identical, activity as the complete sequence. In a particular aspect, 'fragment' may refer to a oligonucleotide comprising a nucleic acid sequence of at least 5 nucleic acid residues (preferably, at least 10 nucleic acid residues, at least 15 nucleic acid residues, at least 20 nucleic acid residues, at least 25 nucleic acid

residues, at least 40 nucleic acid residues, at least 50 nucleic acid residues, at least 60 nucleic residues, at least 70 nucleic acid residues, at least 80 nucleic acid residues, at least 90 nucleic acid residues, at least 100 nucleic acid residues, at least 125 nucleic acid residues, at least 150 nucleic acid residues, at least 175 nucleic acid residues, at least 200 nucleic acid residues, or at least 250 nucleic acid residues) of the nucleic acid sequence of said complete sequence.

[0049] The term 'fragment of a polypeptide' relates to peptides, oligopeptides, polypeptides, proteins, monomers, subunits and enzymes that comprise a stretch of contiguous amino acid residues, and exhibit substantially a similar, but not necessarily identical, functional or expression activity as the complete sequence. In a particular aspect, 'fragment' may refer to a peptide or polypeptide comprising an amino acid sequence of at least 5 amino acid residues (preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues) of the amino acid sequence of said complete sequence.

[0050] The term 'hybridization' means any process by which a strand of nucleic acid binds with a complementary strand through base pairing. The term 'hybridization complex' refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (for example,  $C_{0t}$  or  $R_{0t}$  analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (for example, paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed). The term "stringent conditions" refers to conditions that permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, for example, formamide, temperature, and other conditions well known in the art. In particular, reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature can increase stringency. The term 'standard hybridization conditions' refers to salt and temperature conditions substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such 'standard hybridization conditions' are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art

according to well known formulae, wherein hybridization is typically 10-20<sup>N</sup>C below the predicted or determined T<sub>m</sub> with washes of higher stringency, if desired.

[0051] The term 'inhibit' or 'inhibiting', in relationship to the term 'response' means that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

[0052] The term 'inhibition' refers to the reduction, down regulation of a process or the elimination of a stimulus for a process, which results in the absence or minimization of the expression or activity of a protein or polypeptide.

[0053] The term 'induction' refers to the inducing, up-regulation, or stimulation of a process, which results in the expression or activity of a protein or polypeptide.

[0054] The term 'ligand' means a molecule, including an endogenous, naturally occurring or synthetic, non-natural molecules, specific for an endogenous, naturally occurring receptor.

[0055] The term 'pharmaceutically acceptable salts' refers to the non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds which inhibit the expression or activity of TARGETS as disclosed herein. These salts can be prepared *in situ* during the final isolation and purification of compounds useful in the present invention.

[0056] The term 'polypeptide' relates to proteins (such as TARGETS (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein below)), proteinaceous molecules, fragments of proteins, monomers, subunits or portions of polymeric proteins, peptides, oligopeptides and enzymes (such as kinases, proteases, GPCR's etc.).

[0057] The term 'polynucleotide' means a polynucleic acid, in single or double stranded form, and in the sense or antisense orientation, complementary polynucleic acids that hybridize to a particular polynucleic acid under stringent conditions, and polynucleotides that are homologous in at least about 60 percent of its base pairs, and more particularly 70 percent of its base pairs are in common, most particularly 90 per cent, and in a particular embodiment, 100 percent of its base pairs. The polynucleotides include polyribonucleic acids, polydeoxyribonucleic acids, and synthetic analogues thereof. It also includes nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate. The polynucleotides are described by sequences that vary in length, that range from about 10 to about 5000 bases, particularly about 100 to about 4000 bases, more particularly about 250 to about 2500 bases. One polynucleotide embodiment comprises from about 10 to about 30 bases in length. A particular embodiment of polynucleotide is the polyribonucleotide of from about 17 to about 22 nucleotides, more commonly described as small interfering RNAs (siRNAs - double stranded siRNA molecules or self-complementary single-stranded siRNA molecules (shRNA)). Another particular embodiment are nucleic acids with modified backbones such as peptide nucleic acid (PNA), polysiloxane, and 2'-O-(2-methoxy)ethylphosphorothioate, or including non-naturally occurring nucleic acid residues, or one or

more nucleic acid substituents, such as methyl-, thio-, sulphate, benzoyl-, phenyl-, amino-, propyl-, chloro-, and methanocarbanucleosides, or a reporter molecule to facilitate its detection.

Polynucleotides herein are selected to be 'substantially' complementary to different strands of a particular target DNA sequence. This means that the polynucleotides must be sufficiently complementary to hybridize with their respective strands. Therefore, the polynucleotide sequence need not reflect the exact sequence of the target sequence. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the polynucleotide, with the remainder of the polynucleotide sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the polynucleotide, provided that the polynucleotide sequence has sufficient complementarity with the sequence of the strand to hybridize therewith under stringent conditions or to form the template for the synthesis of an extension product.

**[0058]** The term 'preventing' or 'prevention' refers to a reduction in risk of acquiring or developing a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop) in a subject that may be exposed to a disease-causing agent, or predisposed to the disease in advance of disease onset.

**[0059]** The term 'prophylaxis' is related to and encompassed in the term 'prevention', and refers to a measure or procedure the purpose of which is to prevent, rather than to treat or cure a disease. Non-limiting examples of prophylactic measures may include the administration of vaccines; the administration of low molecular weight heparin to hospital patients at risk for thrombosis due, for example, to immobilization; and the administration of an anti-malarial agent such as chloroquine, in advance of a visit to a geographical region where malaria is endemic or the risk of contracting malaria is high.

**[0060]** The term 'solvate' means a physical association of a compound useful in this invention with one or more solvent molecules. This physical association includes hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Representative solvates include hydrates, ethanolates and methanolates.

**[0061]** The term 'subject' includes humans and other mammals. In the context of this invention, it is particularly envisaged that mammals are to be treated which are economically, agronomically or scientifically important. Scientifically important organisms include, but are not limited to, mice, rats, and rabbits. Non-limiting examples of agronomically important animals are sheep, cattle and pigs, while, for example, cats and dogs may be considered as economically important animals. Preferably, the subject is a human.

**[0062]** 'Therapeutically effective amount' means that amount of a drug, compound, expression inhibitory agent, or pharmaceutical agent that will elicit the biological or medical response of a subject that is being sought by a medical doctor or other clinician.

[0063] The term ‘treating’ or ‘treatment’ of any disease or disorder refers, in one embodiment, to ameliorating the disease or disorder (i.e., arresting the disease or reducing the manifestation, extent or severity of at least one of the clinical symptoms thereof). In another embodiment ‘treating’ or ‘treatment’ refers to ameliorating at least one physical parameter, which may not be discernible by the subject. In yet another embodiment, ‘treating’ or ‘treatment’ refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In a further embodiment, ‘treating’ or ‘treatment’ relates to slowing the progression of the disease.

[0064] The term “vectors” also relates to plasmids as well as to viral vectors, such as recombinant viruses, or the nucleic acid encoding the recombinant virus.

[0065] The term “vertebrate cells” means cells derived from animals having vertebrate structure, including fish, avian, reptilian, amphibian, marsupial, and mammalian species. Preferred cells are derived from mammalian species, and most preferred cells are human cells. Mammalian cells include feline, canine, bovine, equine, caprine, ovine, porcine murine, such as mice and rats, and rabbits.

[0066] The term ‘TARGET’ or ‘TARGETS’ means the protein(s) identified in accordance with the assays and methods described herein and determined to be involved in the modulation and/or prevention, treatment or alleviation of cachexia and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, reduction of WAT, and reduction of fat. The term TARGET or TARGETS includes and contemplates lipases responsible for or contributing to the hydrolysis of triacylglycerides and lipolysis, particularly adipose triacylglycerol (TG), including ATGL and/or HSL, alternative species forms, isoforms, fragments and variants, such as splice variants, allelic variants, alternate in frame exons, and alternative or premature termination or start sites, including known or recognized isoforms or variants. Preferably, alternative species forms of ATGL, isoforms of ATGL, fragments of ATGL and variants of ATGL, such as splice variants, allelic variants, alternate in frame exons, and alternative or premature termination or start sites, are enzymatically or lipolytically active, e.g., are capable of removing the first fatty acid from a triacylglycerol (TG), thus generating free fatty acid (FFA) and diacylglycerol (DG). Alternative species forms of HSL, isoforms of HSL, fragments of HSL and variants of HSL, such as splice variants, allelic variants, alternate in frame exons, and alternative or premature termination or start sites, may be enzymatically or lipolytically active, e.g., capable of hydrolyzing triacylglycerol (TG). Lipolytic activity may be detected by assay methods known to those of skill in the art. Preferably, methods such as described hereinunder in Examples 1 and 2 are used. In a preferred embodiment of the invention, the method as described herein in paragraph [0172] to [0176] in Example 2 hereinunder, including references made therein, is used. A preferred embodiment of the invention includes measurement of lipase activity according to Jenkins et al. and Chung et al. as described in Example 2 herein. A further preferred embodiment of the invention includes measurement of lipase activity as

described in paragraph [0175] in Example 2 herein and references made therein. A further preferred embodiment of the invention includes measurement of lipase activity as described in paragraph [0175] in Example 2 herein and references made therein. Any such assays and tests are described for the purpose of illustration and guidance; variations, alterations, adaptations and modifications will be obvious and possible to the person of skill in the art. For instance, the test described in paragraphs [0175] or [0176] may be used to test the lipolytic activity and if desired the inhibition thereof by a given inhibitor, among others, of variants, fragments, variants due to premature termination or the like, of a lipase, preferably of ATGL lipase, without deviating from the scope of the invention.

[0067] The TARGET is preferably a lipase, more particularly ATGL, and particularly human ATGL. Exemplary human ATGL sequence is set out in Figure 7 as well as in SEQ ID NO: 1 (amino acid sequence of human ATGL) and SEQ ID NO: 2 (cDNA including the coding sequence of human ATGL).

[0068] The term “cachexia and its associated or related disorders and conditions” or “cachexia and its related conditions or physiology” or variants thereof, refers to a disease or condition which involves, results at least in part from, or includes loss of weight, muscle atrophy, fatigue, weakness and significant loss of appetite in someone who is not actively trying to lose weight. It can be associated with or result from (directly or indirectly) various underlying disorders including cancer, metabolic acidosis (from decreased protein synthesis and increased protein catabolism), certain infectious diseases (e.g. tuberculosis, AIDS), some autoimmune disorders, addiction to drugs such as amphetamines or cocaine, chronic alcoholism and cirrhosis of the liver, chronic inflammatory disorders, anorexia, and neurodegenerative disease. In a particular aspect, cachexia is cancer cachexia. In other such aspects, muscle wasting and/or unintended body weight loss associated with neurological conditions, immobility or impaired mobility due to various diseases such as neurodegenerative disease, MS, spinal cord injury, is included in the term.

## TARGETS

[0069] The present invention is based on the present inventors' discovery that the TARGETS (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as further described herein) are involved in cachexia and its associated disorders or conditions, whereby inhibition of the TARGETS results in correction, alleviation, or prevention of cachexia and its associated disorders or conditions or fat or muscle pathology. The present invention extends to the recognition that inhibition of lipase activity provides a novel approach to prevention, treatment and/or alleviation of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, and reduction of WAT. Thus inhibition or reduction of the activity of TARGETS, particularly lipases, particularly inhibition of ATGL alone or in combination with inhibition of HSL, is provided for the prevention, treatment and/or alleviation of cachexia, and its associated or related disorders and

conditions, including weight loss, muscle atrophy or wasting and reduction of WAT. The TARGETS are factors or protein molecules involved in the cachexia response and/or serve to facilitate, mediate, or otherwise participate such that their inhibition results in the alleviation, treatment and/or prevention of cachexia and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting and reduction of WAT. The TARGETS may also serve a role in correcting low body weight or fat or muscle atrophy associated with various conditions or disorders.

[0070] Lipase inhibitors, including pancreatic lipase inhibitors have been described and are known, although these are generally broad class inhibitors and may not be specific or particularly effective against the TARGET as described herein, particularly against ATGL. Known and/or recognized and described lipase inhibitors include but are not limited to: lipstatin and its saturated derivative Orlistat (Xenical®) which is described in US Patent 4,598,089; benzoquinone-derived compounds described in US Patent 7,355,055 B2; oxetanones or substituted  $\beta$ -lactones as described in US Patent 7,074,822, US Patent 6,852,865, US Patent 4,931,463, and US Patent 5,175,186; lipolytic enzyme inhibitors purothionins, protamines, polylysines described in US Patent 5,411,956 and US Patent 5,376,640; and thiophene compounds described in US Patent 7,064,122. Compounds with lipase-inhibiting activity, including these above as noted and referenced, and incorporated herein by reference, are described as suitable for the treatment and/or prophylaxis of obesity and of associated accompanying and/or concomitant diseases involved therewith, including in particular metabolic syndromes (hypertension, insulin resistance, Type II diabetes, hyperglyceridemia) and cardiovascular diseases (coronary heart disease, cerebrovascular disease, peripheral occlusive arterial disease). These compounds have been described as capable of reducing the proportion of edible fats actually digested by the body in the total edible fats ingested. These compounds are not described or suggested as involved in or mediating the prevention, alleviation or treatment of a pathological condition including cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting and reduction of WAT,. Therefore, the use of lipase inhibitors, including as described here above in the prevention, alleviation or treatment of a pathological condition including cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, reduction of WAT, is an aspect of the invention.

[0071] Therefore, in one aspect, the present invention relates to a method for assaying for drug candidate compounds that alleviate, prevent or treat cachexia and its associated disorders, conditions and/or physiology comprising contacting the compound with a polypeptide TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above), or enzymatically active fragment thereof, under conditions that allow said polypeptide to bind to the compound or be modulated by the compound, and detecting the formation of a complex between the polypeptide and the compound or an alteration in the activity or expression of the polypeptide TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described



herein above). In particular said method is used to identify an agent that inhibits the TARGET, particularly a lipase inhibitor, particularly and ATGL inhibitor. In particular said method may be used to identify drug candidate compounds that inhibit the excessive release of free FA from TG and/or lipolysis. One means of measuring the complex formation is to determine the binding affinity of said compound to said polypeptide. One means of measuring the alteration in the activity or expression of the polypeptide TARGET is to determine the expression of the TARGET or the enzymatic activity of the TARGET or the release of the product(s) resulting from the enzymatic activity of the TARGET.

[0072] More particularly, the invention relates to a method for identifying an agent or compound that alleviates cachexia or its associated physiology whereby said agent or compound inhibits TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) said method comprising:

- (a) contacting a population of mammalian cells with one or more compound that exhibits binding affinity for a TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) polypeptide, or fragment thereof, and
- (b) measuring a compound-polypeptide property related to TG hydrolysis.

[0073] In a further aspect of the present invention said method is used to identify a compound that inhibits lipolysis, particularly inhibits ATGL activity or expression. In particular the inhibition of the release of free fatty acid from TG may be assessed.

[0074] In a further aspect, the present invention relates to a method for assaying for drug candidate compounds that alleviate cachexia and/or its physiology comprising contacting the compound with a polypeptide TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above), or a fragment thereof, under conditions that allow said compound to modulate the activity or expression of the polypeptide, and determining the activity or expression of the polypeptide. In particular said method may be used to identify drug candidate compounds capable of suppressing the release of free fatty acid from TG. One particular means of measuring the activity or expression of the polypeptide is to determine the amount of said polypeptide using a polypeptide binding agent, such as an antibody, or to determine the activity of said polypeptide in a biological or biochemical measure, for instance the amount of lipolysis or TG hydrolysis.

[0075] The compound-polypeptide property referred to above is related to the expression and/or activity of the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above), and is a measurable phenomenon chosen by the person of ordinary skill in the art. The measurable property may be, for example, the binding affinity of said compound for a peptide domain of the polypeptide TARGET, a property related to the folding or activity of the disease-related protein or the level of any one of a number of biochemical marker levels of cachexia, fatty acids, or

muscle wasting or atrophy. In a preferred method, TG hydrolysis is measured by measuring release of free fatty acid from a TG substrate.

[0076] In an additional aspect, the present invention relates to a method for assaying for drug candidate compounds that inhibit ATGL and/or HSL, comprising contacting the compound with a nucleic acid encoding a TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) polypeptide, or fragment thereof, under conditions that allow said nucleic acid to bind to or otherwise associate with the compound, and detecting the formation of a complex between the nucleic acid and the compound. In particular, said method may be used to identify drug candidate compounds able to suppress the release of inflammatory mediators from mast cells. One particular means of measuring the complex formation is to determine the binding affinity of said compound to said nucleic acid or the presence of a complex by virtue of resistance to nucleases or by gel mobility assays. Alternatively, complex formation may be determined by inhibition of nucleic acid transcription or translation.

[0077] Depending on the choice of the skilled artisan, the present assay method may be designed to function as a series of measurements, each of which is designed to determine whether the drug candidate compound is indeed acting on the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) to thereby inhibit TG hydrolysis. For example, an assay designed to determine the binding affinity of a compound to the TARGET, or fragment thereof, may be necessary, but not sufficient, to ascertain whether the test compound would be useful for alleviating cachexia or its associated diseases, conditions or physiology when administered to a subject. Nonetheless, such binding information would be useful in identifying a set of test compounds for use in an assay that would measure a different property, further down the biochemical pathway, for example suppression of the body weight reduction in a cachexia model or infection model, such as AIDS.

[0078] Suitable controls should always be in place to insure against false positive readings. In a particular embodiment of the present invention the screening method comprises the additional step of comparing the compound to a suitable control. In one embodiment, the control may be a cell or a sample that has not been in contact with the test compound. In an alternative embodiment, the control may be a cell that does not express the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above); for example in one aspect of such an embodiment the test cell may naturally express the TARGET and the control cell may have been contacted with an agent, e.g. an siRNA, which inhibits or prevents expression of the TARGET. Alternatively, in another aspect of such an embodiment, the cell in its native state does not express the TARGET and the test cell has been engineered so as to express the TARGET, so that in this embodiment, the control could be the untransformed native cell. The control may also or alternatively utilize a known lipase inhibitor, including a broad or general lipase inhibitor. Whilst exemplary controls are described

herein, and known in the art, this should not be taken as limiting; it is within the scope of a person of skill in the art to select appropriate controls for the experimental conditions being used.

[0079] The order of taking these measurements is not believed to be critical to the practice of the present invention, which may be practiced in any order. For example, one may first perform a screening assay of a set of compounds for which no information is known respecting the compounds' binding affinity for the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) or capability of modulating the expression or activity of the TARGET. Alternatively, one may screen a set of compounds identified as having binding affinity for a TARGET protein domain, or a class of compounds identified as being an inhibitor of the TARGET. However, for the present assay to be meaningful to the ultimate use of the drug candidate compounds in cachexia or related diseases or physiology, a measurement of the stabilization of weight loss and/or inhibition of cachexia is necessary. Validation studies, including controls, and measurements of binding affinity to the polypeptides and/or specific or particular inhibition of the TARGET of the invention are nonetheless useful in identifying a compound useful in any therapeutic or diagnostic application.

[0080] Analogous approaches based on art-recognized methods and assays may be applicable with respect to the TARGETS (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) and compounds in any of various disease(s) characterized by body weight loss, fat loss, WAT reduction, muscle wasting or atrophy. An assay or assays may be designed to confirm that the test compound, having binding affinity for the TARGET or modulating the TARGET, inhibits the cachexia or its related conditions or physiology. In one such method the correction of body weight loss, fat loss, or muscle loss is measured.

[0081] The present assay method may be practiced *in vitro*, using one or more of the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) proteins, or fragments thereof, including monomers, portions or subunits of polymeric proteins, peptides, oligopeptides and enzymatically active portions thereof.

[0082] The binding affinity of the compound with the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) or a fragment thereof can be measured by methods known in the art, such as using surface plasmon resonance biosensors (Biacore), by saturation binding analysis with a labeled compound (e.g. Scatchard and Lindmo analysis), by differential UV spectrophotometer, fluorescence polarization assay, Fluorometric Imaging Plate Reader (FLIPR<sup>®</sup>) system, Fluorescence resonance energy transfer, and Bioluminescence resonance energy transfer. The binding affinity of compounds can also be expressed in dissociation constant (Kd) or as IC<sub>50</sub> or EC<sub>50</sub>. The IC<sub>50</sub> represents the concentration of a compound that is required for 50% inhibition of binding of another ligand to the polypeptide. The EC<sub>50</sub> represents the concentration required for obtaining 50% of the maximum effect in any assay that measures the TARGET function.

The dissociation constant,  $K_d$ , is a measure of how well a ligand binds to the polypeptide, it is equivalent to the ligand concentration required to saturate exactly half of the binding-sites on the polypeptide. Compounds with a high affinity binding have low  $K_d$ ,  $IC_{50}$  and  $EC_{50}$  values, i.e. in the range of 100 nM to 1 pM; a moderate to low affinity binding relates to a high  $K_d$ ,  $IC_{50}$  and  $EC_{50}$  values, i.e. in the micromolar range.

[0083] The present assay method may also be practiced in a cellular assay. A host cell expressing the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) can be a cell with endogenous expression or a cell over-expressing the TARGET e.g. by transduction. When the endogenous expression of the polypeptide is not sufficient to determine a baseline that can easily be measured, one may use host cells that over-express the TARGET. Over-expression has the advantage that the level of the TARGET substrate end products is higher than the activity level by endogenous expression. Accordingly, measuring such levels using presently available techniques is easier. In one such cellular assay, the biological activity of the TARGET may be measured by measuring the release of free FA from TG.

[0084] One embodiment of the present method for identifying a compound that inhibits cachexia, and its associated or related disorders and conditions, comprises culturing a population of mammalian cells expressing a TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) polypeptide, or a functional fragment or derivative thereof; determining a first level of FA release from TG or of ATGL activity or expression in said population of cells; exposing said population of cells to a compound, or a mixture of compounds; determining a second level of FA release from TG or of ATGL activity or expression in said population of cells under the same or commensurate conditions, during or after exposure of said population of cells to said compound, or the mixture of said compounds; and identifying the compound(s) that suppress TG hydrolysis and/or ATGL activity or expression. In a specific embodiment, the cells are adipose cells. In a specific embodiment the cells are human cells.

[0085] The release of FA from TG or lipolysis or lipase activity or ATGL activity can be determined by methods known in the art such as the methods as described herein.

[0086] The assay method may be based on the particular expression or activity of the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) polypeptide, including but not limited to an enzyme activity. Thus, assays for the enzyme TARGETs may be based on enzymatic activity or enzyme expression. The measurable phenomenon, activity or property may be selected or chosen by the skilled artisan. The person of ordinary skill in the art may select from any of a number of assay formats, systems or design one using his knowledge and expertise in the art.

[0087] Specific methods to determine the inhibition by a compound by measuring the cleavage of the substrate by the polypeptide TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms,

and variants as described herein above), which is a lipase, are well known in the art. Classically, substrates are used in which a fluorescent group is linked to a quencher through a peptide sequence that is a substrate that can be cleaved by the target protease. Cleavage of the linker separates the fluorescent group and quencher, giving rise to an increase in fluorescence.

[0088] It should be understood that the cells expressing the polypeptides, may be cells naturally expressing the polypeptides, or the cells may be transfected to express the polypeptides, as described above. Also, the cells may be transduced to overexpress the polypeptide, or may be transfected to express a non-endogenous form of the polypeptide, which can be differentially assayed or assessed.

[0089] In one particular embodiment the methods of the present invention further comprise the step of contacting the population of cells with an agonist of the polypeptide. This is useful in methods wherein the expression of the polypeptide in a certain chosen population of cells is too low for a proper detection of its activity. By using an agonist the polypeptide may be triggered, enabling a proper read-out if the compound inhibits the polypeptide. Similar considerations apply to the measurement of the release of FA from TG. In a particular embodiment, the cells used in the present method are mammalian adipose cells.

[0090] In a particular aspect of the present invention the methods include the additional step of comparing the compound to be tested to a control, where the control is a population of cells that have not been contacted with the test compound. In a particular aspect of the present invention the methods described above include the additional step of comparing the compound to be tested to a control, where the control is a population of cells that do not express said polypeptide.

[0091] In a particular aspect of the present invention the methods described above include the additional step of comparing the compound to be tested to a control, where the control is a general lipase inhibitor such as a pancreatic lipase inhibitor.

[0092] For high-throughput purposes, libraries of compounds may be used such as antibody fragment libraries, peptide phage display libraries, peptide libraries (e.g. LOPAP<sup>TM</sup>, Sigma Aldrich), lipid libraries (BioMol), synthetic compound libraries (e.g. LOPAC<sup>TM</sup>, Sigma Aldrich, BioFocus DPI) or natural compound libraries (Specs, TimTec).

[0093] Preferred drug candidate compounds are low molecular weight compounds. Low molecular weight compounds, i.e. with a molecular weight of 500 Dalton or less, are likely to have good absorption and permeation in biological systems and are consequently more likely to be successful drug candidates than compounds with a molecular weight above 500 Dalton (Lipinski et al. (1997) *Adv Drug Del Rev* 23: 3–25). Peptides comprise another preferred class of drug candidate compounds. Peptides may be excellent drug candidates and there are multiple examples of commercially valuable peptides such as fertility hormones and platelet aggregation inhibitors. Natural compounds are another preferred class of drug candidate compound. Such compounds are found in

and extracted from natural sources, and which may thereafter be synthesized. The lipids are another preferred class of drug candidate compound.

[0094] Another preferred class of drug candidate compounds is an antibody. The present invention also provides antibodies directed against the TARGETS (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above). These antibodies may be endogenously produced to bind to the the TARGETS within the cell, or added to the tissue to bind to the TARGET polypeptide present outside the cell. These antibodies may be monoclonal antibodies or polyclonal antibodies. The present invention includes chimeric, single chain, and humanized antibodies, as well as FAb fragments and the products of a FAb expression library, and Fv fragments and the products of an Fv expression library.

[0095] In certain embodiments, polyclonal antibodies may be used in the practice of the invention. The skilled artisan knows methods of preparing polyclonal antibodies. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. Antibodies may also be generated against the intact TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) protein or polypeptide, or against a fragment, derivatives including conjugates, or other epitope of the the TARGET protein or polypeptide, such as the TARGET embedded in a cellular membrane, or a library of antibody variable regions, such as a phage display library.

[0096] It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants that may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). One skilled in the art without undue experimentation may select the immunization protocol.

[0097] In some embodiments, the antibodies may be monoclonal antibodies. Monoclonal antibodies may be prepared using methods known in the art. The monoclonal antibodies of the present invention may be "humanized" to prevent the host from mounting an immune response to the antibodies. A "humanized antibody" is one in which the complementarity determining regions (CDRs) and/or other portions of the light and/or heavy variable domain framework are derived from a non-human immunoglobulin, but the remaining portions of the molecule are derived from one or more human immunoglobulins. Humanized antibodies also include antibodies characterized by a humanized heavy chain associated with a donor or acceptor unmodified light chain or a chimeric light chain, or vice versa. The humanization of antibodies may be accomplished by methods known in the art (see, e.g. Mark and Padlan, (1994) "Chapter 4. Humanization of Monoclonal Antibodies", The Handbook of

Experimental Pharmacology Vol. 113, Springer-Verlag, New York). Transgenic animals may be used to express humanized antibodies.

[0098] Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Hoogenboom and Winter, (1991) *J. Mol. Biol.* 227:381-8; Marks *et al.* (1991). *J. Mol. Biol.* 222:581-97). The techniques of Cole, *et al.* and Boerner, *et al.* are also available for the preparation of human monoclonal antibodies (Cole, *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77; Boerner, *et al.* (1991). *J. Immunol.*, 147(1):86-95).

[0099] Techniques known in the art for the production of single chain antibodies can be adapted to produce single chain antibodies to the TARGETS (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above). The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain cross-linking. Alternatively; the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent cross-linking.

[00100] Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens and preferably for a cell-surface protein or receptor or receptor subunit. In one such embodiment, one of the binding specificities is for one domain of the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above); the other one is for another domain of the the TARGET.

[00101] Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, (1983) *Nature* 305:537-9). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. Affinity chromatography steps usually accomplish the purification of the correct molecule. Similar procedures are disclosed in Traunecker, *et al.* (1991) *EMBO J.* 10:3655-9.

[00102] According to another preferred embodiment, the assay method uses a drug candidate compound identified as having a binding affinity for the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above), and/or has already been identified as having down-regulating activity such as antagonist activity for the TARGET.

[00103] In vivo animal models of cachexia or wasting conditions or infections or other disorders wherein body weight loss or muscle atrophy is seen may be utilized by the skilled artisan to further or additionally screen, assess, and/or verify the agents or compounds identified in the present invention,

including further assessing TARGET modulation in vivo. Such animal models include, but are not limited to cachexia models, cancer or tumor models, AIDS models.

[00104] The present invention further relates to a method for inhibiting lipolysis, particularly inhibiting cachexia, comprising contacting said cells with an expression inhibitory agent comprising a polynucleotide sequence that complements at least about 15 to about 30, particularly at least 17 to about 30, most particularly at least 17 to about 25 contiguous nucleotides of a nucleotide sequence encoding a polypeptide TARGET or portion thereof including the TARGET ATGL sequences set out herein and in FIGURES 7 (human ATGL; SEQ ID NO: 1 shows the amino acid sequence and SEQ ID NO: 2 shows the cDNA including the coding sequence) and 8 (mouse ATGL; SEQ ID NO: 3 shows the amino acid sequence and SEQ ID NO: 4 shows the cDNA including the coding sequence).

[00105] Another aspect of the present invention relates to a method for modulating cachexia or associated disorders, conditions or physiology in a mammal, comprising contacting said mammal with an expression-inhibiting agent that inhibits the translation in the cell of a polyribonucleotide encoding the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above). A particular embodiment relates to a composition comprising a polynucleotide including at least one antisense strand that functions to pair the agent with the the TARGET mRNA, and thereby down-regulate or block the expression of the TARGET. The inhibitory agent preferably comprises antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said agent comprises a nucleic acid sequence complementary to, or engineered from, a naturally-occurring polynucleotide sequence encoding a portion of a polypeptide TARGET, including ATGL as set out in Figures 7 (human ATGL; SEQ ID NO: 1 shows the amino acid sequence and SEQ ID NO: 2 shows the cDNA including the coding sequence) or 8 (mouse ATGL; SEQ ID NO: 3 shows the amino acid sequence and SEQ ID NO: 4 shows the cDNA including the coding sequence), or as known in the art.

[00106] The down regulation of gene expression using antisense nucleic acids can be achieved at the translational or transcriptional level. Antisense nucleic acids of the invention are preferably nucleic acid fragments capable of specifically hybridizing with all or part of a nucleic acid encoding the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) or the corresponding messenger RNA. In addition, antisense nucleic acids may be designed which decrease expression of the nucleic acid sequence capable of encoding the TARGET by inhibiting splicing of its primary transcript. Any length of antisense sequence is suitable for practice of the invention so long as it is capable of down-regulating or blocking expression of a nucleic acid coding for the TARGETS. Preferably, the antisense sequence is at least about 17 nucleotides in length. The preparation and use of antisense nucleic acids, DNA encoding antisense RNAs and the use of oligo and genetic antisense is known in the art.

[00107] The skilled artisan can readily utilize any of several strategies to facilitate and simplify the selection process for antisense nucleic acids and oligonucleotides effective in inhibition of TARGET



(e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) expression and/or inhibition of cachexia or related conditions or physiology. Predictions of the binding energy or calculation of thermodynamic indices between an oligonucleotide and a complementary sequence in an mRNA molecule may be utilized (Chiang et al. (1991) *J. Biol. Chem.* 266:18162-18171; Stull et al. (1992) *Nucl. Acids Res.* 20:3501-3508). Antisense oligonucleotides may be selected on the basis of secondary structure (Wickstrom et al (1991) in *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, Wickstrom, ed., Wiley-Liss, Inc., New York, pp. 7-24; Lima et al. (1992) *Biochem.* 31:12055-12061). Schmidt and Thompson (U.S. Patent 6,416,951) describe a method for identifying a functional antisense agent comprising hybridizing an RNA with an oligonucleotide and measuring in real time the kinetics of hybridization by hybridizing in the presence of an intercalation dye or incorporating a label and measuring the spectroscopic properties of the dye or the label's signal in the presence of unlabelled oligonucleotide. In addition, any of a variety of computer programs may be utilized which predict suitable antisense oligonucleotide sequences or antisense targets utilizing various criteria recognized by the skilled artisan, including for example the absence of self-complementarity, the absence hairpin loops, the absence of stable homodimer and duplex formation (stability being assessed by predicted energy in kcal/mol). Examples of such computer programs are readily available and known to the skilled artisan and include the OLIGO 4 or OLIGO 6 program (Molecular Biology Insights, Inc., Cascade, CO) and the Oligo Tech program (Oligo Therapeutics Inc., Wilsonville, OR). In addition, antisense oligonucleotides suitable in the present invention may be identified by screening an oligonucleotide library, or a library of nucleic acid molecules, under hybridization conditions and selecting for those which hybridize to the target RNA or nucleic acid (see for example U.S. Patent 6,500,615). Mishra and Toulme have also developed a selection procedure based on selective amplification of oligonucleotides that bind target (Mishra et al (1994) *Life Sciences* 317:977-982). Oligonucleotides may also be selected by their ability to mediate cleavage of target RNA by RNase H, by selection and characterization of the cleavage fragments (Ho et al (1996) *Nucl Acids Res* 24:1901-1907; Ho et al (1998) *Nature Biotechnology* 16:59-630). Generation and targeting of oligonucleotides to GGGA motifs of RNA molecules has also been described (U.S. Patent 6,277,981).

**[00108]** The antisense nucleic acids are preferably oligonucleotides and may consist entirely of deoxyribo-nucleotides, modified deoxyribonucleotides, or some combination of both. The antisense nucleic acids can be synthetic oligonucleotides. The oligonucleotides may be chemically modified, if desired, to improve stability and/or selectivity. Since oligonucleotides are susceptible to degradation by intracellular nucleases, the modifications can include, for example, the use of a sulfur group to replace the free oxygen of the phosphodiester bond. This modification is called a phosphorothioate linkage. Phosphorothioate antisense oligonucleotides are water soluble, polyanionic, and resistant to endogenous nucleases. In addition, when a phosphorothioate antisense oligonucleotide hybridizes to

its target site, the RNA-DNA duplex activates the endogenous enzyme ribonuclease (RNase) H, which cleaves the mRNA component of the hybrid molecule. Oligonucleotides may also contain one or more substituted sugar moieties. Particular oligonucleotides comprise one of the following at the 2' position: OH, SH, SCH<sub>3</sub>, F, OCN, heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide.

[00109] In addition, antisense oligonucleotides with phosphoramidite and polyamide (peptide) linkages can be synthesized. These molecules should be very resistant to nuclease degradation. Furthermore, chemical groups can be added to the 2' carbon of the sugar moiety and the 5 carbon (C-5) of pyrimidines to enhance stability and facilitate the binding of the antisense oligonucleotide to its target site. Modifications may include 2'-deoxy, O-pentoxy, O-propoxy, O-methoxy, fluoro, methoxyethoxy phosphorothioates, modified bases, as well as other modifications known to those of skill in the art.

[00110] Another type of expression-inhibitory agent that can reduce the level of the TARGETS (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) is the ribozyme. Ribozymes are catalytic RNA molecules (RNA enzymes) that have separate catalytic and substrate binding domains. The substrate binding sequence combines by nucleotide complementarity and, possibly, non-hydrogen bond interactions with its target sequence. The catalytic portion cleaves the target RNA at a specific site. The substrate domain of a ribozyme can be engineered to direct it to a specified mRNA sequence. The ribozyme recognizes and then binds a target mRNA through complementary base pairing. Once it is bound to the correct target site, the ribozyme acts enzymatically to cut the target mRNA. Cleavage of the mRNA by a ribozyme destroys its ability to direct synthesis of the corresponding polypeptide. Once the ribozyme has cleaved its target sequence, it is released and can repeatedly bind and cleave at other mRNAs.

[00111] Ribozyme forms include a hammerhead motif, a hairpin motif, a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) motif or Neurospora VS RNA motif. Ribozymes possessing a hammerhead or hairpin structure are readily prepared since these catalytic RNA molecules can be expressed within cells from eukaryotic promoters (Chen, et al. (1992) *Nucleic Acids Res.* 20:4581-9). A ribozyme of the present invention can be expressed in eukaryotic cells from the appropriate DNA vector. If desired, the activity of the ribozyme may be augmented by its release from the primary transcript by a second ribozyme (Ventura, et al. (1993) *Nucleic Acids Res.* 21:3249-55).

[00112] Ribozymes may be chemically synthesized by combining an oligodeoxyribonucleotide with a ribozyme catalytic domain (20 nucleotides) flanked by sequences that hybridize to the target mRNA after transcription. The oligodeoxyribonucleotide is amplified by using the substrate binding sequences as primers. The amplification product is cloned into a eukaryotic expression vector.

[00113] Ribozymes are expressed from transcription units inserted into DNA, RNA, or viral vectors. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on nearby gene regulatory sequences. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Gao and Huang, (1993) *Nucleic Acids Res.* 21:2867-72). It has been demonstrated that ribozymes expressed from these promoters can function in mammalian cells (Kashani-Sabet, et al. (1992) *Antisense Res. Dev.* 2:3-15).

[00114] Analogous to antisense RNA, the siRNA can be modified to confer resistance to nucleolytic degradation, or to enhance activity, or to enhance cellular distribution, or to enhance cellular uptake, such modifications may consist of modified internucleoside linkages, modified nucleic acid bases, modified sugars and/or chemical linkage of the siRNA to one or more moieties or conjugates. The nucleotide sequences are selected according to siRNA designing rules that give an improved reduction of the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) sequences compared to nucleotide sequences that do not comply with these siRNA designing rules (For a discussion of these rules and examples of the preparation of siRNA, WO 2004/094636, and US 2003/0198627, are hereby incorporated by reference).

[00115] The present invention also relates to compositions, and methods using said compositions, comprising a DNA expression vector capable of expressing a polynucleotide capable of stabilizing adipose cells or tissue, or muscle mass, in particular capable of inhibiting unintended loss of adipose tissue or muscle mass, and described hereinabove as an expression inhibition agent.

[00116] A particular aspect of these compositions and methods relates to the down-regulation or blocking of the expression of the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) by the induced expression of a polynucleotide encoding an intracellular binding protein that is capable of selectively interacting with the TARGET. An intracellular binding protein includes any protein capable of selectively interacting, or binding, with the polypeptide in the cell in which it is expressed and neutralizing the function of the polypeptide. Preferably, the intracellular binding protein is a neutralizing antibody or a fragment of a neutralizing antibody having binding affinity to an epitope of a TARGET. More preferably, the intracellular binding protein is a single chain antibody.

[00117] The polynucleotide expressing the expression-inhibiting agent, or a polynucleotide expressing the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) polypeptide in cells, is particularly included within a vector. The polynucleic acid is operably linked to signals enabling expression of the nucleic acid sequence and is introduced into a cell utilizing, preferably, recombinant vector constructs, which will express the antisense nucleic acid once the vector is introduced into the cell. A variety of viral-based systems are available, including adenoviral, retroviral, adeno-associated viral, lentiviral, herpes simplex viral or a sendaiviral vector systems, and all may be used to introduce and express polynucleotide sequence for the expression-inhibiting agents or the polynucleotide expressing the TARGET polypeptide in the target cells.

[00118] Particularly, the viral vectors used in the methods of the present invention are replication defective. Such replication defective vectors will usually pack at least one region that is necessary for the replication of the virus in the infected cell. These regions can either be eliminated (in whole or in part), or be rendered non-functional by any technique known to a person skilled in the art. These techniques include the total removal, substitution, partial deletion or addition of one or more bases to an essential (for replication) region. Such techniques may be performed in vitro (on the isolated DNA) or in situ, using the techniques of genetic manipulation or by treatment with mutagenic agents. Preferably, the replication defective virus retains the sequences of its genome, which are necessary for encapsidating, the viral particles.

[00119] In a preferred embodiment, the viral element is derived from an adenovirus. Preferably, the vehicle includes an adenoviral vector packaged into an adenoviral capsid, or a functional part, derivative, and/or analogue thereof. Adenovirus biology is also comparatively well known on the molecular level. Many tools for adenoviral vectors have been and continue to be developed, thus making an adenoviral capsid a preferred vehicle for incorporating in a library of the invention. An adenovirus is capable of infecting a wide variety of cells. However, different adenoviral serotypes have different preferences for cells. To combine and widen the target cell population that an adenoviral capsid of the invention can enter in a preferred embodiment, the vehicle includes adenoviral fiber proteins from at least two adenoviruses. Preferred adenoviral fiber protein sequences are serotype 17, 45 and 51. Techniques or construction and expression of these chimeric vectors are disclosed in US 2003/0180258 and US 2004/0071660, hereby incorporated by reference.

[00120] In a preferred embodiment, the nucleic acid derived from an adenovirus includes the nucleic acid encoding an adenoviral late protein or a functional part, derivative, and/or analogue thereof. An adenoviral late protein, for instance an adenoviral fiber protein, may be favorably used to target the vehicle to a certain cell or to induce enhanced delivery of the vehicle to the cell. Preferably, the nucleic acid derived from an adenovirus encodes for essentially all adenoviral late proteins, enabling the formation of entire adenoviral capsids or functional parts, analogues, and/or derivatives thereof. Preferably, the nucleic acid derived from an adenovirus includes the nucleic acid encoding adenovirus

E2A or a functional part, derivative, and/or analogue thereof. Preferably, the nucleic acid derived from an adenovirus includes the nucleic acid encoding at least one E4-region protein or a functional part, derivative, and/or analogue thereof, which facilitates, at least in part, replication of an adenoviral derived nucleic acid in a cell. The adenoviral vectors used in the examples of this application are exemplary of the vectors useful in the present method of treatment invention.

[00121] Certain embodiments of the present invention use retroviral vector systems. Retroviruses are integrating viruses that infect dividing cells, and their construction is known in the art. Retroviral vectors can be constructed from different types of retrovirus, such as, MoMuLV (“murine Moloney leukemia virus”) MSV (“murine Moloney sarcoma virus”), HaSV (“Harvey sarcoma virus”); SNV (“spleen necrosis virus”); RSV (“Rous sarcoma virus”) and Friend virus. Lentiviral vector systems may also be used in the practice of the present invention.

[00122] In other embodiments of the present invention, adeno-associated viruses (“AAV”) are utilized. The AAV viruses are DNA viruses of relatively small size that integrate, in a stable and site-specific manner, into the genome of the infected cells. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and they do not appear to be involved in human pathologies.

[00123] In the vector construction, the polynucleotide agents of the present invention may be linked to one or more regulatory regions. Selection of the appropriate regulatory region or regions is a routine matter, within the level of ordinary skill in the art. Regulatory regions include promoters, and may include enhancers, suppressors, etc.

[00124] Promoters that may be used in the expression vectors of the present invention include both constitutive promoters and regulated (inducible) promoters. The promoters may be prokaryotic or eukaryotic depending on the host. Among the prokaryotic (including bacteriophage) promoters useful for practice of this invention are lac, lacZ, T3, T7, lambda P<sub>r</sub>, P<sub>i</sub>, and trp promoters. Among the eukaryotic (including viral) promoters useful for practice of this invention are ubiquitous promoters (e.g. HPRT, vimentin, actin, tubulin), therapeutic gene promoters (e.g. MDR type, CFTR, factor VIII), tissue-specific promoters, including animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals, e.g. chymase gene control region which is active in mast cells (Liao *et al.*, (1997), *Journal of Biological Chemistry*, 272,; 2969-2976), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl, et al. (1984) *Cell* 38:647-58; Adames, et al. (1985) *Nature* 318:533-8; Alexander, et al. (1987) *Mol. Cell. Biol.* 7:1436-44), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder, et al. (1986) *Cell* 45:485-95), and beta-globin gene control region which is active in myeloid cells (Mogram, et al. (1985) *Nature* 315:338-40; Kollias, et al. (1986) *Cell* 46:89-94).

[00125] Other promoters which may be used in the practice of the invention include promoters which are preferentially activated in dividing cells, promoters which respond to a stimulus (e.g. steroid hormone receptor, retinoic acid receptor), tetracycline-regulated transcriptional modulators, cytomegalovirus immediate-early, retroviral LTR, metallothionein, SV-40, E1a, and MLP promoters. Further promoters which may be of use in the practice of the invention include promoters which are active and/or expressed in lipase expressing cells, ATGL expressing cells, adipocytes, hepatocytes.

[00126] Additional vector systems include the non-viral systems that facilitate introduction of polynucleotide agents into a patient. For example, a DNA vector encoding a desired sequence can be introduced *in vivo* by lipofection. Synthetic cationic lipids designed to limit the difficulties encountered with liposome-mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner, et al. (1987) Proc. Natl. Acad. Sci. USA 84:7413-7); see Mackey, et al. (1988) Proc. Natl. Acad. Sci. USA 85:8027-31; Ulmer, et al. (1993) Science 259:1745-8). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, (1989) Nature 337:387-8). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in International Patent Publications WO 95/18863 and WO 96/17823, and in U.S. Pat. No. 5,459,127. The use of lipofection to introduce exogenous genes into the specific organs *in vivo* has certain practical advantages and directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, for example, pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides, e.g., hormones or neurotransmitters, and proteins for example, antibodies, or non-peptide molecules could be coupled to liposomes chemically. Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, for example, a cationic oligopeptide (e.g., International Patent Publication WO 95/21931), peptides derived from DNA binding proteins (e.g., International Patent Publication WO 96/25508), or a cationic polymer (e.g., International Patent Publication WO 95/21931).

[00127] It is also possible to introduce a DNA vector *in vivo* as a naked DNA plasmid (see U.S. Pat. Nos. 5,693,622, 5,589,466 and 5,580,859). Naked DNA vectors for therapeutic purposes can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (see, e.g., Wilson, et al. (1992) J. Biol. Chem. 267:963-7; Wu and Wu, (1988) J. Biol. Chem. 263:14621-4; Hartmut, et al. Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990; Williams, et al (1991). Proc. Natl. Acad. Sci. USA 88:2726-30). Receptor-mediated DNA delivery approaches can also be used (Curiel, et al. (1992) Hum. Gene Ther. 3:147-54; Wu and Wu, (1987) J. Biol. Chem. 262:4429-32).

[00128] The present invention also provides biologically compatible, cachexia inhibiting or modulating compositions comprising an effective amount of one or more compounds identified as TARGET inhibitors, and/or the expression-inhibiting agents as described hereinabove.

[00129] A biologically compatible composition is a composition, that may be solid, liquid, gel, or other form, in which the compound, polynucleotide, vector, and antibody of the invention is maintained in an active form, e.g., in a form able to effect a biological activity. For example, a compound of the invention would have inverse agonist or antagonist activity on the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above); a nucleic acid would be able to replicate, translate a message, or hybridize to a complementary mRNA of the TARGET; a vector would be able to transfect a target cell and express the antisense, antibody, ribozyme or siRNA as described hereinabove; an antibody would bind a the TARGET polypeptide domain.

[00130] A particular biologically compatible composition is an aqueous solution that is buffered using, e.g., Tris, phosphate, or HEPES buffer, containing salt ions. Usually the concentration of salt ions will be similar to physiological levels. Biologically compatible solutions may include stabilizing agents and preservatives. In a more preferred embodiment, the biocompatible composition is a pharmaceutically acceptable composition. Such compositions can be formulated for administration by topical, oral, parenteral, intranasal, subcutaneous, and intraocular, routes. Parenteral administration is meant to include intravenous injection, intramuscular injection, intraarterial injection or infusion techniques. The composition may be administered parenterally in dosage unit formulations containing standard, well-known non-toxic physiologically acceptable carriers, adjuvants and vehicles as desired.

[00131] A particular embodiment of the present composition invention is a pharmaceutical composition comprising a therapeutically effective amount of an expression-inhibiting agent as described hereinabove, in admixture with a pharmaceutically acceptable carrier. Another particular embodiment is a pharmaceutical composition for the treatment or prevention of a disease or condition involving cachexia, and its associated or related disorders and conditions, or a susceptibility to such conditions, comprising an effective amount of the TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) antagonist or inverse agonist, its pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof in admixture with a pharmaceutically acceptable carrier. A further particular embodiment is a pharmaceutical composition for the treatment or prevention of a disease or condition involving cachexia, or a susceptibility to the condition, comprising an effective amount of the TARGET antagonist or inverse agonist, its pharmaceutically acceptable salts, hydrates, solvates, or prodrugs thereof in admixture with a pharmaceutically acceptable carrier.

[00132] Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration.

Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical compositions for oral use can be prepared by combining active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl-cellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate. Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinyl-pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

[00133] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

[00134] Preferred sterile injectable preparations can be a solution or suspension in a non-toxic parenterally acceptable solvent or diluent. Examples of pharmaceutically acceptable carriers are saline, buffered saline, isotonic saline (e.g. monosodium or disodium phosphate, sodium, potassium; calcium or magnesium chloride, or mixtures of such salts), Ringer's solution, dextrose, water, sterile water, glycerol, ethanol, and combinations thereof 1,3-butanediol and sterile fixed oils are conveniently employed as solvents or suspending media. Any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid also find use in the preparation of injectables.

[00135] The agents or compositions of the invention may be combined for administration with or embedded in polymeric carrier(s), biodegradable or biomimetic matrices or in a scaffold. The carrier, matrix or scaffold may be of any material that will allow composition to be incorporated and expressed and will be compatible with the addition of cells or in the presence of cells. Particularly, the carrier matrix or scaffold is predominantly non-immunogenic and is biodegradable. Examples of biodegradable materials include, but are not limited to, polyglycolic acid (PGA), polylactic acid (PLA), hyaluronic acid, catgut suture material, gelatin, cellulose, nitrocellulose, collagen, albumin,



fibrin, alginate, cotton, or other naturally-occurring biodegradable materials. It may be preferable to sterilize the matrix or scaffold material prior to administration or implantation, e.g., by treatment with ethylene oxide or by gamma irradiation or irradiation with an electron beam. In addition, a number of other materials may be used to form the scaffold or framework structure, including but not limited to: nylon (polyamides), dacron (polyesters), polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g., polyvinylchloride), polycarbonate (PVC), polytetrafluorethylene (PTFE, teflon), thermanox (TPX), polymers of hydroxy acids such as polylactic acid (PLA), polyglycolic acid (PGA), and polylactic acid-glycolic acid (PLGA), polyorthoesters, polyanhydrides, polyphosphazenes, and a variety of polyhydroxyalkanoates, and combinations thereof. Matrices suitable include a polymeric mesh or sponge and a polymeric hydrogel. In the particular embodiment, the matrix is biodegradable over a time period of less than a year, more particularly less than six months, most particularly over two to ten weeks. The polymer composition, as well as method of manufacture, can be used to determine the rate of degradation. For example, mixing increasing amounts of polylactic acid with polyglycolic acid decreases the degradation time. Meshes of polyglycolic acid that can be used can be obtained commercially, for instance, from surgical supply companies (e.g., Ethicon, N.J.). In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions that have charged side groups, or a monovalent ionic salt thereof.

**[00136]** The composition medium can also be a hydrogel, which is prepared from any biocompatible or non-cytotoxic homo- or hetero-polymer, such as a hydrophilic polyacrylic acid polymer that can act as a drug absorbing sponge. Certain of them, such as, in particular, those obtained from ethylene and/or propylene oxide are commercially available. A hydrogel can be deposited directly onto the surface of the tissue to be treated, for example during surgical intervention.

**[00137]** Embodiments of pharmaceutical compositions of the present invention comprise a vector encoding an agent of the present invention, particularly a recombinant replication defective vector, and a transfection enhancer, such as poloxamer. An example of a poloxamer is Poloxamer 407, which is commercially available (BASF, Parsippany, N.J.) and is a non-toxic, biocompatible polyol. A poloxamer impregnated with recombinant viruses may be deposited directly on the surface of the tissue to be treated, for example during a surgical intervention. Poloxamer possesses essentially the same advantages as hydrogel while having a lower viscosity.

**[00138]** The active expression-inhibiting agents may also be entrapped in microcapsules prepared, for example, by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.

[00139] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™. (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[00140] As defined above, therapeutically effective dose means that amount of protein, polynucleotide, peptide, or its antibodies, agonists or antagonists, which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED<sub>50</sub> (the dose therapeutically effective in 50% of the population) and LD<sub>50</sub> (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD<sub>50</sub>/ED<sub>50</sub>.

Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[00141] For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state, age, weight and gender of the patient; diet, desired duration of treatment, method of administration, time and frequency

of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[00142] The pharmaceutical compositions according to this invention may be administered to a subject by a variety of methods. They may be added directly to target tissues, complexed with cationic lipids, packaged within liposomes, or delivered to target cells by other methods known in the art. Localized administration to the desired tissues may be done by direct injection, transdermal absorption, catheter, infusion pump or stent. The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment. Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. Examples of ribozyme delivery and administration are provided in Sullivan et al. WO 94/02595.

[00143] Antibodies according to the invention may be delivered as a bolus only, infused over time or both administered as a bolus and infused over time. Those skilled in the art may employ different formulations for polynucleotides than for proteins. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

[00144] As discussed hereinabove, recombinant viruses may be used to introduce DNA encoding polynucleotide agents useful in the present invention. Recombinant viruses according to the invention are generally formulated and administered in the form of doses of between about  $10^4$  and about  $10^{14}$  pfu. In the case of AAVs and adenoviruses, doses of from about  $10^6$  to about  $10^{11}$  pfu are preferably used. The term pfu ("plaque-forming unit") corresponds to the infective power of a suspension of virions and is determined by infecting an appropriate cell culture and measuring the number of plaques formed. The techniques for determining the pfu titre of a viral solution are well documented in the prior art.

[00145] In a further aspect the present invention provides a method of preventing and/or treating cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, reduction of WAT, and reduction of fat, said method comprising administering to a subject a therapeutically effective amount of an agent as disclosed herein. In a particular embodiment, the agent is selected from an expression-inhibiting agent and an antibody.

[00146] The invention also relates to the use of an agent as described above for the preparation of a medicament for treating or preventing cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, and reduction of WAT,. In a particular embodiment, the disease is cancer cachexia, wasting disease associated with AIDS or other infectious disease or condition, chronic substance abuse, alcoholism, cirrhosis of the liver or low body weight associated with anorexia or other disorders.

[00147] The present invention also provides a method of treating and/or preventing cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, and reduction of WAT, a pharmaceutical composition or compound as described herein, particularly a therapeutically effective amount of an agent which inhibits the expression or activity of a TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) as identified herein. In a particular embodiment, the disease is cancer cachexia, wasting disease associated with AIDS or other infectious disease or condition, chronic substance abuse, alcoholism, cirrhosis of the liver, or low body weight associated with anorexia or other disorders.

[00148] The invention also relates to an agent or a pharmaceutical composition as described above for use in the treatment and/or prevention of cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting and reduction of WAT.

[00149] Administration of the agent or pharmaceutical composition of the present invention to the subject patient includes both self-administration and administration by another person. The patient may be in need of treatment for an existing disease or medical condition, or may desire prophylactic treatment to prevent or reduce the risk for diseases and medical conditions characterized by cachexia, and its associated or related disorders and conditions. The agent of the present invention may be delivered to the subject patient orally, transdermally, via inhalation, injection, nasally, rectally or via a sustained release formulation.

[00150] Still another aspect of the invention relates to a method for diagnosing a pathological condition including cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting and reduction of WAT, comprising determining the amount or activity of a polypeptide TARGET (e.g., ATGL and/or HSL, alternative species forms, isoforms, and variants as described herein above) in a biological sample, and comparing the amount with the amount of the polypeptide in a healthy subject, wherein an increase of the amount of or activity of polypeptide compared to the healthy subject is indicative of the presence of the pathological condition. In a particular embodiment, the disease is cancer cachexia, wasting disease associated with AIDS or other infectious disease or condition, substance abuse, chronic alcoholism, cirrhosis of the liver or low body weight associated with anorexia or other disorders.

[00151] Another aspect of the invention relates to a method for diagnosing a pathological condition including cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting and reduction of WAT, comprising determining the amount, expression, or activity of a ATGL in a biological sample, and comparing the amount with the amount of the polypeptide in a healthy subject, wherein an increase of the amount of or activity of or expression of ATGL compared to the healthy subject is indicative of the presence of the pathological condition. In a particular embodiment, the disease is cancer cachexia, wasting disease associated with AIDS or other

infectious disease or condition, substance abuse, chronic alcoholism, cirrhosis of the liver or low body weight associated with anorexia or other disorders.

[00152] The polypeptides or the polynucleotides of the present invention employed in the methods described herein may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. To perform the methods it is feasible to immobilize either the polypeptide of the present invention or the compound to facilitate separation of complexes from uncomplexed forms of the polypeptide, as well as to accommodate automation of the assay. Interaction (e.g., binding) of the polypeptide of the present invention with a compound can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the polypeptide to be bound to a matrix. For example, the polypeptide of the present invention can be "His" tagged, and subsequently adsorbed onto Ni-NTA microtitre plates, or ProtA fusions with the polypeptides of the present invention can be adsorbed to IgG, which are then combined with the cell lysates (e.g., <sup>35</sup>S-labelled) and the candidate compound, and the mixture incubated under conditions favorable for complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the plates are washed to remove any unbound label, and the matrix is immobilized. The amount of radioactivity can be determined directly, or in the supernatant after dissociation of the complexes. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of the protein binding to the protein of the present invention quantitated from the gel using standard electrophoretic techniques.

[00153] Other techniques for immobilizing protein on matrices can also be used in the method of identifying compounds. For example, either the polypeptide of the present invention or the compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated protein molecules of the present invention can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with the polypeptides of the present invention but which do not interfere with binding of the polypeptide to the compound can be derivatized to the wells of the plate, and the polypeptide of the present invention can be trapped in the wells by antibody conjugation. As described above, preparations of a labeled candidate compound are incubated in the wells of the plate presenting the polypeptide of the present invention, and the amount of complex trapped in the well can be quantitated.

[00154] The polynucleotides encoding the TARGET polypeptides include ATGL and HSL encoding polynucleotides, particularly ATGL encoding nucleotides, such as human or mouse ATGL as known in the art and/or set out herein in Figures 7 (human ATGL; SEQ ID NO: 1 shows the amino acid sequence and SEQ ID NO: 2 shows the cDNA including the coding sequence) and 8 (mouse ATGL;

SEQ ID NO: 3 shows the amino acid sequence and SEQ ID NO: 4 shows the cDNA including the coding sequence).

[00155] The invention is further illustrated in the following examples.

## EXPERIMENTAL SECTION

### EXAMPLE 1 - Animal Model Studies

[00156] A murine animal model of tumor associated cachexia was utilized to examine the role of lipases in cachexia. Lewis lung carcinoma cells are used in mice to generate tumors and cachexia effects. Lewis lung carcinoma cells were obtained from the European Collection of Cell Cultures (ECACC) and maintained in cell culture before injecting  $\sim 4 \times 10^6$  cells subcutaneously in the neck area of C57BL/6J mice. Lewis lung carcinoma is a cell line established from the lung of a C57BL mouse bearing a tumor resulting from an implantation of primary Lewis lung carcinoma. The cells are reported to be highly tumorigenic, but weakly metastatic in mice (Laster, W.R. et al (1971) Proc of the Amer Assoc for Cancer Research 12(NMAR):7). Average tumor weight after 21 days in this model was 3.67g. Numerous mitoses seen in HE stained histological sections indicated a highly proliferating tumor in the mice. Average mouse body weight, however, decreased by 3.0g after 21 days. In this model system, tumor bearing wild type animals lose on average 3.84g in body weight (excluding the tumor) whereas wild type animals without tumor gain 2.2g in the same time period (20 days). The difference is highly significant.

[00157] Knockouts were generated in mice for both ATGL (Lipase A) and for HSL (Lipase B), using the method as previously reported (Haemmerle, G et al (2006) Science 312(5774):734-737; Schweiger, M et al (2006) J Biol Chem 281(52):40236-40241). Lewis lung carcinoma cells were injected  $\sim 4 \times 10^6$  cells subcutaneously in the neck area of wildtype and knockout mice. After subcutaneous injection of tumor cells or mock injection, mice were kept under standard conditions and had free access to food and water. After 21 days the animal were sacrificed, weighed and the tumor tissue was dissected. There was no significant difference in ATGL ko animals (Lipase A) between control (weight gain 1.84) and tumor bearing animals (weight gain 0.97) (FIGURE 1A), demonstrating the cachexia is prevented in these ATGL ko animals. Tumor bearing HSL (Lipase B) ko animals did show a loss of body weight, but the loss was less pronounced than wild type animals (FIGURE 1A). These changes cannot be attributed to differences in food intake (FIGURE 1B).

[00158] Upon visual inspection, white adipose tissue (WAT) is completely lost 20 days after injection of tumor cells in wild type animals (FIGURE 2A). WAT is preserved, however, in ATGL ko mice (FIGURE 2B). This is also evident in the WAT weight (normalized to body weight) (FIGURE 3A). Loss of HSL (Lipase B) partially protects against loss of WAT. This effect is even better demonstrated in nuclear magnetic resonance (NMR) measurements. Twenty-one days after injection of tumor cells

or mock injections, mice were sacrificed and organs and WAT were dissected. WAT weight was determined and normalized to body weight (mg/g) (FIGURE 3A). NMR analysis for the assessment of total body fat content was performed before injection and immediately before sacrificing the animals respectively. Using NMR analysis, fat content is recorded as percentage and total fat weight is calculated from the animals' weight. Change (in g) is reported compared to the initial values before injection (FIGURE 3B). Average fat change by NMR was negative and significant in wildtype and HSL (Lipase B) ko mice, ie it was significantly reduced from before injection, while mock infected animals gained fat. Lipase A (ATGL) ko mice showed only a slight reduction versus mock infected mice, and a significant gain overall during the tumor experiment.

[00159] Further studies looked at lipase activity upon tumor-induced loss of adipose tissue. Animals were sacrificed 14 days after injection of tumor cells or mock injection, a time point where adipose tissue still can be dissected from wild type tumor bearing mice. Lipase activity was measured in homogenates of adipose tissue without (total lipase activity) or with inhibition of HSL by the HSL inhibitor 76-0079 (NNC 0076-0000-0079, provided by NovoNordisk, Denmark) (Lipase B inhibitor) (Schweiger M et al (2006) J Biol Chem 281(52):40236-40241). Total lipase activity is increased approximately 3-fold in tumor bearing wild type animals (FIGURE 4, WT, white bar) vs control wild type animals (FIGURE 4, WT, black bar). This is in part due to an increase of ATGL activity as seen after inhibition of HSL (FIGURE 4, WT + Lipase B inhibitor, black vs. white bar). ATGL tumor bearing mice show only a modest (40%) increase of total lipase activity (FIGURE 4, lipase A ko, black vs. white bar). No lipase activity is seen (as expected) in ATGL ko also inhibited for HSL (FIGURE 4, Lipase A ko + Lipase B inhibitor). Tumor bearing HSL ko animals show an over 2-fold induction of total lipase activity (FIGURE 4, Lipase B ko), which is attributable to ATGL induction.

[00160] Changes in muscle mass were determined in mock infected and tumor bearing mice including wildtype, Lipase A (ATGL) ko mice, and Lipase B (HSL) ko mice. Twenty-one days after injection of tumor cells or mock injections, mice were sacrificed. *M. gastrocnemius* and *M. soleus* were dissected from both hind legs and the weight recorded. Muscle weight normalized to body weight is compared to the mock injected animals. In tumor-bearing animals, 50% of *M. gastrocnemius* (fast twitching skeletal muscle) muscle mass is lost in wild type animals and HSL ko animals (FIGURE 5A, WT and Lipase B ko respectively). No loss of *M. gastrocnemius* muscle mass is seen in ATGL ko mice (FIGURE 5A, Lipase A ko). No significant changes are seen in *M. soleus* (slow twitching skeletal muscle) mass in wildtype, Lipase A ko or Lipase B ko animals (FIGURE 5B). These results confirm the inhibition of skeletal muscle loss in mice lacking ATGL.

[00161] Proteasome activity is a measure of intracellular and myofibrillar protein breakdown. Proteasome activity was measured in mock infected and tumor bearing mice including wildtype, Lipase A (ATGL) ko mice, and Lipase B (HSL) ko mice (FIGURE 6A). Proteasome activity is two-fold higher in tumor bearing wild type mice compared to control (WT, highly significant) and a 25%

increase is seen in HSL ko animals (Lipase B ko, significant). No proteasome activity increase is seen in ATGL (Lipase A) ko animals.

[00162] Caspase activity, an indicator of apoptotic cell death was measured in mock infected and tumor bearing mice including wildtype, Lipase A (ATGL) ko mice, and Lipase B (HSL) ko mice (FIGURE 6B). An incremental increase in caspase activity is seen in tumor bearing wild type mice (FIGURE 6B, wt\_8, wt\_14 and wt\_21 days). No significant changes are recorded in ATGL (Lipase A) ko animals, wither at 14 or 21 days.

## MATERIALS AND METHODS

### **Lipase Assay (Triacylglycerol hydrolase assay):**

[00163] Mesenteric, retroperitoneal, omental and, gonadal white adipose tissues (WAT) of wild-type (wt), Adipose Tri-Glyceride Lipase-knockout (ATGL-ko) and, Hormone Sensitive Lipase-knockout (HSL-ko) mice were removed surgically and, washed in phosphate-buffered saline (PBS) containing 1 mM ethylenediaminetetraacetic-acid(EDTA). The tissue was homogenized in lysis buffer (0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol, 20 µg/ml leupeptin, 2 µg/ml antipain, 1 µg/ml pepstatin, pH 7.0) using a Magna Lyser (Roche diagnostics GmbH, Mannheim, Germany). The WAT lysate was centrifuged at 100,000g for 1 hour (h) at 4°C. The lipid-free infranant (cytosolic fraction) was collected and, used for triacyl-glycerol (TG) hydrolase assays. The substrate for the measurement of lipase activity containing triolein and [9,10-3H(N)-triolein] (NEN Life Science Products, Boston, MA) as radioactive tracer was emulsified with phosphatidylcholine/phosphatidylinositol using a conventional ultrasound sonicator. The cytosolic fractions supplemented with or, without a specific inhibitor for HSL were incubated at 37°C for 60 min under constant shaking. The reaction was terminated by addition of 3.25 ml methanol/chloroform/heptane (10:9:7) and, 1 ml of 0.1 M potassium carbonate and, 0.1 M boric acid (pH 10.5). After centrifugation at 800 g for 20 minutes(min) the radioactivity in 1 ml of the upper phase was determined by liquid scintillation counting in a LS 6500 Multi-Purpose Scintillation Counter from Beckman Coulter Inc. (Fullerton, CA).

### **Proteasome assay:**

[00164] *Musculus gastrocnemius* was homogenized in lysis buffer (20 mM Tris-HCl [pH 7.2], 0.1 mM EDTA, 1mM 2-mercaptoethanol, 1mM Dithiothreitol [DTT], 5 mM ATP, 20% glycerol, 0.04% (v/v) Triton X-100) using a magna lyser (Roche diagnostics GmbH, Mannheim, Germany) at 4°C. The lysate was centrifuged at 13,000g for 15 mins at 4°C. The supernatant was collected and, protein concentration was determined with the RC DC Protein assay kit (BioRad, Hercules, CA). The chymotrypsin like proteasome activity was determined by incubating 40 µg protein with 0.167 µg/µl N-succinyl-Leu-Leu-Val-Try-7-amido-4-methylcoumarin (N-Suc LLVY-AMC, Sigma, St. Louis,



MO) in incubation buffer (100mM Tris-HCl [pH 7.4], 50 mM [HEPES pH 8.0] and 5 mmol/l Ethyleneglycoltetraacetic-acid[EGTA]) for 60 mins at 37°C. Fluorescence was read with a conventional spectrofluorometer at 380 nm excitation and, 460 nm emission. Adapted from (Busquets et al., 2004; Ventrucchi et al., 2004)

**Caspase Assay:**

[00165] About 15 mg to 25 mg tissue from *Musculus gastrocnemius* was homogenized at 4°C in 300µl homogenisation buffer (25mM HEPES pH 7.5; 5mM MgCl<sub>2</sub>; 1mM EGTA; 1mM Pefabloc SC; 1% Protease-Inhibitor-cocktail [v/v] [P8340 from Sigma, St.Louis, MO]. The lysate was centrifuged at 13,000g for 20 min at 4°C. The supernatant was collected and the protein-content was measured using the DC Protein Assay from Bio-Rad, Hercules, CA. Caspase activity was determined by incubating 50µg protein (in 100µl solution) with 100µl of Caspase-Glo® 3/7 Reagent (Promega Corp., Madison, WI) for 1.5 hrs at 20°C and, luminescence was measured using a luminometer (LUMIstar Optima BMG Labtec, Offenburg, GERMANY).

**Animal care:**

[00166] Female C57BL/6J mice were maintained on a regular light-dark cycle (12 h light, 12 h dark) and, kept on a standard laboratory chow diet (4.5% w/w fat). HSL-ko and, ATGL-ko mice were generated by targeted homologous recombination, as described by (Haemmerle et al., 2006) (Haemmerle et al., 2002). Mice used for experiments were 8-9 weeks old. During the experiments, food intake (g) as well as mouse body weight (g) were measured daily using a conventional laboratory weighing balance.

**Animal treatment and tissue harvesting:**

[00167] Mice were randomly divided into two groups: control non-tumour-bearing mice and, lewis lung carcinoma (LLC)-bearing mice. Mice were fasted for 12 h and subsequently anesthetized using isoflurane. Thereafter blood samples were collected by retro-orbital puncture from each mouse. Subsequently, 4x10<sup>6</sup> LLC-cells obtained from an exponentially proliferating cell-culture, maintained in conventional DMEM high glucose cell culture medium, containing 10% Fetal bovine serine [v/v], were subcutaneously injected at dorsum just below the neck. After different time periods (8 days, 14 days, 21 days) the mice were anaesthetized using isoflurane, blood samples were taken, as described above and, mice were kept in an unconscious state using 1% isoflurane. *Musculus.gastrocnemius* and, *Musculus soleus* were excised rapidly, and either frozen in liquid nitrogen, or stored in 4% neutral buffered formalin for subsequent preparation of paraffin blocks. Thereafter, mice were sacrificed by cervical dislocation, and tissues were rapidly excised, weighed, and either frozen in liquid nitrogen, or stored in formalin.

**[00168] Blood and plasma parameters:**

[00169] Serum levels of TG, glycerol, and FA were determined using commercial kits (Thermo Electron corp., Victoria, Australia; Sigma, St. Louis, MO; Wako Chemicals, Neuss, Germany). Blood glucose concentration was determined using Accu-Check glucometer (Roche Diagnostics, Vienna, Austria).

**NMR**

[00170] The mice were anesthetized using isoflurane, and whole body fat mass was measured using the Minispec mq NMR analyzer (Brucker Optics, Woodlands, TX) as described by (Tinsley et al., 2004).

**H&E**

[00171] Paraffin-embedded, formalin-fixed specimen cross-sections were stained by hematoxylin, and counter-stained by eosin using standard methods.

**EXAMPLE 2 - Screening**

[00172] The activity of ATGL can primarily be measured by cleavage of triglycerides (exemplary assay provided in Example 1 above Lipase Assay), because the enzyme does not recognize water-soluble substrates. Using this method, about 300 measurements can be carried out per day by one person in a throughput assay.

[00173] ATGL activity may also be measured using cell culture. Preadipocyte-lines, e.g. 3T3-L1 cells, are differentiated to adipocytes. Then after beta-adrenergic stimulation, these cells secrete large amounts of glycerol and fatty acids into the culture medium, which can be measured easily using commercial enzymatic kits. By incubation with inhibitors, this secretion can be inhibited. In the absence of ATGL, the secretion is inhibited by about 70%. In the absence of HSL and ATGL, there is almost no beta-adrenergic stimulation of lipolysis in adipocytes (Schweiger M et al (2006) J Biol Chem 281(52):40236-40241). Therefore, in the presence of an HSL inhibitor (such as 76-0079, Novo Nordisk), specific ATGL activity can be determined in living adipocytes (Schweiger M et al (2006) J Biol Chem 281(52):40236-40241).

[00174] ATGL inhibition activity of a phospholipase-inhibitor (R)-Bromo-enol lactone (Cayman Chemicals, Cat. No. 10006800, CAS No. 478288-90-3) has been demonstrated with expressed ATGL (Jenkins CM et al (2004) J Biol Chem 279(47):48968-48975) or native ATGL in hepatocytes (Chung C et al (2008) J Hepatol 48:471-478). This lipase inhibitor also inhibits other lipases including iPLA<sub>2</sub>β

and iPLA<sub>2</sub> $\gamma$  (Mancuso DJ et al (2000) J Biol Chem 275:9937-9945; Hazen S et al (1991) J Biol Chem 266:7227-7232; Zupan LA et al (1993) J Med Chem 36:95-100). This provides an ATGL inhibiting compound for studies, assessment, and as a control in further screening.

[00175] To further assess ATGL hydrolysis of neutral lipids, His-tagged ATGL can be transiently expressed in COS-7 cells using a eukaryotic expression vector. For comparison, COS-7 cells are also transfected with a similar construction expressing His-tagged HSL. Extracts from transfected cells are preincubated with an inhibitor or candidate compound or compound library. When extracts are preincubated with the fluorescent lipase inhibitor (NBD-HEHP) and subsequently subjected to SDS-PAGE analysis and fluorography, fluorescent signals can be observed in positions corresponding to the expected molecular weight of ATGL and HSL providing evidence that ATGL is enzymatically active in transfected COS cells. To confirm ATGL activity, TG-hydrolase activity assays can be performed using a radioactively labelled [9,10-<sup>3</sup>H(N)]-triolein substrate. As controls, no enzymatic activities should be observed when radioactively labeled retinyl palmitate, cholesteryl oleate or phosphatidylcholine are used as lipid substrates.

[00176] To determine ATGL function in adipocytes, including in assays in the presence or absence of one or more candidate compounds, modulators, or inhibitors, a recombinant adenovirus encoding His-tagged full length mouse or human ATGL cDNA is constructed and used to infect mouse 3T3-L1 adipocytes at day 6 of differentiation. Western blotting analysis of cell-extracts of infected adipocytes reveals expression of His-tagged ATGL at the appropriate molecular weight. Overexpression of ATGL in adipocytes can markedly augment both basal and isoproterenol-stimulated lipolysis, indicative of a functional ATGL lipase in adipose tissue.

## Material and Methods

*cDNA cloning and transient expression of recombinant His-tagged proteins in COS-7 cells and 3T3-L1 adipocytes.*

[00177] The coding sequences of mouse ATGL and HSL are amplified by PCR from cDNA prepared from mRNA of mouse white adipose tissue by reverse transcription. The open reading frame, flanked by *KpnI/XhoI* sites for ATGL and HSL were cloned into the eucaryotic expression vector pcDNA4/HisMax (Invitrogen). Transfection of COS-7 cells was performed with Metafectene™ (Biontex) according to the manufacturer's description. The PCR primers used to generate these probes were as follows.

ATGL forward 5'-TGGTACCGTTCCCGAGGGAGACCAAGTGGA-3',

ATGL revers 5'-CCTCGAGCGCAAGGCGGGAGGCCAGGT-3'.

HSL forward 5'-TGGTACCT-ATGGATTTACGCACGATGACACA-3',

HSL revers 5'-CCTCGAGCGTTCAGTGGTGCAGCAGGCG-3'.

*Construction of the recombinant adenovirus for ATGL expression (ATGL-Ad) and infection of 3T3-L1 cells:*

[00178] The recombinant adenovirus coding for mouse ATGL is prepared by cotransfection of the shuttle plasmid pAvCvSv containing the ATGL cDNA and pJM 17 into HEK-293 cells. The 1.65 kb Mlu I – Cla I flanked mouse ATGL cDNA fragment (His-tag included) is amplified by PCR from the eucaryotic expression vector pcDNA4/HisMax containing mouse ATGL cDNA and subcloned into Mlu I – Cla I digested pAvCvSv. The resulting shuttle plasmid is cotransfected with pJM 17 into HEK-293 cells using the calcium phosphate coprecipitation method. Large scale production of high titer recombinant ATGL-Ad is performed as described elsewhere. 3T3-L1 fibroblasts were cultured in DMEM containing 10% FCS and differentiated using a standard protocol (Bernlohr, D.A. et al (1985) J Biol Chem 260: 5563-7). Adipocytes are infected on day 8 of differentiation with a multiplicity of infection (moi) of ~400 plaque forming units/cell. For that purpose appropriate pfu are preactivated in DMEM containing 0.5 µg/ml of polylysine for 100 min and afterwards the cells are incubated with this virus suspension for 24 hours. After 24 h the medium is removed and the cells are incubated for further 24 h with complete medium. For most of the experiments, recombinant adenovirus expressing β-galactosidase was used as a control (LacZ-Ad).

*Western analysis.*

[00179] Cellular proteins are separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Schleicher & Schuell, Germany). For detection of His-tagged proteins, blots were incubated with 1/10000 diluted Anti-His monoclonal antibody (6xHis, Clontech). Perilipin is detected using a guinea pig polyclonal antibody against Perilipin A and B (PROGEN). Bound immunoglobulins are detected with a HRP-labeled IgG conjugates (Vector Inc.) and visualized by ECL detection (ECL plus, Amersham Pharmacia Biotech, Germany) on a Storm Image Analysis system. Quantitation is performed using ImageQuant Software.

[00180] *Reaction of ATGL and HSL with the fluorescent lipase inhibitor NBD-HEHP.* Transfected COS-7 cells are washed twice with PBS, scraped into lysis buffer (0.25 M sucrose, 1 mM EDTA, 1 mM dithioerythritol, 20 µg/ml leupeptin, 2 µg/ml antipain, 1 µg/ml pepstatin) and disrupted on ice by sonication. Nuclei and unbroken materials are removed by centrifugation at 1.000 g at 4°C for 15 min to obtain cytoplasmic extracts. 50 µg of protein is incubated with 1 nmol fluorescently labelled lipase inhibitor O-((6-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoyl)aminoethyl-O-(n-hexyl)phosphonic acid p-nitrophenyl ester (NBD-HEHP) (Oskolkova, O.V. et al (2003) Chem Phys Lipids 125:103-14.) and 1 mM Triton X-100 (especially purified for membrane research, Hofmann LaRoche) at 37°C for 2 hours under shaking. Protein is precipitated with 10% TCA for 1h on ice, washed with acetone and separated by 10% SDS-PAGE. Gels are fixed in 10% ethanol and 7% acetic

acid. Fluorescence is detected with a BioRad FX Pro Laserscanner (excitation 488 nm, emission 530 nm).

*Northern analysis.*

[00181] The cDNA probe for northern blot analysis of mouse ATGL is prepared by RT-PCR by use of first-strand cDNA from mouse fat mRNA. PCR primers used to generate this probe are as follows: forward 5'-TGGAACATCTCATTCGCTGG-3', reverse 5'-AATGCCGCCATCCACATAG-3'. Total RNA was isolated from various mouse tissues using the TRI Reagent procedure according to manufacturer's protocol (Molecular Research Center, Karlsruhe, Germany). Specific mRNAs were detected using standard Northern blotting techniques with 10 µg total RNA. <sup>32</sup>P-labeled probes for hybridization were generated using random priming. Northern blots are visualized by exposure to a PhosphorImager Screen (Apbiotech, Freiburg, Germany) and analyzed using ImageQuant Software.

*Assay for TG lipase, cholesteryl esterase, retinyl esterase and phospholipase activity.*

[00182] For determination of lipase activity 0.1 ml of cytosolic extracts and 0.1 ml substrate are incubated in a water bath at 37 °C for 60 min. The reaction is terminated by adding 3.25 ml of methanol/chloroform/heptane (10:9:7) and 1 ml of 0.1 M potassium carbonate, 0.1 M boric acid, pH 10.5. After centrifugation (800 g, 20 min) the radioactivity in 1 ml of the upper phase is determined by liquid scintillation counting. Neutral lipase activity is measured in 50 mM potassium phosphate buffer, pH 7.0 and 2.5% defatted BSA. The substrate for neutral TG lipase activity contained 33 nmol triolein/assay with [9,10-<sup>3</sup>H(N)]-triolein (40.000 cpm/nmol, NEN Life Science Products) as radioactive tracer for COS-7 cells and 167 nmol/assay for 3T3-L1 adipocytes (7300 cpm/nmol). The substrates for cholesteryl esterase and retinyl esterase activity contained 10 nmol/assay of cholesteryl oleate or retinyl palmitate and the corresponding tracers cholesteryl [9,10-<sup>3</sup>H]-oleate or retinyl [9,10-<sup>3</sup>H(N)]-palmitate (50.000 cpm/nmol). For determination of phospholipase activity in cytosolic extracts the substrate contained 20 nmol/assay phosphatidylcholine and [dipalmitoyl-1-<sup>14</sup>C]-phosphatidylcholine (12.000 cpm/nmol). All substrates are prepared by sonication (Virsonic 475) essentially as described (30).

*Determination of FA and glycerol release from 3T3-L1 adipocytes.*

[00183] Cells are incubated in DMEM medium (GIBCO) containing 2% fatty acid free BSA (Sigma) with or without 10 µM isoproterenol (Sigma) at 37 °C. Aliquots of the medium were collected and investigated for the FFA and glycerol content by using commercial kits (WAKO).

*Animal Models of Cachexia*

[00184] Lewis lung carcinoma cells are used in mice to generate tumors and cachexia effects as described above herein. Lewis lung carcinoma cells were obtained from the European Collection of Cell Cultures (ECACC) and maintained in cell culture before injecting  $\sim 4 \times 10^6$  cells subcutaneously in the neck area of mice. Lewis lung carcinoma is a cell line established from the lung of a C57BL mouse bearing a tumor resulting from an implantation of primary Lewis lung carcinoma. The cells are reported to be highly tumorigenic, but weakly metastatic in mice (Laster, W.R. et al (1971) Proc of the Amer Assoc for Cancer Research 12(NMAR):7).

#### [00185] REFERENCES

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Haemmerle, G., Lass, A., Zimmermann, R., Gorkiewicz, G., Meyer, C., Rozman, J., Heldmaier, G., Maier, R., Theussl, C., Eder, S., et al. (2006). Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science* 312, 734-737.

Haemmerle, G., Zimmermann, R., Hayn, M., Theussl, C., Waeg, G., Wagner, E., Sattler, W., Magin, T.M., Wagner, E.F., and Zechner, R. (2002). Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. *J Biol Chem* 277, 4806-4815.

Tinsley, F.C., Taicher, G.Z., and Heiman, M.L. (2004). Evaluation of a quantitative magnetic resonance method for mouse whole body composition analysis. *Obes Res* 12, 150-160.

Ventrucci, G., Mello, M.A., and Gomes-Marcondes, M.C. (2004). Proteasome activity is altered in skeletal muscle tissue of tumour-bearing rats a leucine-rich diet. *Endocr Relat Cancer* 11, 887-895.

[00186] From the foregoing description, various modifications and changes in the compositions and methods of this invention will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein.

[00187] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

**WE CLAIM:**

1. A method for identifying a compound that prevents, ameliorates and/or inhibits cachexia and/or its associated or related disorders and conditions, comprising:
  - (a) contacting a compound with a cell expressing ATGL polypeptide; and
  - (b) measuring the expression and/or activity of ATGL in the presence or absence of said compound,wherein a compound that blocks or reduces the expression and/or activity of ATGL is identified as a compound that inhibits cachexia.
2. The method of claim 1 wherein said cell is modified to express ATGL by transfection, transduction or transformation with a nucleic acid encoding the amino acid sequence set out in Figures 7 or 8 or enzymatically active fragments thereof.
3. The method of claim 1 wherein said cell naturally expresses a mammalian ATGL comprising the amino acid sequence set out in Figures 7 or 8, species variants thereof, or enzymatically or lipolytically active fragments thereof.
4. A method for identifying a compound that prevents, ameliorates and/or inhibits cachexia, and its associated or related disorders and conditions, comprising:
  - (a) contacting a compound with a polypeptide comprising an amino acid sequence selected from the group consisting of Figures 7 and/or 8, and fragments thereof; and
  - (b) measuring a compound-polypeptide property related to cachexia.
5. The method according to claim 4, wherein said polypeptide is in an *in vitro* cell-free preparation.
6. The method according to claims 1, 2 or 4, wherein said polypeptide is present in a mammalian cell.
7. The method of claim 5, wherein said property is a binding affinity of said compound to said polypeptide.
8. The method of claim 7, which additionally comprises the steps of:
  - c) contacting a population of mammalian cells expressing said polypeptide with the compound that exhibits a binding affinity of at least 10 micromolar; and
  - d) identifying a compound that inhibits hydrolysis of TG.
9. The method according to any one of claims 4 to 6, wherein said property is the activity of said polypeptide.

10. The method according to either of claims 4 or 6, wherein said property is the expression of said polypeptide.
11. The method according to either of claims 9 or 10, which additionally comprises the steps of:
  - c) contacting a population of mammalian cells expressing said polypeptide with the compound that significantly inhibits the expression or activity of the polypeptide; and
  - d) identifying the compound that inhibits cachexia.
12. The method according to any one of claims 1 to 11, which additionally comprises the step of comparing the compound to be tested to a control.
13. The method according to claim 12, wherein said control is where the polypeptide has not been contacted with said compound.
14. The method according to any one of claims 8 to 13, which additionally comprises the step of comparing the compound to a control, wherein said control is a population of mammalian cells that does not express said polypeptide.
15. The method according to any one of claims 1 to 14, wherein said compound is selected from the group consisting of compounds of a commercially available screening library and compounds having binding affinity for a polypeptide comprising an amino acid sequence selected from the group consisting of Figures 7 and/or 8.
16. The method according to any one of claims 1 to 15, wherein said compound is a peptide in a phage display library or an antibody fragment library.
17. The method according to any one of claims 1 to 16 wherein the compound is further contacted with a cell expressing HSL and at least one other lipase an HSL polypeptide and at least one other lipase polypeptide, further measuring the expression or activity of HSL and at least one other lipase or measuring a compound-HSL polypeptide or compound-other lipase-polypeptide property in the presence of the compound.
18. The method of claim 17 wherein a compound is selected which does not inhibit the expression or activity of the other lipase or does not demonstrate a compound-other lipase polypeptide property.
19. The method of claim 17 wherein a compound is selected which does not inhibit the expression or activity of both HSL and the other lipase or does not demonstrate both a compound-HSL polypeptide and a compound-other lipase polypeptide property.
20. An agent effective in treating and /or inhibiting cachexia and/or related disorders or conditions, selected from the group consisting of an antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA), wherein said agent comprises a nucleic acid sequence complementary to, or engineered from, a naturally-occurring polynucleotide sequence of about 17 to about 30 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of Figures 7 and/or 8.



21. The agent according to claim 20, wherein a vector in a mammalian cell expresses said agent.
22. The agent according to claim 20 or 21, which inhibits cachexia, and its associated or related disorders and conditions.
23. The agent according to claim 21 or 22, wherein said vector is an adenoviral, retroviral, adeno-associated viral, lentiviral, a herpes simplex viral or a sendaiviral vector.
24. The agent according to any one of claims 20 to 23, wherein said antisense polynucleotide and said siRNA comprise an antisense strand of 17-25 nucleotides complementary to a sense strand, wherein said sense strand is selected from 17-25 continuous nucleotides of a nucleic acid sequence selected from the group consisting of Figures 7 and/or 8.
25. The agent according to claim 24, wherein said siRNA further comprises said sense strand.
26. The agent according to any one of claims 20 to 25, wherein said agent is an antisense polynucleotide, ribozyme, or siRNA comprising a nucleic acid sequence complementary to a nucleic acid sequence selected from the group consisting of Figures 7 and /or 8.
27. A pharmaceutical composition comprising a therapeutically effective amount of an agent according to any one of claims 20 to 26 in admixture with a pharmaceutically acceptable carrier.
28. The pharmaceutical composition according to claim 27 for treatment of a cachexia associated with a disease selected from cancer, AIDS, autoimmune disorders, drug addiction, alcoholism, chronic inflammatory disorders, cirrhosis of the liver, anorexia and neurodegenerative disease.
29. The pharmaceutical composition of claim 27 or 28, wherein said agent is selected from the group consisting of a mixture of one or more compounds with each other, and an admixture of at least one of said compounds with a cytokine inhibitor, said cytokine selected from TNF- $\alpha$ , IL-1, IL-6, IFN- $\gamma$ , and LAF.
30. The agent according to any one of claims 20 to 26 for use in the treatment and/or prevention of a disease involving cachexia, and its associated or related disorders and conditions.
31. The agent according to claim 30, wherein the disease is selected from cancer, AIDS, autoimmune disorders, drug addiction, alcoholism, chronic inflammatory disorders, cirrhosis of the liver, anorexia and neurodegenerative disease.
32. A method for treatment and/or prevention of a pathological condition involving cachexia, and its associated or related disorders and conditions in a subject comprising administering to said subject a therapeutically effective amount of agent selected from the group consisting of an agent having binding affinity with a polypeptide comprising an amino acid sequence selected from the group consisting of Figures 7 and /or 8 and fragments thereof, and an antisense polynucleotide, a ribozyme, and a small interfering RNA (siRNA) comprising a nucleic acid sequence complementary to, or engineered from, a naturally-occurring polynucleotide

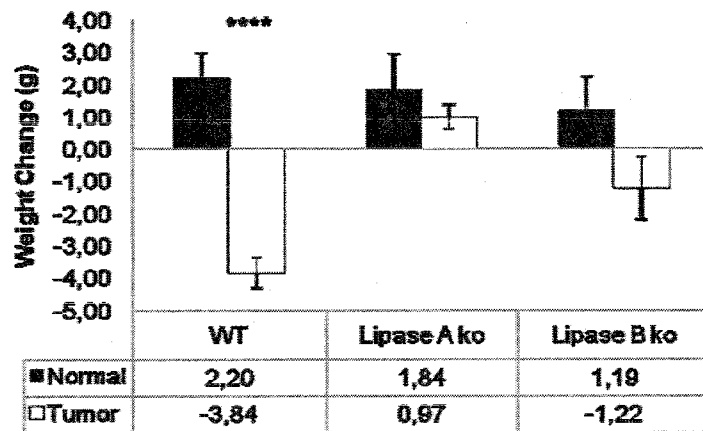
- sequence of about 17 to about 30 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of Figures 7 and /or 8.
33. A method for diagnosing a pathological condition involving cachexia, and its associated or related disorders and conditions, comprising determining a first amount or activity of polypeptide comprising an amino acid sequence selected from the group consisting of Figures 7 and /or 8, present in a biological sample obtained from said subject, and comparing said first amount or activity with the ranges of amounts or activities of the polypeptide determined in a population of healthy subjects, wherein an increase of the amount or activity of polypeptide in said biological sample compared to the range of amounts or activities determined for healthy subjects is indicative of the presence of the pathological condition.
  34. The method of claim 32 or 33 wherein the pathological condition is selected from cancer, AIDS, autoimmune disorders, drug addiction, alcoholism, chronic inflammatory disorders, cirrhosis of the liver, anorexia and neurodegenerative disease.
  35. A method for treatment and/or prevention of a pathological condition involving cachexia, and its associated or related disorders and conditions in a subject comprising administering to said subject a therapeutically effective amount of agent capable of inhibiting the expression or activity of ATGL.
  36. The method of claim 35 wherein the agent specifically inhibits the expression or activity of ATGL or specifically inhibits the activity of ATGL and HSL and does not significantly inhibit other lipases.
  37. A method for treatment and/or prevention of a pathological condition involving cachexia, and its associated or related disorders and conditions in a subject comprising administering to said subject a therapeutically effective amount of agent selected from the group consisting of an agent having binding affinity with a polypeptide comprising an amino acid sequence selected from the group consisting of Figures 7 and /or 8, and fragments thereof.
  38. A method for diagnosing a pathological condition involving cachexia, and its associated or related disorders and conditions, comprising determining a first amount or activity of polypeptide comprising an amino acid sequence selected from the group consisting of Figures 7 and /or 8, present in a biological sample obtained from said subject, and comparing said first amount or activity with the ranges of amounts or activities of the polypeptide determined in a population of healthy subjects, wherein an increase of the amount or activity of polypeptide in said biological sample compared to the range of amounts or activities determined for healthy subjects is indicative of the presence of the pathological condition.
  39. The method of any one of claims 35 to 38, wherein the pathological condition is selected from cachexia, and its associated or related disorders and conditions, including weight loss, muscle atrophy or wasting, and reduction of WAT, as found in cancer, AIDS, autoimmune disorders,

drug addiction, alcoholism, chronic inflammatory disorders, cirrhosis of the liver, anorexia and neurodegenerative disease.

# FIGURE 1

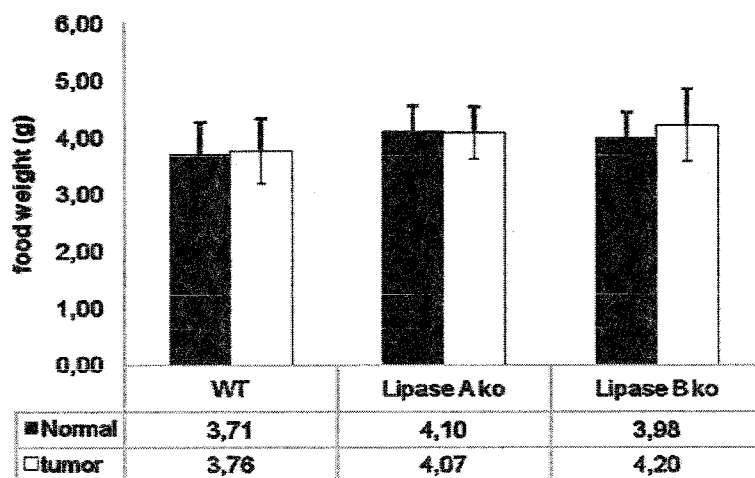
A

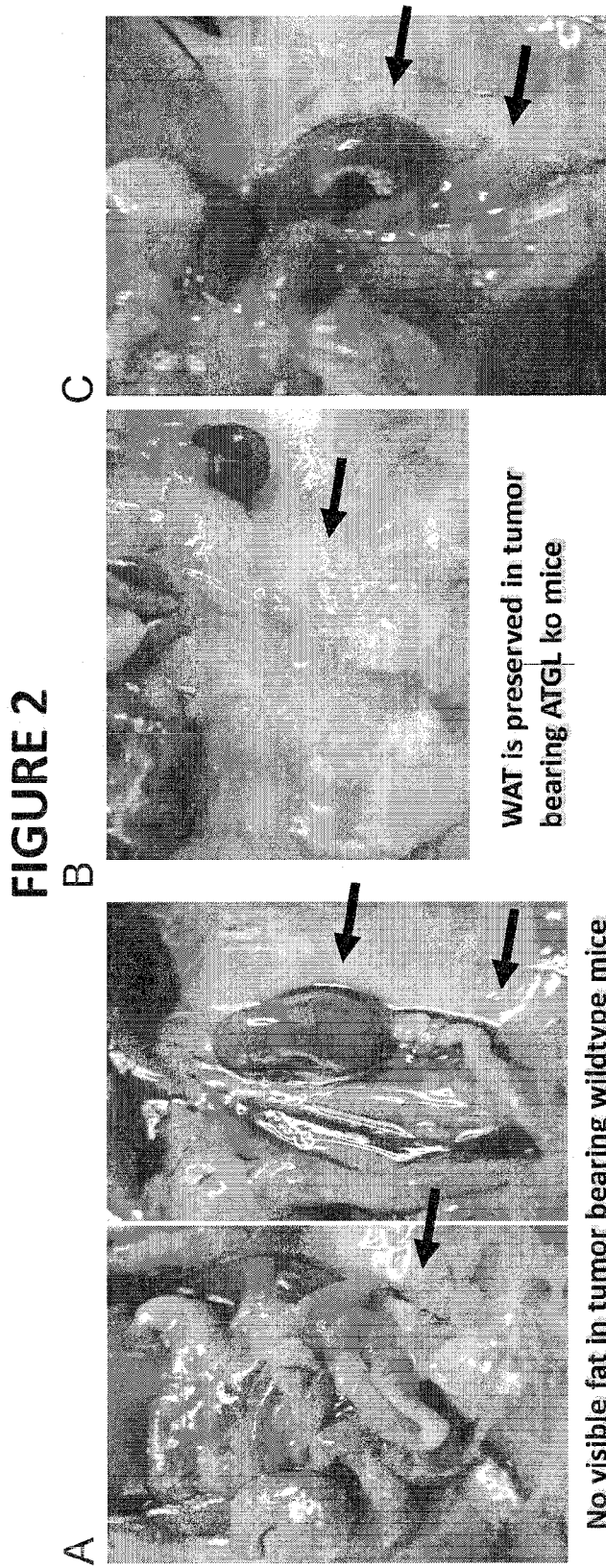
**Average Weight Change**



B

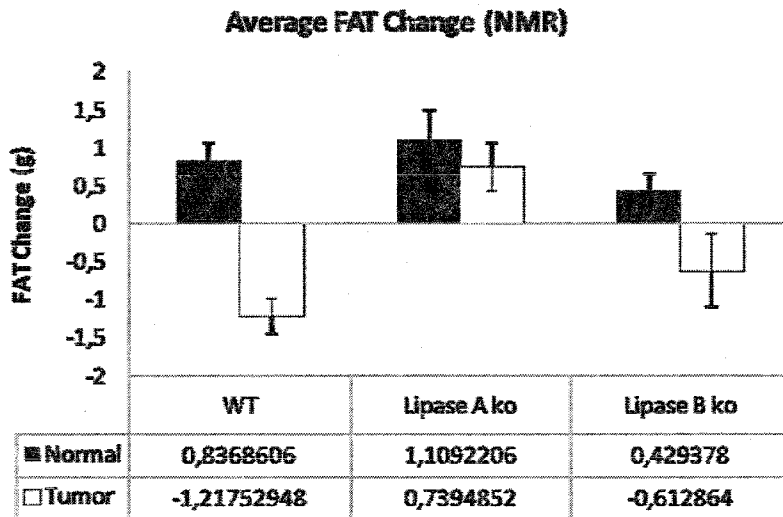
**Average Food Weight per Day**



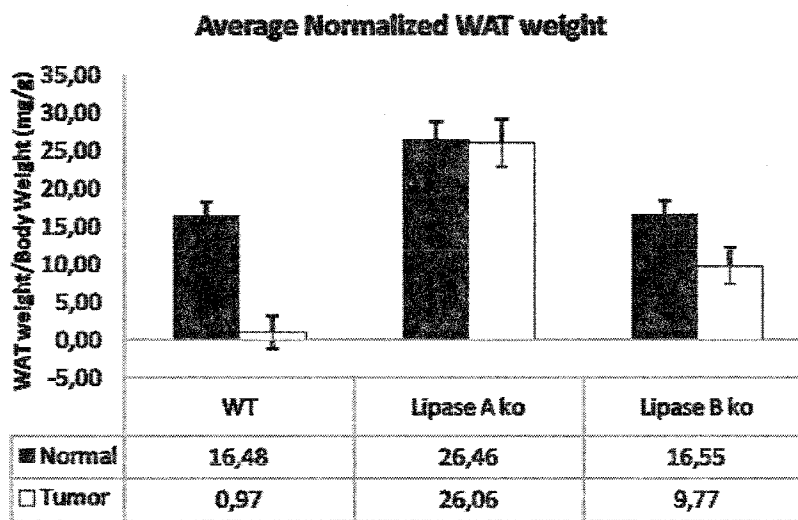


### FIGURE 3

A

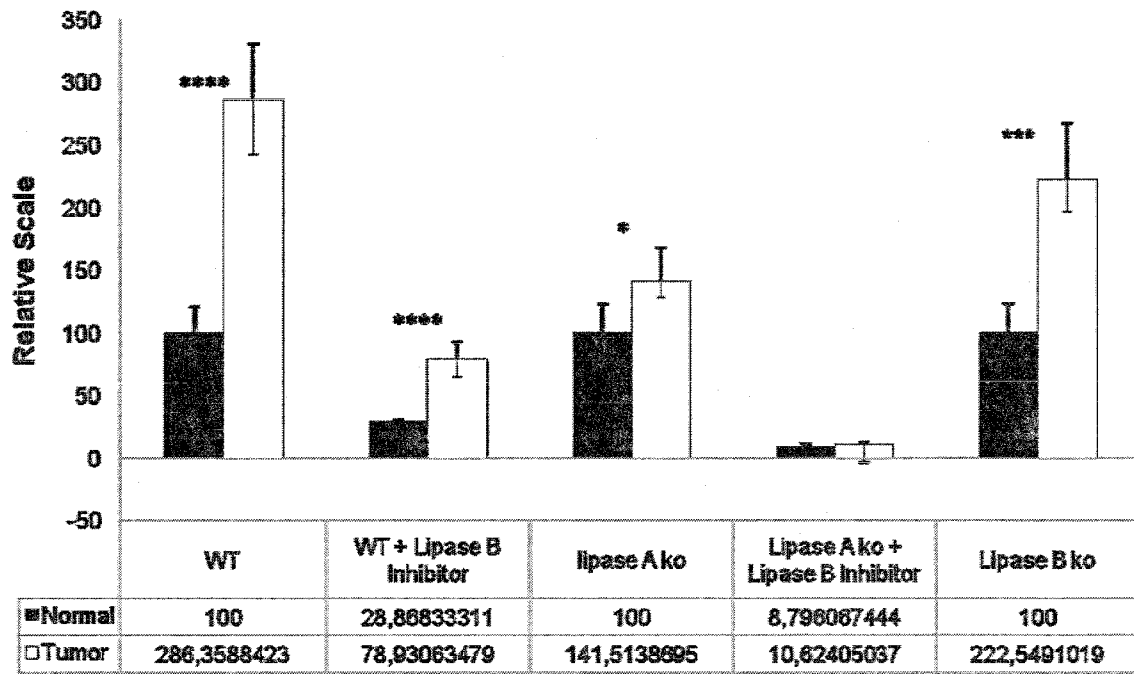


B



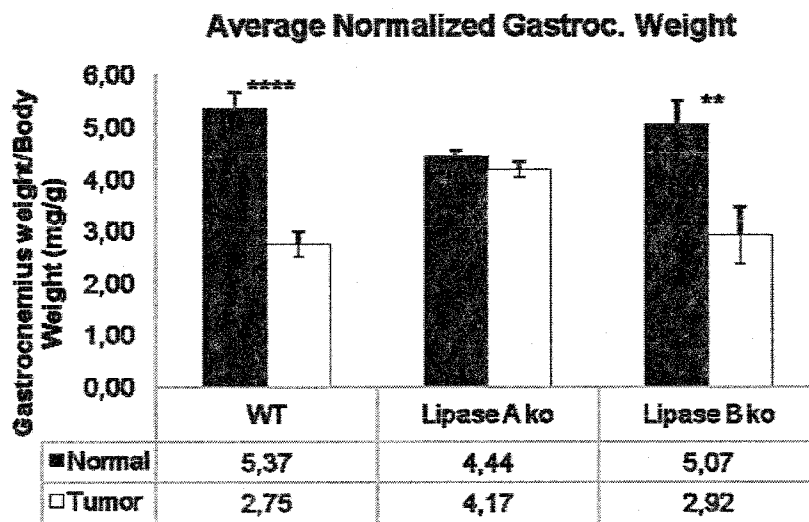
**FIGURE 4**

**Lipase Assay**

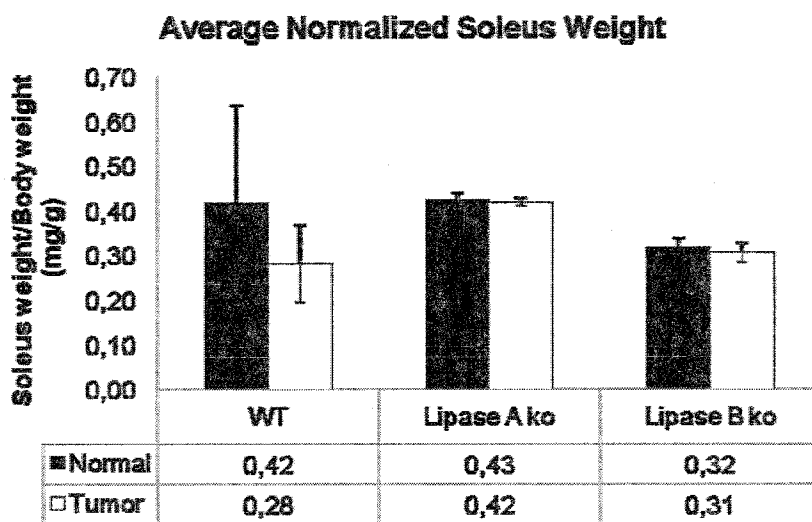


**FIGURE 5**

**A**



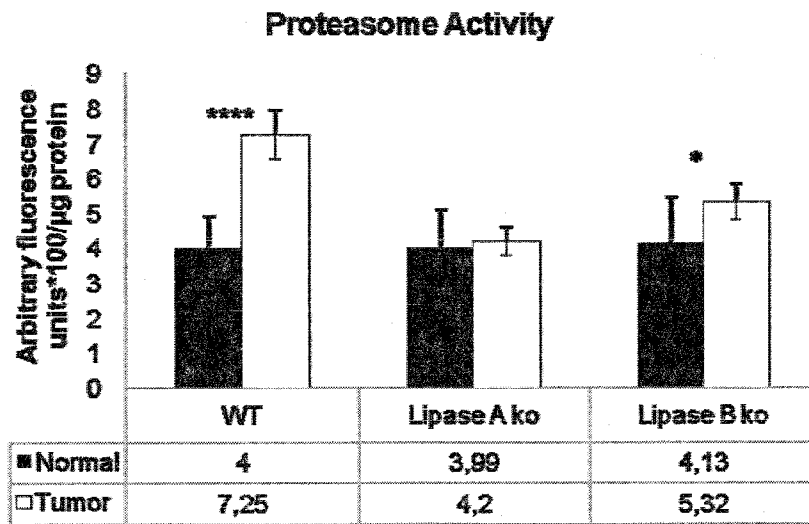
**B**



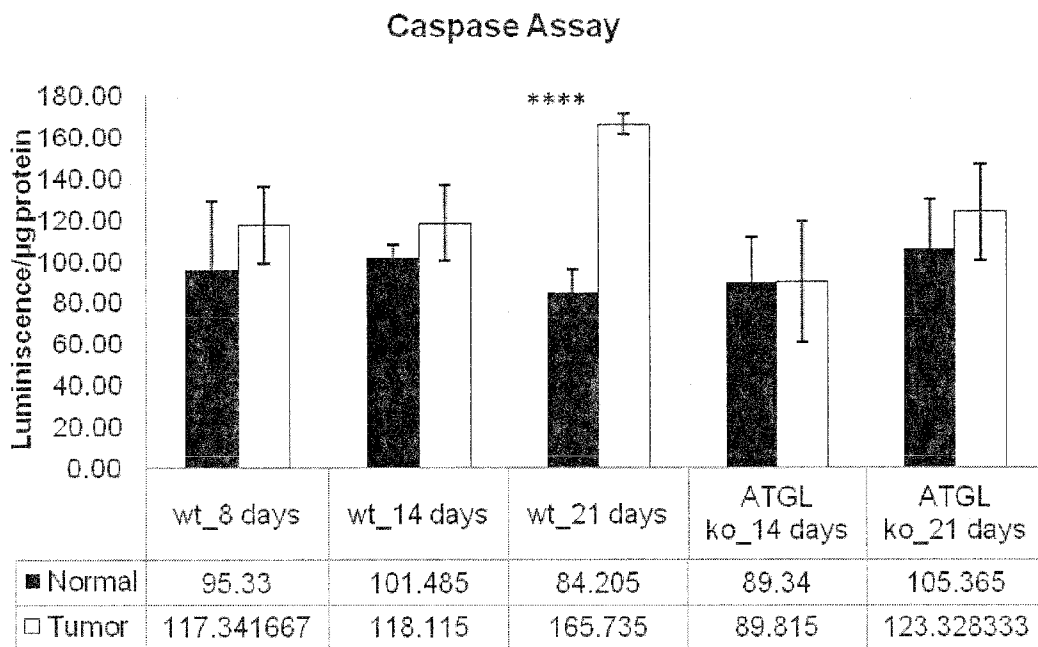


# FIGURE 6

**A**



**B**



# FIGURE 7

## Human ATGL amino acid sequence

```

1 mfprektwni sfagcgflgv yyvgvasclr ehapflvana thiygasaga ltatalvtgv
61 clgeagakfi evskearkrf lgplhpsfnl vkiirsflk vlpadsheha sgrlgisltr
121 vsdgenviis hfnskdeliq anvcsgfipv ycglippslq gvryvdggis dnlplyelkn
181 titvspfsge sdiqpqdsst nihelrvtnt siqfnlrnly rlskalfppe plvlremckq
241 gyrdglrflq rngllnrpnp llalpparph gpedkdqave saqaedysql pgedhilehl
301 parlnealle acveptdllt tlnmlpvrl atammvpytl plesalsfti rllewlpdvp
361 edirwmkeqt gsicqylvmr akrklgrhlp srlpeqvelr rvqslpsvpl scaayrealp
421 gwmrnlnslg dalakweecq rqlllglfct nvafppealr mrpadpapa padpaspqhq
481 lagpapllst papearpvig algl
    
```

## Human ATGL nucleic acid

```

1 ggggccccag tcagacgcag gcagcccaaa agcctgaaca ggcagggcca gaccagctt
61 cttgcctcc gccagcgggg accccgagct agagccgcag cgggacctgc ccggcccccg
121 gctccagcga gcgagcggcg agcagggcgc tcacagagggc ctggcgcgcc acggaacccg
181 gggccccggc gccgcgcgcg cgatgtttcc ccgcgagaag acgtggaaca tctogttcgc
241 gggctgcggc ttctctggcg totactacgt cggcgtggcc tctgcctcc gcgagcacgc
301 gcccttcctg gtggccaacg ccacgcacat ctacggcgcc toggccgggg cgctcacgcg
361 cacggcgtg gtcaccgggg tctgcctggg tgaggctggt gccaagttca ttgaggtatc
421 taaagaggcc cggaaagcgg tctctgggcc cctgcacccc tcttcaacc tggtaaagat
481 catccgcagt ttctgctga aggtcctgcc tgctgtagc catgagcatg ccagtgggcg
541 cctgggcatc tccctgacct gcgtgtcaga cggcgagaat gtcattatat ccacttcaa
601 ctccaaggac gagctcatoc aggccaatgt ctgcagcggg tcatccccg tgtactgtgg
661 gctcatccct cctccctcc agggggtgcg ctacgtggat ggtggcattt cagacaacct
721 gccactctat gagcttaaga acaccatcac agtgtcccc ttctcggggc agagtgcacat
781 ctgtccgcag gacagctcca ccaacatcca cgagctgcgg gtcaccaaca ccagatcca
841 gttcaacctg atgtgcaage agggataccg gtagggcctg cgtttctgc agcggaacgg
901 gctgcgagag ctgtgcaage agggataccg gtagggcctg cgtttctgc agcggaacgg
961 cctcctgaac cggcccaacc ccttgctggc gttgcccccc gccgcgcccc acggcccaga
1021 ggacaaggac caggcagtgg agagcgccca agcggaggat tactgcgagc tgccgggaga
1081 agatcacatc ctggagcacc tgcccgcggc gctcaatgag gccctgctgg aggcctgctg
1141 ggagcccacg gacctgctga ccacctctc caacatgctg cctgtgcgtc tggccacggc
1201 catgatggtg cctacaacgc tgccgctgga gagcgtctg tcttcaacca tccgcttctg
1261 ggagtggctg cccgacgttc ccgaggacat ccggtggatg aaggagcaga cgggcagcat
1321 ctgccagtac ctggtgatgc gcgccaagag gaagctgggc aggcacctgc cctccaggct
1381 gccggagcag gtggagctgc gcgcgtcca gtcgctgcg tocgtgcgcg tgtcctgocg
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1501 ggccaagtgg gaggagtgcc agcgcagct gctgctcggc ctcttctgca ccaacgtggc
1561 cttcccggcc gaagctctgc gcctgcgcgc acccgcgcgc cggctcccg ccccgcgga
1621 cccagcatcc ccgcagcacc agctggccgg gccctgcccc ttgctgagca cccctgctcc
1681 cgaggcccgg ccctgatcg gggcctggg gctgtgagac cccgacctc tcgaggaacc
1741 ctgcctgaga gcctccatt accactgcgc agtgagatga ggggactcac agttgccaag
1801 aggggtcttt gcctggggcc cctcgcagc cactcaacca gctgcatgca ctgagagggg
1861 aggtttccac accctcccc tgggcgcgtg aggccccgcg caoctgtgcc ttaatcttcc
1921 ctcccctgtg ctgcccagc acctccccg ccccttact cctgagaact ttgcagctgc
1981 ccttccctcc cgtttttca tggcctgctg aaatatgtgt gtgaagaatt atttattttc
2041 gccaagcac atgtaataaa tgctgcagcc caaaaaaaaa aaaaaaaaaa aaaaaaaaaa
2101 aaaaaaaaaa aaa
    
```

# FIGURE 8

## Mouse ATGL amino acid sequence

```

1 mftretkwni sfagcgflgv yhigvasclr ehapflvana thiygasaga ltatalvtga
61 clgeaganii evskearkrf lgplhpsfnl vktirgcllk tlpadchera ngrlgisltr
121 vsdgenviis hfsskdeliq anvctstfipv ycglipptlq gvryvdggis dnlplyelkn
181 titvspfsge sdicpqdsst nihelrvtnt siqfnlrnly rlskalfppe pmvlremckq
241 gyrdglrflr rnalleacve pkdlmttln mlpvrlatam mvpytlples avsftirle
301 wlpdvpedir wmkeqtgsic qylvmrakrk lgdhlp srls eqvelrraqs lpsvplscat
361 ysealpnwvr nnlslgdala kweecqrql lglfctnvaf ppdalrmrap asptaadpat
421 pqdppglppc
    
```

## Mouse ATGL nucleic acid

```

1 acagcgtctc cgctccgcc ggcggagacc ccaaggtatc gagactgagg gaccactgac
61 ccgcaggaca tcgagtcacg atgttcacga gggagaccaa gtggaacatc tcattcgtcg
121 gctgaggcgt cctcggggtc taccacattg gcgtggcctc ctgcctcctg gagcacgcgc
181 ccttcctggg gcccaacgcc actcacatct acggagcctc ggcagggggc ctcaccgcca
241 cagcgtgggt cactggggcc tgcctgggtg aagcagggtc caacattatt gaggtgtcca
301 aggaggcccc gaagcgggtc ctgggtcctc tgcatccctc cttcaacctg gtgaagacca
361 tccgtggctg tctactaaag aacctgcctg ctgattgcca tgagcgcgcc aatggcagcc
421 tgggcatctc cctgactcgt gtttcagacg gagagaaagt catcatatcc cactttagct
481 ccaaggatga gctcatccag gccaatgtct gcagcacatt tatcccgggt tactgtggcc
541 tcattcctcc taccctccaa ggggtgcgct atgtggatgg cggcatttca gacaactgac
601 cactttatga gctgaagaat accatcacag tgcctccatt ctcaggcgag agtgacatct
661 gccctcagga cagctccacc aacatccacg agcttcgctg caccacacc agcatccagt
721 tcaaccttcg caatctctac gcctctctga aggetctctt ccggccagag cccatggctc
781 tccgagagat gtgcaaacag ggctacagag atggacttog attccttagg aggaatgccc
841 tgctggaggc ctgtgtggaa ccaaaggacc tgatgaccac cctttccaac atgctaccag
901 tgcgcctggc aacggccatg atgggtccct atactctgcc gctggagagt gcagtgtcct
961 tcaccatccg cttgttgagg tggctgcctg atgtccctga agatatacgg tggatgaaag
1021 agcagacggg tagcatctgc cagtatctgg tgatgagggc caagaggaaa ttgggtgacc
1081 atctgccttc cagactgtct gagcagggtg aactgcgaag tgcccagctc ctgccctctg
1141 tgccactgtc ttgcgccacc tacagtgagg ccctacccaa ctgggtacga aacaacctct
1201 cactggggga cgcgtggcc aagtgggaag aatgccagcg tcagctactg ctgggtctct
1261 totgcaccaa tgtggccttc ccgcgggatg ccttgcgcat gcgcgcacct gccagcccca
1321 ctgcgcgaga tctgcccacc ccacaggatc cacctggcct ccgccttgc tgagaatcac
1381 cattcccaca tgcocggct accagccaag ctccaagttg tctgcccc aataagaggag
1441 ccccggggtg gaacaagatc ctgtctgccc cggctctccc ccttacatgc tgtggaatga
1501 ggacatagga cctgcacag ctgcaagtgg gcttctgatg tgaaacctt caccagccac
1561 tcaactatgct actcctgggt gggagggatg gggagtcgcc ctccccogga gccacagag
1621 cctcccccg tcacgtcacc tgtgccttac tctgcccac caocttttca gtgcagggtc
1681 agtettaaga actccacatc tgctgctgct ccctggtgtc caagtttctc tgacaggtgt
1741 gtgaagaatt atttattttt gccaaagcag atctaataaa agccacagct cagcttctg
    
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**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/EP2010/054360

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
INV. C12Q1/44	G01N33/574	G01N33/68
ADD.	A61K39/00	A61K48/00
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) C12Q G01N A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/039232 A2 (UNIV INNSBRUCK [AT]; KRONENBERG FLORIAN [AT]; HEID IRIS [DE]; SCHOENBO) 12 April 2007 (2007-04-12)	20-31
A	p.2, line 24-p.3, line 18; p.12, par.2; p.14, lines 25-33	1-19, 32-39
X	WO 2005/115461 A2 (KARL FRANZENS UNI GRAZ; ZECHNER RUDOLF [AT]; ZIMMERMANN ROBERT [AT]; S) 8 December 2005 (2005-12-08)	20-31
A	p.7, par.2	1-19, 32-39
X	US 2006/051841 A1 (GROSS RICHARD W [US] ET AL) 9 March 2006 (2006-03-09)	20-31
A	par.0103; claim 4	1-19, 32-39
	----- -/--	
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Date of the actual completion of the international search	Date of mailing of the international search report	
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International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] 7 October 2000 (2000-10-07), "1M0275012F Mouse 10kb plasmid UJGC1M library Mus musculus genomic clone UJGC1M0275012 F, DNA sequence." XP002583593 retrieved from EBI accession no. EMBL:AZ465350 Database accession no. AZ465350 the whole document -----	20-31

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2010/054360

## Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
  - a. (means)
    - on paper
    - in electronic form
  - b. (time)
    - in the international application as filed
    - together with the international application in electronic form
    - subsequently to this Authority for the purpose of search
2.  In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/054360

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007039232	A2	12-04-2007	NONE
WO 2005115461	A2	08-12-2005	EP 1765997 A2 28-03-2007 US 2009018066 A1 15-01-2009
US 2006051841	A1	09-03-2006	US 2009181443 A1 16-07-2009