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(71) Applicant *(for all designated States except US)*: **BAYER HEALTHCARE AG** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GOLZ, Stefan** [DE/DE]; Bückmannsmühle 46, 45326 Essen (DE). **SUMMER, Holger** [DE/DE]; Katernberger Schulweg 3, 42113 Wuppertal (DE). **GEERTS, Andreas** [DE/DE]; Schuckertstr. 29, 42113 Wuppertal (DE). **WILMEN, Andreas** [DE/DE]; Arndtstrasse 5, 50676 Köln (DE). **GRUSDAT, Gerda** [DE/DE]; Habichtweg 19, 42115 Wuppertal (DE). **BRÜGGEMEIER, Ulf** [DE/DE]; Leysiefen 20, 42799 Leichligen (DE).

(74) Common Representative: **BAYER HEALTHCARE AG**; Law and Patents, Patents and Licensing, 51368 Leverkusen (DE).

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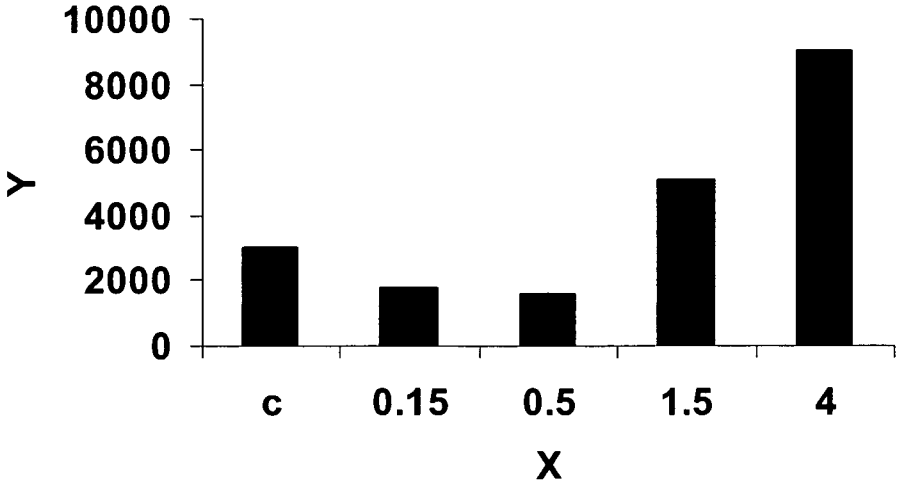
(54) Title: MYG AS A BIOMARKER FOR PPARA MODULATORS

Fig. 7



(57) Abstract: The invention provides MYG as a biomarker for PPARa modulators. The invention relates to the identification of a biomarker or a set of biomarkers or a combination of molecular and clinical markers to monitor or identify or quantify a treatment with a PPARa modulator or a combination of one or more PPARa modulators with other pharmacological compounds.

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MYG as a Biomarker for PPARa modulators

Technical field of the invention

The present invention is in the field of molecular biology, more particularly, the present invention relates to nucleic acid sequences and amino acid sequences of MYG and its regulation use as a biomarker of PPARa activity in mammals.

Background of the invention

TaqMan-Technology / expression profiling

TaqMan is a recently developed technique, in which the release of a fluorescent reporter dye from a hybridisation probe in real-time during a polymerase chain reaction (PCR) is proportional to the accumulation of the PCR product. Quantification is based on the early, linear part of the reaction, and by determining the threshold cycle (CT), at which fluorescence above background is first detected.

Gene expression technologies may be useful in several areas of drug discovery and development, such as target identification, lead optimization, and identification of mechanisms of action. The TaqMan technology can be used to compare differences between expression profiles of normal tissue and diseased tissue. Expression profiling has been used in identifying genes, which are up- or downregulated in a variety of diseases. An interesting application of expression profiling is temporal monitoring of changes in gene expression during disease progression and drug treatment or in patients versus healthy individuals. The premise in this approach is that changes in pattern of gene expression in response to physiological or environmental stimuli (e.g., drugs) may serve as indirect clues about disease-causing genes or drug targets. Moreover, the effects of drugs with established efficacy on global gene expression patterns may provide a guidepost, or a genetic signature, against which a new drug candidate can be compared.

MYG

MYG (Myoglobin)

Myoglobin is a single-chain globular protein of 153 amino acids in human, containing a heme (iron-containing porphyrin) prosthetic group in the center around which the

remaining apoprotein folds. With a molecular weight of 16,700 Daltons, it is the primary oxygen-carrying pigment of muscle tissues. Unlike the blood-borne hemoglobin, to which it is structurally related , this protein does not exhibit cooperative binding of oxygen, since positive cooperativity is a property reserved for multimeric proteins. Instead, the binding of oxygen by myoglobin is unaffected by the oxygen pressure in the surrounding tissue.

Garry et al. (1998) created mice without myoglobin by targeted disruption of the myoglobin gene. The mice were fertile and exhibited normal exercise capacity and a normal ventilatory response to hypoxia. Although heart and soleus muscles were depigmented, they functioned normally in standard in vitro assays of muscle performance across a range of work conditions and oxygen availability. These data demonstrated that myoglobin is not essential to meet the metabolic requirements of pregnancy or exercise in a terrestrial mammal, and raise new questions about oxygen transport and metabolic regulation in working muscles. Whether simple diffusion is enough or other mechanisms exist to facilitate high rates of oxygen transport to working myocytes remained to be elucidated.

Godecke et al. (1999) used homologous recombination to delete exon 2 of the mouse Mb gene. They found that Mb -/- mice appeared phenotypically normal but had significantly elevated levels of hemoglobin and an increase in coronary flow and coronary reserve. Histologic analysis demonstrated increased capillary density in the hearts of Mb-deficient mice. Godecke et al. (1999) concluded that disruption of myoglobin results in the activation of multiple compensatory mechanisms that steepen the pO2 gradient to mitochondria, suggesting that myoglobin is important for the delivery of oxygen to mitochondria

Myoglobin is a common biomarker for acute myocardial infarction and early cardiac deseases (Wuh, 2005; Hasic et al. 2006).

The ammount of myoglobin can be meassured with Innotrac Aio! Immunoassays as described in Weber et al. (2005).

Parameters in research and clinical studies

Common clinical parameters (but not limited to)

Physical Examination, Vital Signs, Clinical Chemistry, Hematology, Urinalysis, Electrocardiogram, Opthalmologic Evaluation

Hematology (but not limited to):

Hemoglobin, hematocrit, red blood count (RBC), white blood count (WBC), differential WBC, platelet count

Clinical Chemistry (but not limited to):

Sodium, potassium, chloride, glucose, urea nitrogen, total bilirubin, calcium, creatinine, magnesium, total cholesterol, HDL, LDL (calculated), total triglycerides, uric acid, alkaline phosphatase, AST (SGOT), LDH, GGT, ALT (SGPT), total protein, albumin, inorganic phosphorus, CO2

Thyroid function (but not limited to):

T-4 (thyroxine)

Urinalysis (but not limited to):

specific gravity, pH, glucose, ketones, protein, WBC, RBC

Coagulation (but not limited to):

prothrombin time, partial thromboplastin time

Imaging (but not limited to):

PET (Positron Emission Tomography), CT (Computed Tomography), ultrasonic, SPECT (Single Photon Emission Computed Tomography), Echocardiography, or Impedance Cardiography, X-ray

Other:

electroencephalography (EEG),

Pharmacokinetic

Pharmacokinetics is a branch of pharmacology dedicated to the study of the time course of substances and their relationship with an organism or system. In practice, this discipline is applied mainly to drug substances, though in principle it concerns itself with all manner of compounds residing within an organism or system, such as nutrients, metabolites, endogenous hormones, toxins, etc. So, in basic terms, while pharmacodynamics explores what a drug does to the body, pharmacokinetics explores what the body does to the drug.

Absorption and disposition

Pharmacokinetics has been broadly divided into two categories of study: absorption and disposition. Once a drug is administered as a dose, these processes begin simultaneously.

Absorption

The process of absorption can be seen as increasing the amount of a compound or dose x introduced into a system. Absorption studies seek to define the rate of input, dx/dt, of the dose x. For example, a constant rate infusion, R, of a drug might be 1 mg/hr, while the integral over time of dx/dt is referred to as the extent of drug input, x(t), ie. the total amount of drug x administered up to that particular time t. Sometimes the drug is assumed to be absorbed from the gastrointestinal tract in the form of a 1st order process with a 1st-order rate of absorption designated as Ka. Complex absorption profiles can be created by the use of controlled, extended, delayed or timed release of drugs from a dosage form.

Pharmacokinetics has many applications in drug therapy. By studying absorption -- the amount of a drug which gets into the system (bloodstream) following administration -- pharmacokinetics may guide the formulation of drug products. The amount of drug released from different formulations may vary; for example, two different tablets containing the same amount of drug chemical may not release the same amount into the bloodstream; a pharmacokinetic absorption study can determine whether or not the two tablets are equivalent and can be used interchangeably.

Disposition

Disposition is further subdivided into the study of the distribution, metabolism and elimination or excretion of a drug. Thus, pharmacokinetics is sometimes referred to as ADME.

The processes of disposition can be seen as clearing the system of a dose, or disposing of the dose. The disposition process distributes the compound or substance within the system, converts or metabolizes it, and eliminates the parent compound or products of the parent compound by passing them from the system into the urine, feces, sweat, exhalation or other routes of elimination. Sometimes compounds or their products may remain essentially indefinitely in the system by incorporation into the system.

Physiological Factors Affecting Oral Drug Absorption (but not limited to)

1. Gatrointestinal Motility (Decreased stomach emptying slows drug absorption; can be decreased by food, disease, drugs (opioids))

2. Gastrointestinal Blood Flow (Removes drug from site of absorption (conc. gradient); Limiting factor for highly absorbed drugs (e.g. ethanol))

3. Surface area (Approx 250 m2 (adult male); 1000x > stomach; Most drug absorption occurs in small intestine (esp. duodenum))

4. Metabolism and Efflux (Many drugs are metabolized in the intestinal wall; Many drugs are effluxed from enterocytes to gut lumen by transport proteins)

5. Changes in pH of Gastrointestinal Tract (Affects polarity of drug; Can be altered by food, disease, other drugs (e.g. antacids))

Dose-response relationship

The Dose-response relationship describes the change in effect on an organism caused by differing levels of exposure (or doses) to a substance. This may apply to individuals (eg: a small amount has no observable effect, a large amount is fatal), or to populations (eg: how many people are affected at different levels of exposure).

Studying dose response, and developing dose response models, is central to determining "safe" and "hazardous" levels and dosages for drugs, potential pollutants, and other substances that humans are exposed to. These conclusions are often the basis for public policy.

Toxicokinetics

Toxicokinetics is the application of pharmacokinetics to determine the relationship between the systemic exposure of a compound in experimental animals and its toxicity. It is used primarily for establishing relationships between exposures in toxicology experiments in animals and the corresponding exposures in humans.

Methods and parameters usesd in pharmacokinetic (but not limited to)

HPLC (UV/Vis, DAD, EC, RI, fluorescence) isocratic and gradient, LC-MS/MS, Plasma Protein Binding, biotransformation and excretion, Adverse Drug Interactions, Interindividual Differences in Drug Metabolism, Drug Excretion, Elimination Half-Life, Clearance (Cl), Maintenance Dosage Calculations, Bioavailability

CLINICAL DIAGNOSTICS

A principle of diagnostic testing is the correlation of the results of a procedure (e.g. blood test, urine test, CSF, test, sputum test, tissue biopsy, radiologic examination, measurement of one or more biomarkers, and the like) with particular clinical parameters. The correlation necessarily involves a comparison between two or more groups distinguished by the clinical parameter. A clinical parameter could be, for example, presence or absence of disease, risk of disease, stage of disease, severity of disease, class of disease or response to treatment of disease.

Accordingly, the diagnostician uses this correlation to qualify the status of a subject with respect to the clinical parameter. That is, the diagnostician uses the results of a procedure on a subject to classify or diagnose a subject status with respect to a clinical parameter, the confidence of the diagnosis/classification being related to the classifying or splitting power of the signs or symptoms used in the test.

Biomarkers having the most diagnostic utility, such as those of this invention, show a statistical difference between treatment and untreatment of at least $p < 0.05$, $p < 10\text{-}2$, $p < 10\text{-}3$, $p < 10\text{-}4$ or $p < 10\text{-}5$.

Examples (but nor limited to) for cardiovascular markers:

HEMODYNAMIC PARAMETERS

Blood Pressure Systolic (SBP), Blood Pressure Diastolic (DBP), Arterial Pressure (MAP), Cardiac Index (CI), Cardiac Output (CO), Central Venous Pressure (CVP), Pulmonary Artery Pressure (PA), Systolic (PAS), Diastolic (PAD), Pulmonary Capillary Wedge Pressure (PWCP), Pulmonary Vascular Resistance (PVR), Right Ventricular Pressure (RV), Systolic-, Diastolic-, Stroke Index (S1), Systemic Vascular Resistance (SVR)

NUCLEAR MEDICINE:

Myocardial Perfusion Scintigraphy, Myocardial Wall Motion Studies with Ejection Fraction, Thyroid Scintigraphy, Biliary Scintigraphy, Liver and Spleen Scintigraphy, Dynamic Renal Imaging, Computer Assessed Renal Function, Repeat Computer Assessed Renal Function after Pharmacological Intervention, Static Renal Scintigraphy, Bone Scintigraphy, Gallium Scintigraphy, Bone Mineral Content by Dual Photon Absorptiometry (Bone Densitometry), Brain Scintigraphy with Single - Photon Emission Computed Tomography, Perfusion and Ventilation Scintigraphy, Single Photon Emission Computed Tomography (SPECT)

The invention relates to the identification of a biomarker or a set of biomarkers or a combination of molecular and clinical markers to monitor or identify or quantificate a treatment with a PPARa modulator or a combination of one or more PPARa modulators with other pharmacological compounds.

Summary of the invention

The invention relates to the use of MYG polypeptides and polynucleotides as a biomarker for PPARa activity. The invention further comprises methods of diagnosing of PPARa activity in mammals.

Brief Description of the Drawings

Fig.1

Figure 1 shows the nucleotide sequence of a MYG polynucleotide rat (SEQ ID NO:1).

Fig.2

Figure 2 shows the amino acid sequence of a MYG polypeptide rat (SEQ ID NO:2).

Fig.3

Figure 3 shows the nucleotide sequence of a primer useful for the invention (SEQ ID NO:3).

Fig.4

Figure 4 shows the nucleotide sequence of a primer useful for the invention (SEQ ID NO:4).

Fig.5

Figure 5 shows the nucleotide sequence of a primer useful for the invention (SEQ ID NO:5).

Fig.6

Figure 6 shows the results of real-time PCR expression analysis of MYG in PPARa modulator treated rat hearts after oral compound administration. X : treatment; Y : relative expression; c : control (untreated); numbers : treated with PPARa in mg/kg

Fig.7

Figure 7 shows the results of real-time PCR expression analysis of MYG in PPARa modulator rat oral mucosa after oral compound administration. X : treatment; Y : relative expression; c : control (untreated); numbers : treated with PPARa in mg/kg

Fig.8

Figure 8 shows the result of real-time PCR expression analysis of MYG in PPARa modulator rat heart after IP compound administration. X : treatment; Y : relative expression; c : control (untreated); numbers : treated with PPARa in mg/kg

Fig.9

Figure 9 shows the result of real-time PCR expression analysis of MYG in PPARa modulator rat oral mucosa after IP compound administration. X : treatment; Y : relative expression; c : control (untreated); numbers : treated with PPARa in mg/kg

Fig.10

Figure 10 shows the results of microarray expression analysis of MYG in PPARa treated rat oral mucosa after oral compound administration. X : treatment, Y : relative expression ; A= Control, B= 0.5 mg/kg PPARa agonist; C= 1 mg/kg PPARa agonist; D= 4 mg/kg PPARa agonist

Fig.11

Figure 11 shows the nucleotide sequence of a MYG polynucleotide human (SEQ ID NO:6).

Fig.12

Figure 12 shows the amino acid sequence of a MYG polypeptide human (SEQ ID NO:7).

Detailed description of the invention

PPARa

Peroxisome proliferators are a diverse group of chemicals which includes hypolipidemic drugs, herbicides, leukotriene antagonists, and plasticizers. Two major categories of

peroxisome proliferator chemicals play a significant role in current society: the fibrate class of hypolipidemic drugs, which are used to reduce triglycerides and cholesterol in patients with hyperlipidemia; and phthalate ester plasticizers, which are used in the production of highly versatile, flexible vinyl plastics. Peroxisome proliferators induce hepatomegaly as a result of liver hyperplasia and an increase in the size and number of peroxisomes. Peroxisome Proliferative Activated Receptor alpha (PPARa) is described in the literature with synonyms hPPAR, NR1C1, PPARA or PPAR-alpha and genebank accession number NM_001001928 (human) and NM_013196 (rat) - but not limited to. Sher et al. (1993) cloned a cDNA for human peroxisome proliferator-activated receptor from a human liver cDNA library. The PPAR cDNA exhibited 85% and 91% DNA and deduced amino acid sequence identity, respectively, with mouse PPARa.

Kersten et al. (2000) reviewed the roles of PPARs in health and disease.

Using RNase protection and in situ hybridization, Michalik et al. (2001) showed that the alpha, delta (PPARD) (which they called beta), and gamma (PPARG) isotypes of PPAR are expressed in the mouse epidermis during fetal development and that they disappear progressively from the interfollicular epithelium after birth. A low level of Ppara expression was reactivated in the adult epidermis after various stimuli resulting in keratinocyte proliferation and differentiation, such as tetradecanoylphorbol acetate topical application, hair plucking, or skin wound healing.

Fu et al. (2003) presented evidence that oleylethanolamide (OEA), a naturally occurring lipid that regulates satiety and body weight, is a natural endogenous ligand for PPARA. In vitro, OEA showed high affinity binding to PPARA. In vivo, OEA reduced total food intake and suppressed body weight gain in wildtype mice, whereas it had no effect in Ppara-null mice. In the small intestine of wildtype mice, OEA regulated the expression of several PPARA target genes, initiating the transcription of proteins involved in lipid metabolism and repressing inducible nitric oxide synthase (iNOS), an enzyme which may contribute to feeding stimulation.

PPARa can be activated by a wide variety of saturated and unsaturated fatty acids, including palmitic acid, oleic acid, linoleic acid, and arachidonic acid include, but are not limited to, Oleylethanolamide, Palmitoylethanolamide.

Ligands for PPARs include dietary fatty acids, and a number of drugs used in the treatment of different cardiovascular and/or metabolic diseases. For example, fibrates, a group of serum triglyceride lowering agents, are synthetic agonists for PPAR. Examples of fibrates include, but are not limited to, are gemfibrozil, bezafibrate, clofibrate, fenofibrate, micronized fenofibrate.

Other synthetic modulators for PPARa include, but are not limited to, GW2331, WY14643, Tesaglitazar, Muraglitazar, Ragaglitazar, Naveglitazar MK-767 (KRP-297, L-410198), TZD18, L-65041, ETYA, LY 171883, GW 6471, GW 7647, GW 0742, MK 886, DRF 2519 AZD 6610, Netoglitazone, Rosiglitazone, Troglitazone, Isaglitazone, Ragaglitazar, NC-2100, SB-219994, GW-409544, GW-544, AR-H039242, CLX-0940, LR-90, LY-510929, LY-929, EMD-336340, EML-4156, LM-4156, DRF-4158, DRF-MDX8, BM-17.0744, DRF-4832, KT6-207, BMS-298585, GW-590735, LY-518674, NS-220, LSN-862, GSK-677954, TY-51501, TY-12780, ONO-5129, KRP-101, PPM-204, PLX-203, AVE-8134, AVE-0847, DRL-10945, DRF-10945, Peliglitazar, E-3030, GFT-505, Chiglitazar, CS-00098, MC-3002, LBM-642, AVE-0897, Plexxikon-2.

Other PPARa modulators are described also in patent applications WO 2006/032384, WO 2005/097784 and WO 2006/040002 and are herby incorporated by reference.

Forms of administration (but not limited to):

1. Oral

2. Mucosal: buccal or sublingual, nasal, ocular, vaginal, rectal

3. Parental: intravenous, intramuscular, subcutaneous

4. Inhalation

5. Percutaneous (transdermal)

The compounds or modulators can be administered by different routes including intravenous, intrapentoneal, subcutaneous, intramuscular, oral, transmucosal, rectal, transdermal, or inhalant. In some embodiments, oral administration is preferred. For oral administration, for example, the compounds can be formulated into conventional oral dosage forms such as capsules, tablets, and liquid preparations such as syrups, elixirs, and concentrated drops.

Pharmaceutical preparations for oral use can be obtained, for example, by combining the active compounds with solid excipients, 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, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid, or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain, for example, gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin ("gelcaps"), as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or 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 paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

Alternatively, injection (parenteral administration) may be used, e.g., intramuscular, intravenous, intrapentoneal, and/or subcutaneous. For injection, the PPARa modulators are formulated in sterile liquid solutions, preferably in physiologically compatible buffers or solutions, such as saline solution, Hank's solution, or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

Administration can-also be by transmucosal, topical, transdermal, or inhalant means. For transmucosal, topical or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation.

Transmucosal administration, for example, may be through nasal sprays or suppositories (rectal or vaginal).

The PPARa modulators could be administrated by formulated preferably as oils, creams, lotions, ointments and the like by choice of appropriate carriers known in the art.

Suitable carriers include vegetable or mineral oils, white petrolatum (white soft paraffin), branched chain fats or oils, animal fats and high molecular weight alcohol (greater than C12). The preferred carriers are those in which the active ingredient is soluble.

Emulsifiers, stabilizers, humectants and antioxidants may also be included as well as agents imparting color or fragrance, if desired. Creams for topical application are preferably formulated from a mixture of mineral oil, self-emulsifying beeswax and water in which mixture the active ingredient, dissolved in a small amount solvent (e.g., an oil), is admixed. Additionally, administration by transdermal means may comprise a transdermal patch or dressing such as a bandage impregnated with an active ingredient and optionally one or more carriers or diluents known in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For inhalants, PPARa modulators may be formulated as dry powder or a suitable solution, suspension, or aerosol. Powders and solutions may be formulated with suitable additives known in the art. For example, powders may include a suitable powder base such as lacatose or starch, and solutions may comprise propylene glycol, sterile water, ethanol, sodium chloride and other additives, such as acid, alkali and buffer salts.

Such solutions or suspensions may be administered by inhaling via spray, pump, atomizer, or nebulizer, and the like. The PPAR modulator may also be used in combination with other inhaled therapies, for example corticosteroids such as fluticasone proprionate, beclomethasone dipropionate, triamcinolone acetonide, budesonide, and mometasone furoate; beta agonists such as albuterol, salmeterol, and formoterol; anticholinergic agents such as ipratroprium bromide or tiotropium; vasodilators such as treprostinal and iloprost; enzymes such as DNAase; therapeutic proteins; immunoglobulin antibodies; an oligonucleotide, such as single or double stranded DNA or RNA, siRNA; antibiotics such as tobramycin; muscarinic receptor antagonists; leukotriene antagonists; cytokine antagonists; protease inhibitors; cromolyn sodium; nedocril sodium; and sodium cromoglycate.

The amounts of various compounds to be administered can be determined by standard procedures taking into account factors such as the compound EC50, the biological half-life of the compound, the age, size, and weight of the patient, and the disorder associated with the patient. The importance of these and other factors are well known to those of ordinary skill in the art. Generally, a dose will be between about 0.01 and 50 mg/kg, preferably 0.1 and 20 mg/kg of the patient being treated. Multiple doses may be used.

The PPAR modulator may also be used in combination with other therapies for treating the same disease. Such combination use includes administration of the compounds and one or more other therapeutics at different times, or co-administration of the compound and one or more other therapies. In certain embodiments, dosage may be modified for one or more of the compounds of the invention or other therapeutics used in combination, e.g., reduction in the amount dosed relative to a compound or therapy used alone, by methods well known to those of ordinary skill in the art.

Definition of terms

The "Biomarker" refers to a gene or a group of genes or a parameter or a group of parameters or a combination of both which could be used as an indicator or to measure or monitor or evaluate a normal or pathogenic or modified processes or pharmacological response to a therapeutic or non non-therapeutic intervention. A biomarker or a combination of biomarkers could be used to measure or evaluate or monitor the activity of an intrinsic or externally induced molecule or process or pathway. A biomarker or a combination of biomarkers could be used to measure or evaluate or monitor effect on etiologic agents or anatomical features. A biomarker or a combination of biomarkers could be used for later measurements thought to be directly related to clinical outcomes. A biomarker or a combination of biomarkers could be a candidate for surrogate endpoint if it is expected to predict clinical benefit or treatment monitoring based on epidemiological, therapeutic, pathophysiological, pharmacological or other scientific evidence.

The "mechanistic biomarker" refers to a gene or a group of genes or a parameter or a group of parameters or a combination of both which could be used to monitor or control or evaluate the involuntary and consistent response of an organism or cell to a given stimulus. The stimulus may be any one or more of the PPAR isoforms, e.g., PPARg, PPARa and PPARd modulators, in particular a PPARa modulator. A mechanistic biomarker could be used in particular as a biomarker.

The "surrogate endpoint" is defined as a laboratory or physical sign that is used in therapeutic or clinical or drug discovery trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient or animal feels, functions, survives, responses and that is expected to predict the effect of a therapy or treatment.

MYG: MG, Myoglobin, Genbank accession no.: NM_005368

MYH7: myosin, heavy chain 7, cardiac muscle, beta, Genbank accession no.: NM_000257

TNNT2: troponin T type 2, Genbank accession no.: NM_000364

CKM: creatine kinase, muscle, Genbank accession no.: NM_001824

MYBPC3: myosin binding protein C, Genbank accession no.: NM_000256

TBX15: T-box 15, Genbank accession no.: NM_152380

H19: H19, imprinted maternally expressed untranslated mRNA, Genbank accession no.: NR_002196

SMPX: small muscle protein, X-linked, Genbank accession no.: NM_014332

LDHB: lactate dehydrogenase B, Genbank accession no.: NM_002300

PDK4: pyruvate dehydrogenase kinase, isoenzyme 4, Genbank accession no.: NM_002612

PYGM: phosphorylase, glycogen; muscle, Genbank accession no.: NM_005609

CPT1A: carnitine palmitoyltransferase 1A, Genbank accession no.: NM_001031847

MTE1: acyl-CoA thioesterase 2, Genbank accession no.: NM_006821

CTE1: cytosolic acyl-CoA thioesterase 1, Genbank accession no.: AB010428

UCP3: uncoupling protein 3 (mitochondrial, proton carrier), Genbank accession no.: NM_003356

FABP3: atty acid binding protein 3, Genbank accession no.: NM_004102

A "clinical event" is an occurrence of clinically discernible signs of a disease or of clinically reportable symptoms of a disease. "Clinically discernible" indicates that the sign can be appreciated by a health care provider. "Clinically reportable" indicates that the symptom is the type of phenomenon that can be described to a health care provider. A clinical event may comprise clinically reportable symptoms even if the particular patient cannot himself or herself report them, as long as these are the types of phenomena that are generally capable of description by a patient to a health care provider.

The term "PPAR-mediated" disease or condition and like terms refer to a disease or condition in which the biological function of a PPAR affects the development and/or course of the disease or condition, and/or in which modulation of PPAR alters the

development, course, and/or symptoms of the disease or condition. Similarly, the phrase "PPAR modulation provides a therapeutic benefit" indicates that modulation of the level of activity of PPAR in a subject indicates that such modulation reduces the severity and/or duration of the disease, reduces the likelihood or delays the onset of the disease or condition, and/or causes an improvement in one or more symptoms of the disease or condition. In some cases the disease or condition may be mediated by any one or more of the PPAR isoforms, e.g. PPARg, PPARa and PPARd.

An "oligonucleotide" is a stretch of nucleotide residues which has a sufficient number of bases to be used as an oligomer, amplimer or probe in a polymerase chain reaction (PCR). Oligonucleotides are prepared from genomic or cDNA sequence and are used to amplify, reveal, or confirm the presence of a similar DNA or RNA in a particular cell or tissue. Oligonucleotides or oligomers comprise portions of a DNA sequence having at least about 10 nucleotides and as many as about 35 nucleotides, preferably about 25 nucleotides.

"Probes" may be derived from naturally occurring or recombinant single- or double-stranded nucleic acids or may be chemically synthesized. They are useful in detecting the presence of identical or similar sequences. Such probes may be labeled with reporter molecules using nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. Nucleic acid probes may be used in southern, northern or in situ hybridizations to determine whether DNA or RNA encoding a certain protein is present in a cell type, tissue, or organ.

A "fragment of a polynucleotide" is a nucleic acid that comprises all or any part of a given nucleotide molecule, the fragment having fewer nucleotides than about 6 kb, preferably fewer than about 1 kb.

"Reporter molecules" are radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents which associate with a particular nucleotide or amino acid sequence, thereby establishing the presence of a certain sequence, or allowing for the quantification of a certain sequence.

"Chimeric" molecules may be constructed by introducing all or part of the nucleotide sequence of this invention into a vector containing additional nucleic acid sequence which

might be expected to change any one or several of the following MYG characteristics: cellular location, distribution, ligand-binding affinities, interchain affinities, degradation/turnover rate, signaling, etc.

"Active", with respect to a MYG polypeptide, refers to those forms, fragments, or domains of a MYG polypeptide which retain the biological and/or antigenic activity of a MYG polypeptide.

"Naturally occurring MYG polypeptide" refers to a polypeptide produced by cells which have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including but not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

"Derivative" refers to polypeptides which have been chemically modified by techniques such as ubiquitination, labeling (see above), pegylation (derivatization with polyethylene glycol), and chemical insertion or substitution of amino acids such as ornithine which do not normally occur in human proteins.

"Conservative amino acid substitutions" result from replacing one amino acid with another having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

"Insertions" or "deletions" are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by producing the peptide synthetically while systematically making insertions, deletions, or substitutions of nucleotides in the sequence using recombinant DNA techniques.

A "signal sequence" or "leader sequence" can be used, when desired, to direct the polypeptide through a membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous sources by recombinant DNA techniques.

An "oligopeptide" is a short stretch of amino acid residues and may be expressed from an oligonucleotide. Oligopeptides comprise a stretch of amino acid residues of at least 3, 5,

10 amino acids and at most 10, 15, 25 amino acids, typically of at least 9 to 13 amino acids, and of sufficient length to display biological and/or antigenic activity.

"Inhibitor" (or antagonist) is any substance which retards or prevents a chemical or physiological reaction or response. Common inhibitors include but are not limited to antisense molecules, antibodies, and antagonists.

"Activator" (or agonist) is any agency bringing about activation; a molecule that increases the activity of an enzyme or a protein that increases the production of a gene product in DNA transcription.

The term "modulator" refers to a chemical or biological compound with capacity to either enhance (e.g., "agonist" activity) or partially enhance (e.g., "partial agonist" activity) or inhibit (e.g., "antagonist" activity or "inverse agonist" activity) a functional property of biological activity or process (e.g., enzyme activity or receptor binding); such enhancement or inhibition may be contingent on the occurrence of a specific event, such as activation of a signal transduction pathway, transcriptional regulation or enzymatic modification and/or may be manifest only in particular cell types.

The term 'therapeutically effective amount" as used herein, means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, I veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated.

"Biomarker" are measurable and quantifiable biological parameters (e.g. specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health - and physiology related assessments, such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidemiologic studies, etc.. Parameter that can be used to identify a toxic effect in an individual organism and can be used in extrapolation between species. Indicator signalling an event or condition in a biological system or sample and giving a measure of exposure, effect, or susceptibility.

Biological markers can reflect a variety of disease characteristics, including the level of exposure to an environmental or genetic trigger, an element of the disease process itself, an intermediate stage between exposure and disease onset, or an independent factor associated with the disease state but not causative of pathogenesis. Depending on the specific characteristic, biomarkers can be used to identifying a disease (diagnostic biomarkers), or predict future disease course, including response to therapy (efficacy biomarkers and mechanistic biomarkers).

"Standard expression" is a quantitative or qualitative measurement for comparison. It is based on a statistically appropriate number of normal samples and is created to use as a basis of comparison when performing diagnostic assays, running clinical trials, or following patient treatment profiles.

"Animal" as used herein may be defined to include human, domestic (e.g., cats, dogs, etc.), agricultural (e.g., cows, horses, sheep, etc.) or test species (e.g., mouse, rat, rabbit, etc.).

A "MYG polynucleotide", within the meaning of the invention, shall be understood as being a nucleic acid molecule selected from a group consisting of

(i) nucleic acid molecules encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 7,

(ii) nucleic acid molecules comprising the sequence of SEQ ID NO: 1 or SEQ ID NO: 6,

(iii) nucleic acid molecules having the sequence of SEQ ID NO: 1 or SEQ ID NO: 6,

(iv) nucleic acid molecules the complementary strand of which hybridizes under stringent conditions to a nucleic acid molecule of (i), (ii), or (iii),

(v) nucleic acid molecules the sequence of which differs from the sequence of a nucleic acid molecule of (iii) due to the degeneracy of the genetic code,

(vi) nucleic acid molecules which have a sequence identity of at least 80%, 85%, 90%, 95%, 98% or 99%; and

(vii) wherein the polypeptide encoded by said nucleic acid molecules of (i)-(vi) have MYG characteristics

A "MYG polypeptide", within the meaning of the invention, shall be understood as being a polypeptide selected from a group consisting of

(i) polypeptides having the sequence of SEQ ID NO: 2 or SEQ ID NO: 7,

(ii) polypeptides comprising the sequence of SEQ ID NO: 2 or SEQ ID NO: 7,

(iii) polypeptides encoded by MYG polynucleotides; and

(iv) polypeptides which show at least 99%, 98%, 95%, 90%, or 80% identity with a polypeptide of (i), (ii), or (iii);

wherein said polypeptide has MYG characteristics.

The nucleotide sequences encoding a MYG (or their complement) have numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use in the construction of oligomers for PCR, use for chromosome and gene mapping, use in the recombinant production of MYG, and use in generation of antisense DNA or RNA, their chemical analogs and the like. Uses of nucleotides encoding a MYG disclosed herein are exemplary of known techniques and are not intended to limit their use in any technique known to a person of ordinary skill in the art. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, e.g., the triplet genetic code, specific base pair interactions, etc.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of MYG - encoding nucleotide sequences may be produced. Some of these will only bear minimal homology to the nucleotide sequence of the known and naturally occurring MYG. The invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the

standard triplet genetic code as applied to the nucleotide sequence of naturally occurring MYG, and all such variations are to be considered as being specifically disclosed.

Although the nucleotide sequences which encode a MYG, its derivatives or its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring MYG polynucleotide under stringent conditions, it may be advantageous to produce nucleotide sequences encoding MYG polypeptides or its derivatives possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding a MYG polypeptide and/or its derivatives without altering the encoded amino acid sequence include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

Nucleotide sequences encoding a MYG polypeptide may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA techniques. Useful nucleotide sequences for joining to MYG polynucleotides include an assortment of cloning vectors such as plasmids, cosmids, lambda phage derivatives, phagemids, and the like. Vectors of interest include expression vectors, replication vectors, probe generation vectors, sequencing vectors, etc. In general, vectors of interest may contain an origin of replication functional in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for one or more host cell systems.

Another aspect of the subject invention is to provide for MYG-specific hybridization probes capable of hybridizing with naturally occurring nucleotide sequences encoding MYG. Such probes may also be used for the detection of similar protein encoding sequences and should preferably show at least 40% nucleotide identity to MYG polynucleotides. The hybridization probes of the subject invention may be derived from the nucleotide sequence presented as SEQ ID NO: 1 or SEQ ID NO: 6 or from genomic sequences including promoter, enhancers or introns of the native gene. Hybridization probes may be labelled by a variety of reporter molecules using techniques well known in the art.

It will be recognized that many deletional or mutational analogs of MYG polynucleotides will be effective hybridization probes for MYG polynucleotides. Accordingly, the invention relates to nucleic acid sequences that hybridize with such MYG encoding nucleic acid sequences under stringent conditions.

"Stringent conditions" refers to conditions that allow for the hybridization of substantially related nucleic acid sequences. For instance, such conditions will generally allow hybridization of sequence with at least about 85% sequence identity, preferably with at least about 90% sequence identity, more preferably with at least about 95% sequence identity. Hybridization conditions and probes can be adjusted in well-characterized ways to achieve selective hybridization of human-derived probes. Stringent conditions, within the meaning of the invention are 68°C in a buffer containing 0,2 x SSC (1x standard saline-citrate = 150 mM NaCl, 15 mM Trinatriumcitrat).

Nucleic acid molecules that will hybridize to MYG polynucleotides under stringent conditions can be identified functionally. Without limitation, examples of the uses for hybridization probes include: histochemical uses such as identifying tissues that express MYG; measuring mRNA levels, for instance to identify a sample's tissue type or to identify cells that express abnormal levels of MYG; and detecting polymorphisms of MYG.

PCR provides additional uses for oligonucleotides based upon the nucleotide sequence which encodes MYG. Such probes used in PCR may be of recombinant origin, chemically synthesized, or a mixture of both. Oligomers may comprise discrete nucleotide sequences employed under optimized conditions for identification of MYG in specific tissues or diagnostic use. The same two oligomers, a nested set of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for identification of closely related DNAs or RNAs.

Rules for designing polymerase chain reaction (PCR) primers are now established, as reviewed by PCR Protocols. Degenerate primers, i.e., preparations of primers that are heterogeneous at given sequence locations, can be designed to amplify nucleic acid sequences that are highly homologous to, but not identical with MYG. Strategies are now available that allow for only one of the primers to be required to specifically hybridize with

a known sequence. For example, appropriate nucleic acid primers can be ligated to the nucleic acid sought to be amplified to provide the hybridization partner for one of the primers. In this way, only one of the primers need be based on the sequence of the nucleic acid sought to be amplified.

PCR methods for amplifying nucleic acid will utilize at least two primers. One of these primers will be capable of hybridizing to a first strand of the nucleic acid to be amplified and of priming enzyme-driven nucleic acid synthesis in a first direction. The other will be capable of hybridizing the reciprocal sequence of the first strand (if the sequence to be amplified is single stranded, this sequence will initially be hypothetical, but will be synthesized in the first amplification cycle) and of priming nucleic acid synthesis from that strand in the direction opposite the first direction and towards the site of hybridization for the first primer. Conditions for conducting such amplifications, particularly under preferred stringent hybridization conditions, are well known.

Other means of producing specific hybridization probes for MYG include the cloning of nucleic acid sequences encoding MYG or MYG derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate reporter molecules.

It is possible to produce a DNA sequence, or portions thereof, entirely by synthetic chemistry. After synthesis, the nucleic acid sequence can be inserted into any of the many available DNA vectors and their respective host cells using techniques which are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into the nucleotide sequence. Alternately, a portion of sequence in which a mutation is desired can be synthesized and recombined with longer portion of an existing genomic or recombinant sequence.

MYG polynucleotides may be used to produce a purified oligo-or polypeptide using well known methods of recombinant DNA technology. The oligopeptide may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species from which the nucleotide sequence was derived or from a different species.

Advantages of producing an oligonucleotide by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

Quantitative determinations of nucleic acids

An important step in the molecular genetic analysis of human disease is often the enumeration of the copy number of a nucleis acid or the relative expression of a gene in particular tissues.

Several different approaches are currently available to make quantitative determinations of nucleic acids. Chromosome-based techniques, such as comparative genomic hybridization (CGH) and fluorescent in situ hybridization (FISH) facilitate efforts to cytogenetically localize genomic regions that are altered in tumor cells. Regions of genomic alteration can be narrowed further using loss of heterozygosity analysis (LOH), in which disease DNA is analyzed and compared with normal DNA for the loss of a heterozygous polymorphic marker. The first experiments used restriction fragment length polymorphisms (RFLPs) (Johnson, 1989), or hypervariable minisatellite DNA [Barnes, 2000]. In recent years LOH has been performed primarily using PCR amplification of microsatellite markers and electrophoresis of the radio labelled (Jeffreys, 1985) or fluorescently labelled PCR products and compared between paired normal and disease DNAs.

A number of other methods have also been developed to quantify nucleic acids. More recently, PCR and RT-PCR methods have been developed which are capable of measuring the amount of a nucleic acid in a sample. One approach, for example, measures PCR product quantity in the log phase of the reaction before the formation of reaction products plateaus.

A gene sequence contained in all samples at relatively constant quantity is typically utilized for sample amplification efficiency normalization. This approach, however, suffers from several drawbacks. The method requires that each sample has equal input amounts of the nucleic acid and that the amplification efficiency between samples is identical until the time of analysis. Furthermore, it is difficult using the conventional methods of PCR

quantitation such as gel electrophoresis or plate capture hybridization to determine that all samples are in fact analyzed during the log phase of the reaction as required by the method.

Another method called quantitative competitive (QC)-PCR, as the name implies, relies on the inclusion of an internal control competitor in each reaction. The efficiency of each reaction is normalized to the internal competitor. A known amount of internal competitor is typically added to each sample. The unknown target PCR product is compared with the known competitor PCR product to obtain relative quantitation. A difficulty with this general approach lies in developing an internal control that amplifies with the same efficiency than the target molecule.

5' Fluorogenic Nuclease Assays

Fluorogenic nuclease assays are a real time quantitation method that uses a probe to monitor formation of amplification product. The basis for this method of monitoring the formation of amplification product is to measure continuously PCR product accumulation using a dual-labelled fluorogenic oligonucleotide probe, an approach frequently referred to in the literature simply as the "TaqMan method" (Piatak, 1993), (Heid, 1996), (Gibson, 1996), (Holland, 1991).

The probe used in such assays is typically a short (about 20-25 bases) oligonucleotide that is labeled with two different fluorescent dyes. The 5' terminus of the probe is attached to a reporter dye and the 3' terminus is attached to a quenching dye, although the dyes could be attached at other locations on the probe as well. The probe is designed to have at least substantial sequence complementarity with the probe binding site. Upstream and downstream PCR primers which bind to flanking regions of the locus are added to the reaction mixture. When the probe is intact, energy transfer between the two fluorophors occurs and the quencher quenches emission from the reporter. During the extension phase of PCR, the probe is cleaved by the 5' nuclease activity of a nucleic acid polymerase such as Taq polymerase, thereby releasing the reporter from the oligonucleotide-quencher and resulting in an increase of reporter emission intensity which can be measured by an appropriate detector.

One detector which is specifically adapted for measuring fluorescence emissions such as those created during a fluorogenic assay is the ABI 7700 or 4700 HT manufactured by Applied Biosystems, Inc. in Foster City, Calif. The ABI 7700 uses fiber optics connected with each well in a 96-or 384 well PCR tube arrangement. The instrument includes a laser for exciting the labels and is capable of measuring the fluorescence spectra intensity from each tube with continuous monitoring during PCR amplification. Each tube is re-examined every 8.5 seconds.

Computer software provided with the instrument is capable of recording the fluorescence intensity of reporter and quencher over the course of the amplification. The recorded values will then be used to calculate the increase in normalized reporter emission intensity on a continuous basis. The increase in emission intensity is plotted versus time, i.e., the number of amplification cycles, to produce a continuous measure of amplification. To quantify the locus in each amplification reaction, the amplification plot is examined at a point during the log phase of product accumulation. This is accomplished by assigning a fluorescence threshold intensity above background and determining the point at which each amplification plot crosses the threshold (defined as the threshold cycle number or Ct). Differences in threshold cycle number are used to quantify the relative amount of PCR target contained within each tube. Assuming that each reaction functions at 100% PCR efficiency, a difference of one Ct represents a two-fold difference in the amount of starting template. The fluorescence value can be used in conjunction with a standard curve to determine the amount of amplification product present.

Non-Probe-Based Detection Methods

A variety of options are available for measuring the amplification products as they are formed. One method utilizes labels, such as dyes, which only bind to double stranded DNA. In this type of approach, amplification product (which is double stranded) binds dye molecules in solution to form a complex. With the appropriate dyes, it is possible to distinguish between dye molecules free in solution and dye molecules bound to amplification product. For example, certain dyes fluoresce only when bound to amplification product. Examples of dyes which can be used in methods of this general type include, but are not limited to, Syber Green.TM. and Pico Green from Molecular Probes,

Inc. of Eugene, Oreg., ethidium bromide, propidium iodide, chromomycin, acridine orange, Hoechst 33258, Toto-1, Yoyo-1, DAPI (4',6-diamidino-2-phenylindole hydrochloride).

Another real time detection technique measures alteration in energy fluorescence energy transfer between fluorophors conjugated with PCR primers.

Probe-Based Detection Methods

These detection methods involve some alteration to the structure or conformation of a probe hybridized to the locus between the amplification primer pair. In some instances, the alteration is caused by the template-dependent extension catalyzed by a nucleic acid polymerase during the amplification process. The alteration generates a detectable signal which is an indirect measure of the amount of amplification product formed.

For example, some methods involve the degradation or digestion of the probe during the extension reaction. These methods are a consequence of the 5'-3' nuclease activity associated with some nucleic acid polymerases. Polymerases having this activity cleave mononucleotides or small oligonucleotides from an oligonucleotide probe annealed to its complementary sequence located within the locus.

The 3' end of the upstream primer provides the initial binding site for the nucleic acid polymerase. As the polymerase catalyzes extension of the upstream primer and encounters the bound probe, the nucleic acid polymerase displaces a portion of the 5' end of the probe and through its nuclease activity cleaves mononucleotides or oligonucleotides from the probe.

The upstream primer and the probe can be designed such that they anneal to the complementary strand in close proximity to one another. In fact, the 3' end of the upstream primer and the 5' end of the probe may abut one another. In this situation, extension of the upstream primer is not necessary in order for the nucleic acid polymerase to begin cleaving the probe. In the case in which intervening nucleotides separate the upstream primer and the probe, extension of the primer is necessary before the nucleic acid polymerase encounters the 5' end of the probe. Once contact occurs and polymerization continues, the 5'-3' exonuclease activity of the nucleic acid polymerase begins cleaving mononucleotides

or oligonucleotides from the 5' end of the probe. Digestion of the probe continues until the remaining portion of the probe dissociates from the complementary strand.

In solution, the two end sections can hybridize with each other to form a hairpin loop. In this conformation, the reporter and quencher dye are in sufficiently close proximity that fluorescence from the reporter dye is effectively quenched by the quencher dye. Hybridized probe, in contrast, results in a linearized conformation in which the extent of quenching is decreased. Thus, by monitoring emission changes for the two dyes, it is possible to indirectly monitor the formation of amplification product.

Probes

The labeled probe is selected so that its sequence is substantially complementary to a segment of the test locus or a reference locus. As indicated above, the nucleic acid site to which the probe binds should be located between the primer binding sites for the upstream and downstream amplification primers.

Primers

The primers used in the amplification are selected so as to be capable of hybridizing to sequences at flanking regions of the locus being amplified. The primers are chosen to have at least substantial complementarity with the different strands of the nucleic acid being amplified. When a probe is utilized to detect the formation of amplification products, the primers are selected in such that they flank the probe, i.e. are located upstream and downstream of the probe.

The primer must have sufficient length so that it is capable of priming the synthesis of extension products in the presence of an agent for polymerization. The length and composition of the primer depends on many parameters, including, for example, the temperature at which the annealing reaction is conducted, proximity of the probe binding site to that of the primer, relative concentrations of the primer and probe and the particular nucleic acid composition of the probe. Typically the primer includes 15-30 nucleotides. However, the length of the primer may be more or less depending on the complexity of the primer binding site and the factors listed above.

Labels for Probes and Primers

The labels used for labeling the probes or primers of the current invention and which can provide the signal corresponding to the quantity of amplification product can take a variety of forms. As indicated above with regard to the 5' fluorogenic nuclease method, a fluorescent signal is one signal which can be measured. However, measurements may also be made, for example, by monitoring radioactivity, colorimetry, absorption, magnetic parameters, or enzymatic activity. Thus, labels which can be employed include, but are not limited to, fluorophors, chromophores, radioactive isotopes, electron dense reagents, enzymes, and ligands having specific binding partners (e.g., biotin-avidin).

Monitoring changes in fluorescence is a particularly useful way to monitor the accumulation of amplification products. A number of labels useful for attachment to probes or primers are commercially available including fluorescein and various fluorescein derivatives such as FAM, HEX, TET and JOE (all which are available from Applied Biosystems, Foster City, Calif.); lucifer yellow, and coumarin derivatives.

Labels may be attached to the probe or primer using a variety of techniques and can be attached at the 5' end, and/or the 3' end and/or at an internal nucleotide. The label can also be attached to spacer arms of various sizes which are attached to the probe or primer. These spacer arms are useful for obtaining a desired distance between multiple labels attached to the probe or primer.

In some instances, a single label may be utilized; whereas, in other instances, such as with the 5' fluorogenic nuclease assays for example, two or more labels are attached to the probe. In cases wherein the probe includes multiple labels, it is generally advisable to maintain spacing between the labels which is sufficient to permit separation of the labels during digestion of the probe through the 5'-3' nuclease activity of the nucleic acid polymerase.

Microarray

Nucleic acid arrays that have been used in the present invention are those that are commercially available from Affymetrix (Santa Clara, Calif.) under the brand name

GeneChip Human Genome U133 Plus 2.0 Array.® or Rat Genome 230 2.0 Array respectively which represents the complete coverage of the Human Genome U133 Set plus 9921 probe sets representing approximately 6,500 new genes (with a total of approximately 56 000 transcripts) or the Rat Genome respectively.Affymetrix (Santa Clara, Calif.) GeneChip technology platform which consists of high-density microarrays and tools to help process and analyze those arrays, including standardized assays and reagents, instrumentation, and data management and analysis tools.

GeneChip microarrays consist of small DNA fragments (referred to as probes), chemically synthesized at specific locations on a coated quartz surface. By extracting and labeling nucleic acids from experimental samples, and then hybridizing those prepared samples to the array, the amount of label can be monitored enabling a measurement of gene regulation

The GeneChip rat genome arrays include a set of rat maintenance genes to facilitate the normalization and scaling of array experiments and to perform data comparison. This set of normalization genes shows consistent levels of expression over a diverse set of tissues.

MYG Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of MYG.

"Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of MYG. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acid. An antibody which specifically binds to an epitope of MYG can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the MYG immunogen.

Typically, an antibody which specifically binds to MYG provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to MYG do not detect other proteins in immunochemical assays and can immunoprecipitate MYG from solution.

MYG can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, MYG can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to MYG can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique.

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to MYG. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries. Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template. Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught. A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology.

Antibodies which specifically bind to MYG also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents. Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which MYG is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Diagnostics

One embodiment of the invention describes MYG as a biomarker for diagnostic use. Use of MYG as a biomarker in diagnostics is based by the comparison of MYG level in a biological sample taken from a PPARa modulator treated mammal with the MYG level in a

control sample taken from an untreated or mock treated mammal. A control sample can also be a sample taken from a mammal being subjected a PPARa treatment. Does the MYG level in the treated mammal differs from the MYG level in an untreated mammal then the treated mammal is diagnosed as treated with a PPARa modulator. Furthermore, comparing MYG levels of a biological sample from a treated mammal with MYG levels of control samples from untreated mammals allows the diagnosis of a MYG-associated modulator treatment. The biological sample is taken from the analogue tissue or body fluid than the control sample.

Normal or standard values for MYG expression are established by using control samples taken from untreated or mammals being subjected a treatment. A control sample can be obtained by collecting separate or combined body fluids or cell extracts taken from untreated mammalian subjects, preferably human, achieving statistical relevant numbers. To obtain the normal or standard MYG level of the control samples, the samples were subjected to suitable detection methods to detect MYG polypeptide, polynucleotide or activity. The determination of MYG level in a mammal subjected to diagnosis is performed analogously by collecting a biological sample taken from said mammal. Quantities of MYG levels in biological samples taken from a mammal subjected to diagnosis are compared with the standard or normal values measured from a control sample. Deviation between standard value (determined from control sample) and subject value (determined from biological sample) establishes the parameters for diagnosing PPARa modulator treatment. Absolute quantification of MYG levels measured from biological or control samples may be achieved by comparing those values with values obtained from an experiment in which a known amount of a substantially purified polypeptide or polynucleotide is used.

Antibodies which specifically bind MYG may be used for the diagnosis of PPARa modulator treatment characterized by the expression of the biomarker MYG, or in diagnostic assays to monitor patients being treated with a PPARa modulator. Such a treatment includes medication with PPAR polypeptides or polynucleotides, or agonists, antagonists, and inhibitors of PPAR. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for MYG include methods which utilize the antibody and a label to detect MYG in body

fluids or in extracts of cells or tissues taken from a mammal. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent joining with a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring MYG, including ELISAs, RIAs, Planar Waveguide technology, Real-Time PCR, microarray analysis and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of MYG expression. Planar Waveguide Technology bioassays are designed to perform multiplexed nucleic acid hybridization assays, immunoaffinity reactions and membrane receptor based assays with high sensitivity and selectivity. The recognition elements specific for the analytes of interest are bound onto the surface in small discrete spots; the transfer of the recognition elements onto the surface is performed using an adequate spotting technology, which requires only minute amounts of recognition elements. Such an arrangement of different recognition elements in an array format allows the simultaneous detection and quantification of hundreds to thousands of different analytes per sample including replicates.

Reactions on microarrays usually follow a typical scheme: Recognition elements (e.g. oligonucleotides, cDNAs, or antibodies) are spotted onto the chemically modified planar waveguide surface with typical spot diameters of 100 - 200 μm. The remaining free binding sites on the surface subsequently are being blocked to reduce or eliminate nonspecific binding. In a next step the sample (e.g. fluorescently labeled cDNA or pre-incubated analyte / fluorescently labeled antibody complex) is transferred onto the surface for incubation. The incubation time where a selective recognition and binding between recognition elements and corresponding target molecules (e.g. DNA - DNA hybridization or antigen - antibody interaction) occurs depends on the affinity between the analytes and the immobilized recognition elements. The resulting fluorescing spots can then be detected during readout.

Due to the laterally resolved imaging of the fluorescence signals of the individual spots by a CCD-camera, a large variety of different analytes can be quantified simultaneously, requiring typically sample volumes in the range of 15 μl. Calibration and referencing spots

allow for accurate quantification of analytes using just one chip and enable the establishment of dose response and time dependent activity profiles.

Normal or standard values for MYG expression are established by using control samples taken from untreated mammalian subjects. A control sample can be obtained by collecting separate or combined body fluids or cell extracts taken from normal mammalian subjects, preferably human, achieving statistical relevant numbers. To obtain normal or standard values the control samples are combined with an antibody to MYG under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, preferably by photometric means. The determination of MYG level in a mammal subjected to diagnosis is performed analogously by collecting a biological sample from said mammal, combining said sample with an antibody to MYG and determination of complex formation. Quantities of MYG expressed in biological samples from a mammal subjected to diagnosis are compared with the standard or normal values measured from a control sample. Deviation between standard value (determined from control sample) and subject value (determined from biological sample) establishes the parameters for diagnosing PPARa modulator treatment. Absolute quantification of MYG levels measured from biological or control samples may be achieved by comparing those values with values obtained from an experiment in which a known amount of a substantially purified polypeptide is used.

In another embodiment of the invention, the polynucleotides encoding MYG may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantified gene expression in control and biological samples in which expression of the biomarker MYG may be correlated with disease. The diagnostic assay may be used to distinguish between absence, presence, and excess expression of MYG, and to monitor regulation of MYG levels during therapeutic intervention.

Polynucleotide sequences encoding MYG may be used for the diagnosis of PPARa activity associated with expression of MYG. The polynucleotide sequences encoding MYG may be used in Southern, Northern, or dot-blot analysis, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; bDNA (branched DNA

technology) and Planar Waveguide Technology; and in microarrays utilizing a biological sample from diseased mammals to detect altered MYG expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding MYG may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding MYG may be labeled by standard methods and added to a biological sample from diseased mammals under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is altered from that of a comparable control sample, the nucleotide sequences have hybridized with nucleotide sequences in the sample, and the presence of altered levels of nucleotide sequences encoding MYG in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient.

In order to provide a basis for the diagnosis of PPARa modulator treatment associated with expression of MYG, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding MYG, under conditions suitable for hybridization or amplification. Quantification of MYG levels measured from biological or control samples may be achieved by comparing those values with values obtained from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a PPARa modulator.

Biomarker

Use of MYG as a biomarker

One of ordinary skill in the art knows several methods and devices for the detection and analysis of the markers of the instant invention. With regard to polypeptides or proteins in patient test samples, immunoassay devices and methods are often used. These devices and methods can utilize labelled molecules in various sandwich, competitive, or non-competitive assay formats, to generate a signal that is related to the presence or amount of an analyte of interest. Additionally, certain methods and devices, such as biosensors and optical immunoassays, may be employed to determine the presence or amount of analytes without the need for a labelled molecule.

Preferably the markers are analyzed using an immunoassay, although other methods are well known to those skilled in the art (for example, the measurement of marker RNA levels). The presence or amount of a marker is generally determined using antibodies specific for each marker and detecting specific binding. Any suitable immunoassay may be utilized, for example, enzyme- linked immunoassays (ELISA), radioimmunoassay (RIAs), competitive binding assays, planar waveguide technology, and the like. Specific immunological binding of the antibody to the marker can be detected directly or indirectly. Direct labels include fluorescent or luminescent tags, metals, dyes, radionuclides, and the like, attached to the antibody. Indirect labels include various enzymes well known in the art, such as alkaline phosphatase, horseradish peroxidase and the like. For an example of how this procedure is carried out on a machine, one can use the RAMP Biomedical device, called the Clinical Reader sup™, which uses the fluorescent tag method, though the skilled artisan will know of many different machines and manual protocols to perform the same assay. Diluted whole blood is applied to the sample well. The red blood cells are retained in the sample pad, and the separated plasma migrates along the strip. Fluorescent dyed latex particles bind to the analyte and are immobilized at the detection zone. Additional particles are immobilized at the internal control zone. The fluorescence of the detection and internal control zones are measured on the RAMP Clinical Reader sup™, and the ratio between these values is calculated. This ratio is used to determine the analyte concentration

by interpolation from a lot-specific standard curve supplied by the manufacturer in each test kit for each assay.

The use of immobilized antibodies specific for the markers is also contemplated by the present invention and is well known by one of ordinary skill in the art. The antibodies could be immobilized onto a variety of solid supports, such as magnetic or chromatographic matrix particles, the surface of an assay place (such as microtiter wells) , pieces of a solid substrate material (such as plastic, nylon, paper), and the like. An assay strip could be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip could then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a coloured spot.

The analysis of a plurality of markers may be carried out separately or simultaneously with one test sample. Several markers may be combined into one test for efficient processing of a multiple of samples. In addition, one skilled in the art would recognize the value of testing multiple samples (for example, at successive time points) from the same individual. Such testing of serial samples will allow the identification of changes in marker levels over time. Increases or decreases in marker levels, as well as the absence of change in marker levels, would provide useful information about the disease status that includes, but is not limited to identifying the approximate time from onset of the event, the presence and amount of salvagable tissue, the appropriateness of drug therapies, the effectiveness of various therapies, identification of the severity of the event, identification of the disease severity, and identification of the patient's outcome, including risk of future events.

An assay consisting of a combination of the markers referenced in the instant invention may be constructed to provide relevant information related to differential diagnosis. Such a panel may be constucted using 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or more or individual markers. The analysis of a single marker or subsets of markers comprising a larger panel of markers could be carried out methods described within the instant invention to optimize clinical sensitivity or specificity in various clinical settings.

The analysis of markers could be carried out in a variety of physical formats as well. For example, the use of microtiter plates or automation could be used to facilitate the processing of large numbers of test samples. Alternatively, single sample formats could be developed to facilitate immediate treatment and diagnosis in a timely fashion, for example, in ambulatory transport or emergency room settings. Particularly useful physical formats comprise surfaces having a plurality of discrete, addressable locations for the detection of a plurality of different analytes. Such formats include protein microarrays, or "protein chips" and capillary devices.

Cardiac markers serve an important role in the early detection and monitoring of cardiovascular disease. Markers of disease are typically substances found in a bodily sample that can be easily measured. The measured amount can correlate to underlying disease pathophysiology, presence or absence of a current or imminent cardiac event, probability of a cardiac event in the future. In patients receiving treatment for their condition the measured amount will also correlate with responsiveness to therapy. Markers can include elevated levels of blood pressure, cholesterol, blood sugar, homocysteine and C- reactive protein (CRP). However, current markers, even in combination with other measurements or risk factors, do not adequately identify patients at risk, accurately detect events (i.e., heart attacks), or correlate with therapy. For example, half of patients do not have elevated serum cholesterol or other traditional risk factors.

Use of markers in diagnosis of cardiac conditions is described in, for example, Alpert et al. (2000); Newby et al. (2001); de Lemos et al.(2002); Boersma et al. (2002); Christenson et al. (2001), each of which is incorporated by reference in its entirety.

Cardiovascular biomarker

<u>BNP (as an example for cardiovascular biomarkers)</u>

B-type natriuretic peptide (BNP), also called brain- type natriuretic peptide is a 32 amino acid, 4 kDa peptide that is involved in the natriuresis system to regulate blood pressure and fluid balance. The precursor to BNP is synthesized as a 108-amino acid molecule, referred

to as "pre pro BNP," that is proteolytically processed into a 76-amino acid N-terminal peptide (amino acids 1-76), referred to as "NT pro BNP" and the 32-amino acid mature hormone, referred to as BNP or BNP 32 (amino acids 77-108). It has been suggested that each of these species--NT pro- BNP, BNP-32, and the pre pro BNP--can circulate in human plasma. The 2 forms, pre pro BNP and NT pro BNP, and peptides which are derived from BNP, pre pro BNP and NT pro BNP and which are present in the blood as a result of proteolyses of BNP, NT pro BNP and pre pro BNP, are collectively described as markers related to or associated with BNP. Proteolytic degradation of BNP and of peptides related to BNP have also been described in the literature and these proteolytic fragments are also encompassed it the term "BNP related peptides". BNP and BNP-related peptides are predominantly found in the secretory granules of the cardiac ventricles, and are released from the heart in response to both ventricular volume expansion and pressure overload. Elevations of BNP are associated with raised atrial and pulmonary wedge pressures, reduced ventricular systolic and diastolic function, left ventricular hypertrophy, and myocardial infarction. Furthermore, there are numerous reports of elevated BNP concentration associated with congestive heart failure and renal failure. While BNP and BNP-related peptides are likely not specific for ACS, they may be sensitive markers of ACS because they may indicate not only cellular damage due to ischemia, but also a perturbation of the natriuretic system associated with ACS. The term "BNP" as used herein refers to the mature 32-amino acid BNP molecule itself. As the skilled artisan will recognize, however, other markers related to BNP may also serve as diagnostic or prognostic indicators in patients with ACS. For example, BNP is synthesized as a 108-amino acid pre pro-BNP molecule that is proteolytically processed into a 76-amino acid "NT pro BNP" and the 32- amino acid BNP molecule. Because of its relationship to BNP, the concentration of NT pro-BNP molecule can also provide diagnostic or prognostic information in patients. The phrase "marker related to BNP or BNP related peptide" refers to any polypeptide that originates from the pre pro-BNP molecule, other than the 32-amino acid BNP molecule itself. Thus, a marker related to or associated with BNP includes the NT pro-BNP molecule, the pro domain, a fragment of BNP that is smaller than the entire 32-amino acid sequence, a fragment of pre pro-BNP other than BNP, and a fragment of the pro domain.

Biomarker classes

MYG can be used as a biomarker useful for a disease comprised in a group of diseases consisting of cardiovascular diseases, Congestive Heart Failure, and metabolic diseases. Furthermore, MYG can be used as a biomarker useful for treatments comprised in a group of treatments consisting of of PPAR treatment and PPARa treatment.

A biomarker can be:

Disease Biomarker: a biomarker that relates to a clinical outcome or measure of disease.

Efficacy Biomarker: a biomarker that reflects beneficial effect of a given treatment.

Staging Biomarker: a biomarker that distinguishes between different stages of a chronic disorder.

Surrogate Biomarker: a biomarker that is regarded as a valid substitute for a clinical outcomes measure.

Toxicity Biomarker: a biomarker that reports a toxicological effect of a drug on an *in vitro* or *in vivo* system.

Mechanism Biomarker: a biomarker that reports a downstream effect of a drug.

And Target Biomarker: a biomarker that reports interaction of the drug with its target.

One preferred embodiment of the invention is a method of use of MYG as a biomarker for a PPAR modulator treatment comprising :

(a) obtaining a biological sample from a mammal,

(b) measuring the level of MYG in the biological sample,

(c) obtaining a control sample from a mammal,

(d) measuring the level of MYG in the control sample,

(e) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(f) diagnosing a PPAR modulator treatment based upon the MYG level of the biological sample in comparison to the control sample.

One preferred embodiment of the invention is a method of use of MYG as a biomarker for a PPAR modulator treatment comprising :

(a) measuring the level of MYG in a biological sample taken from a mammal,

(b) measuring the level of MYG in a control sample taken from a mammal,

(c) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(d) diagnosing a PPAR modulator treatment based upon the MYG level of the biological sample in comparison to the control sample.

One preferred embodiment of the invention is a method of use of MYG as a biomarker for a PPAR modulator treatment comprising :

(a) obtaining a biological sample from a mammal,

(b) measuring the level of MYG in the biological sample,

(c) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(d) diagnosing a PPAR modulator treatment based upon the MYG level of the biological sample in comparison to the control sample.

One preferred embodiment of the invention is a method of use of MYG as a biomarker for a PPAR modulator treatment comprising :

(a) measuring the level of MYG in a biological sample taken from a mammal,

(b) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(c) diagnosing a PPAR modulator treatment based upon the MYG level of the biological sample in comparison to the control sample.

The PPAR modulator of the methods is in a preferred embodiment a PPARa modulator. In a preferred embodiement of the invention the PPAR modulator can be useful in the treatment of for example cardiovascular diseases, Congestive Heart Failure, and metabolic diseases.

The biological sample in step (a) of the methods is in a preferred embodiment a biological sample taken from a mammal comprised in a group of samples consisting of a blood sample, a plasma sample, a serum sample, a tissue sample, a oral mucosa sample, a saliva sample, an interstitial fluid sample or an urine sample. The blood sample is for example a whole blood sample, a fractionated blood sample, a platelet sample, a neutrophil sample, a leukocyte sample, a white blood cell sample, a monocyte sample, a red blood cell sample, a granulocyte sample, and an erythrocyte sample. A tissue sample is for example a sample collected from muscle, adipose, heart or skin.

In a preferred embodiment MYG is used as a biomarker diagnosing a PPARa modulator treatment which is associated with altered MYG levels. In another preferred embodiment MYG is used as a biomarker for predicting an adverse outcome in a patient treated with a PPARa modulator.

Use of MYG as a mechanistic biomarker in diagnostics is based by the comparison of MYG level in a biological sample taken from a PPARa modulator treated mammal with the MYG level in a control sample taken from an untreated or placebo treated mammal , the mammal subject to treatment before treatment, a normal mammal or a group of untreated or normal mammals. Does the MYG level in the PPARa modulator treated mammal differs from the MYG level in a normal or untreated mammal then the treated mammal is diagnosed as a PPARa modulator treated patient associated with altered MYG level. Does

the MYG level in the biological sample differs from the MYG level in the control sample then the therapy or drug action can be considered as successful. Does the MYG level in the biological sample does not differ or differs only slightly from the MYG level in the control sample then the therapy can be considered as not successful. If the therapy is considered not successful increased dosages of the same therapy, repeat of the same therapy or an alternative treatment which is different from the first therapy can be considered. Additionally different routes of administration of the PPARa modulator can be compared. An alternative treatment can also be an investigational drug treatment. The method can be used to compare different investigational drugs.

A control sample can be a sample taken from a mammal. A control sample can be a previously taken sample from a mammal, as a MYG level in a control sample can be a predetermined level of MYG measured in a previously taken sample. The level of MYG in a control sample or in a biological sample can be determined for example as a relative value and as an absolute value. A previously measured MYG level from a control sample can be for example stored in a database, in an internet publication, in an electronically accessible form, in a publication. Comparing the level of MYG of a biological sample to a control sample may be comparing relative values or absolute quantified values.

Another embodiment is a method of use of MYG as a biomarker for guiding a therapy with a PPAR modulator comprising:

(a) obtaining a baseline level of MYG in a biological sample from a mammal,

(b) administering to a mammal a PPAR modulator treatment,

(c) obtaining one or more subsequent biological samples from the PPAR modulator treated mammal

(d) measuring the level of MYG in the one or more subsequent biological samples,

(e) comparing the level of MYG in the one or more subsequent biological samples with the baseline sample, and

(f) determining whether increased dosages, additional or alternative treatments are necessary based on MYG levels obtained from one or more subsequent biological samples compared to the baseline MYG level.

A method of use of MYG as a biomarker for guiding a therapy with a PPARa modulator treatment comprising :

(a) administering to a mammal a PPARa modulator treatment,

(b) obtaining one or more subsequent biological samples from the treated mammal

(c) measuring the level of MYG in the one or more subsequent biological samples,

(d) comparing the level of MYG in the one or more subsequent biological samples with the MYG baseline of untreated mammals, and

(e) determining whether increased dosages, additional or alternative treatments are necessary based on MYG levels obtained from one or more subsequent biological samples compared to the baseline MYG level.

Another embodiment is a method of use of MYG as a biomarker for guiding a therapy with a PPAR modulator comprising:

(a) obtaining a baseline level of MYG in biological sample taken from a mammal,

(b) obtaining one or more subsequent biological samples taken from the PPAR modulator treated mammal

(c) measuring the level of MYG in the one or more subsequent biological samples,

(d) comparing the level of MYG in the one or more subsequent biological samples with the baseline sample, and

(e) determining whether increased dosages, additional or alternative treatments are necessary based on MYG levels obtained from one or more subsequent biological samples compared to the baseline MYG level.

A method of use of MYG as a biomarker for guiding a therapy with a PPARa modulator treatment comprising :

(a) measuring the level of MYG in one or more subsequent biological samples taken from a mammal treated with a PPARa modulator,

(b) comparing the level of MYG in the one or more subsequent biological samples with the MYG baseline in samples taken from untreated mammals, and

(c) determining whether increased dosages, additional or alternative treatments are necessary based on MYG levels obtained from one or more subsequent biological samples compared to the baseline MYG level.

The PPAR modulator of the method is in a preferred embodiment a PPARa modulator. In a preferred embodiement of the invention the PPAR modulator can be useful in the treatment of for example cardiovascular diseases, Congestive Heart Failure, and metabolic diseases.

In a preferred embodiment MYG is used as a biomarker for guiding a PPARa modulator treatment which is associated with altered MYG levels.

Use of MYG as a efficacy, mechanistic or surrogate endpoint biomarker in diagnostics is based by the comparison of MYG level in a biological sample taken from a PPARa modulator treated mammal with the MYG level taken from said untreated mammal or with control samples taken from other untreated mammals. Does the MYG level in the baseline sample differs from the MYG level in the subsequent samples then the therapy or drug action can be considered as successful. Does the MYG level in the baseline sample does not differ or differs only slightly from the MYG level in the subsequent samples then the therapy can be considered as not successful. If the therapy is considered not successful increased dosages of the same therapy, repeat of the same therapy or an alternative treatment which is different from the first therapy can be considered. Additionally different routes of administration of the PPARa modulator can be compared. An alternative

treatment can also be an investigational drug treatment. The method can be used to compare different investigational drugs.

The biological sample in step (a) of the methods is in a preferred embodiment a biological sample taken from a mammal comprised in a group of samples consisting of a blood sample, a plasma sample, a serum sample, a tissue sample, a oral mucosa sample, a saliva sample, an interstitial fluid sample or an urine sample. The blood sample is for example a whole blood sample, a fractionated blood sample, a platelet sample, a neutrophil sample, a leukocyte sample, a white blood cell sample, a monocyte sample, a red blood cell sample, a granulocyte sample, and a erythrocyte sample. A tissue sample is for example a sample taken from muscle, adipose, heart, skin or a biopsy.

In a preferred embodiment the level of MYG is determined by determining the level of MYG polynucleotide.

In another preferred embodiment the level of MYG is determined by determining the level of MYG polypeptide.

In a further preferred embodiment the level of MYG is determined by determining the level of MYG activity.

In a preferred embodiment of the invention the mammal is a human.

In a preferred embodiment of the invention the level of MYG of the biological sample is elevated compared to the control sample. In a further preferred embodiment of the invention the level of MYG of the biological sample compared to the control sample is elevated at least by a factor of 1.5, 2, 2.5, 5, 7.5, 10, 15, 20, or >20.

Another embodiment of the present invention prefers the use of MYG in combination with the use of one or more biomarkers, more preferably with biomarkers used in diagnosing PPARa modulator treatment.

In a preferred embodiment of the invention the use of MYG is combined with the use of one or more biomarkers which are comprised in a group of biomarkers consisting of

MYH7, TNNT2, CKM, MYBPC3, TBX15, H19, SMPX, LDHB, PDK4, PYGM, CPT1A, MTE1, CTE1, UCP3 and FABP3.

In a further preferred embodiment the use of MYG is combined with the use of one or more clinical biomarkers which are comprised in a group of biomarkers consisting of blood pressure, heart rate, pulmonary artery pressure, systemic vascular resistance, blood glucose, or other clinical parameters.

In a further preferred embodiment the use of MYG is combined with the use of one or more biomarkers which are comprised in a group of biomarkers consisting of comprised in a group of biomarkers consisting of BNP, ANP, Troponin, CRP, Myoglobin, CK-MB or metabolites.

In a further preferred embodiment the use of MYG is combined with the use of one or more diagnostic imaging methods which are comprised in a group of methods consisting of PET (Positron Emission Tomography), CT (Computed Tomography), ultrasonic, SPECT (Single Photon Emission Computed Tomography), Echocardiography, or Impedance Cardiography.

In a further preferred embodiment the use of MYG is combined with the use of one or more diagnostic methods which are comprised in a group of methods consisting of PET (Positron Emission Tomography), CT (Computed Tomography), ultrasonic, SPECT (Single Photon Emission Computed Tomography), Echocardiography, Impedance Cardiography, blood pressure, heart rate, pulmonary artery pressure, or systemic vascular resistance.

In a further preferred embodiment the use of MYG is combined with the use of one or more diagnostic methods which are comprised in a group of methods consisting of PET (Positron Emission Tomography), CT (Computed Tomography), ultrasonic, SPECT (Single Photon Emission Computed Tomography), Echocardiography, Impedance Cardiography, blood pressure, heart rate, pulmonary artery pressure, systemic vascular resistance, BNP, ANP, Troponin, CRP, Myoglobin, CK-MB or metabolites.

In a further preferred embodiment the use of MYG is combined with the use of one or more diagnostic methods or biomarkers which are comprised in a group of methods or biomarkers consisting of PET (Positron Emission Tomography), CT (Computed Tomography), ultrasonic, SPECT (Single Photon Emission Computed Tomography), Echocardiography, Impedance Cardiography, blood pressure, heart rate, pulmonary artery pressure, systemic vascular resistance, BNP, ANP, Troponin, CRP, Myoglobin, CK-MB metabolites, MYH7, TNNT2, CKM, MYBPC3, TBX15, H19, SMPX, LDHB, PDK4, PYGM, CPT1A, MTE1, CTE1, UCP3 or FABP3.

In a further preferred embodiment the use of MYG is combined with the use of one or more diagnostic imaging methods in combination with one or more of the transcriptional markers which are comprised in a group of genes consisting of MYH7, TNNT2, CKM, MYBPC3, TBX15, H19, SMPX, LDHB, PDK4, PYGM, CPT1A, MTE1, CTE1, UCP3 and FABP3.

In a further preferred embodiment is a kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a PPAR modulator therapy in a patient with a disease, the kit comprising one ore more antibodies which specifically binds MYG, detection means, one or more containers for collecting and or holding the biological sample, and an instruction for its use.

Another preferred embodiment is a kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a PPAR modulator therapy in patients, the kit comprising one or more probes or primers for detecting MYG mRNA, detection means, one or more containers for collecting and or holding the biological sample, and an instruction for its use.

Another preferred embodiment is a kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a PPAR modulator therapy in patients, the kit comprising one or

more substrates for detecting MYG activity, detection means, one or more containers for collecting and or holding the biological sample, and an instruction for its use.

A preferred embodiement of the invention is a kit for identifying a disease, wherein the disease is associated with an altered MYG level.

A preferred embodiement of the invention is a kit for guiding a PPAR modulator therapy, wherein the PPAR modulator is a PPARa modulator.

Another embodiement of the invention is the use of MYG as a tool diagnosing a disease.

One embodiment of the invention is a method of use of MYG as a biomarker for a disease comprising :

(a) obtaining a biological sample taken from a mammal

(b) measuring the level of MYG in the biological sample,

(c) obtaining a control sample taken from a mammal

(d) measuring the level of MYG in the control,

(e) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(f) diagnosing a disease based upon the MYG level of the biological sample in comparison to the control sample.

One embodiment of the invention is a method of use of MYG as a biomarker for a disease comprising :

(a) measuring the level of MYG in a biological sample taken from a mammal,

(b) measuring the level of MYG in a control sample taken from a mammal,

(c) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(d) diagnosing a disease based upon the MYG level of the biological sample in comparison to the control sample.

Use of MYG as a biomarker in disease diagnostics is based by the comparison of MYG level in a biological sample from a diseased mammal with the MYG level in a control sample from a healthy or normal mammal. Does the MYG level in the diseased mammal differs from the MYG level in a normal or healthy mammal then the diseased mammal is diagnosed with a disease associated with an altered MYG level. Furthermore, comparing MYG levels of a biological sample from a diseased mammal with MYG levels of control samples from mammals with a MYG-associated disease already diagnosed with different stages or severity of said disease, allows the diagnose of a MYG-associated disease of said first diseased mammal and specifying the severity of the MYG-associated disease. The biological sample is taken from the analogue tissue or body fluid than the control sample.

The biological sample in step (a) of the methods is in a preferred embodiment a biological sample comprised in a group of samples consisting of a blood sample, a plasma sample, a serum sample, a tissue sample, a oral mucosa sample, a saliva sample, an interstitial fluid sample or an urine sample. The blood sample is for example a whole blood sample, a fractionated blood sample, a platelet sample, a neutrophil sample, a leukocyte sample, a white blood cell sample, a monocyte sample, a red blood cell sample, a granulocyte sample, and a erythrocyte sample. A tissue sample is for example a sample collected from muscle, adipose, heart or skin.

In a preferred embodiment MYG is used as a biomarker diagnosing a disease which is associated with altered MYG levels. Another preferred embodiment MYG is used as a biomarker for identifying an individual risk for developing a disease, or for predicting an adverse outcome in a patient diagnosed with a disease,

Use of MYG as a disease biomarker in diagnostics is based by the comparison of MYG level in a biological sample from a diseased mammal with the MYG level in a control sample from a healthy or normal mammal or a group of healthy or normal mammals. Does

the MYG level in the diseased mammal differs from the MYG level in a normal or healthy mammal then the diseased mammal is diagnosed with a disease associated with altered MYG level.

Furthermore, using MYG as a staging biomarker, the MYG levels of a diseased mammal are compared with MYG levels of a mammal with a MYG-associated disease already diagnosed with different stages or severity of said disease, allows the diagnose of said first diseased mammal specifying the severity of the MYG-associated disease.

A control sample can be a sample taken from a mammal. A control sample can be a previously taken sample from a mammal, as a MYG level in a control sample can be a predetermined level of MYG measured in a previously taken sample. The level of MYG in a control sample or in a biological sample can be determined for example as a relative value and as an absolute value. A previously measured MYG level from a control sample can be for example stored in a database, in an internet publication, in an electronically accessible form, in a publication. Comparing the level of MYG of a biological sample to a control sample may be comparing relative values or absolute quantified values.

Another embodiment is a method of use of MYG as a biomarker for guiding a therapy of a disease comprising:

(a) obtaining a baseline level of MYG in biological sample from a diseased mammal,

(b) administering to the diseased mammal a treatment for the disease,

(c) obtaining one or more subsequent biological samples from the diseased mammal

(d) measuring the level of MYG in the one or more subsequent biological samples,

(e) comparing the level of MYG in the one or more subsequent biological samples with the baseline sample, and

(f) determining whether increased dosages, additional or alternative treatments are necessary based on MYG levels obtained from one or more subsequent biological samples compared to the baseline MYG level.

Another embodiment is a method of use of MYG as a biomarker for guiding a therapy of a disease comprising:

(a) obtaining a baseline level of MYG in biological sample taken from a diseased mammal,

(b) obtaining one or more subsequent biological samples taken from the diseased mammal with subsequent treatment for the disease,

(c) measuring the level of MYG in the one or more subsequent biological samples,

(d) comparing the level of MYG in the one or more subsequent biological samples with the baseline sample, and

(e) determining whether increased dosages, additional or alternative treatments are necessary based on MYG levels obtained from one or more subsequent biological samples compared to the baseline MYG level.

In a preferred embodiment MYG is used as a biomarker for guiding a therapy in a disease which is associated with altered MYG levels.

Use of MYG as a disease, efficacy or surrogate endpoint biomarker in diagnostics is based by the comparison of MYG level in a biological sample from a diseased mammal before treatment (the baseline sample level) with the MYG level in subsequent samples from said mammal receiving a treatment for the disease. Does the MYG level in the baseline sample differs from the MYG level in the subsequent samples then the therapy can be considered as successful. Does the MYG level in the baseline sample does not differ or differs only slightly from the MYG level in the subsequent samples then the therapy can be considered as not successful. If the therapy is considered not successful increased dosages of the same

therapy, repeat of the same therapy or an alternative treatment which is different from the first therapy can be considered.

The biological sample in step (a) of the methods is in a preferred embodiment a biological sample comprised in a group of samples consisting of a blood sample, a plasma sample, a serum sample, a tissue sample, a oral mucosa sample, a saliva sample, an interstitial fluid sample or an urine sample. The blood sample is for example a whole blood sample, a fractionated blood sample, a platelet sample, a neutrophil sample, a leukocyte sample, a white blood cell sample, a monocyte sample, a red blood cell sample, a granulocyte sample, and a erythrocyte sample. A tissue sample is for example a sample collected from muscle, adipose, heart, skin or a biopsy.

In a preferred embodiment the level of MYG is determined by determining the level of MYG polynucleotide.

In another preferred embodiment the level of MYG is determined by determining the level of MYG polypeptide.

In a further preferred embodiment the level of MYG is determined by determining the level of MYG activity.

In a preferred embodiment of the invention the mammal is a human.

In a preferred embodiment of the invention the level of MYG of the biological sample is elevated compared to the control sample.

Another embodiment of the present invention prefers the use of MYG in combination with the use of one or more biomarkers, more preferably with biomarkers used in diagnosing MYG-associated diseases.

In a further preferred embodiment is a kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a therapy in a patient with a disease, the kit comprising one ore more antibodies which specifically binds MYG, detection means, one or more containers for collecting and or holding the biological sample, and an instruction for its use.

Another preferred embodiment is a kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a therapy in a patient with a disease, the kit comprising one or more probes or primers for detecting MYG mRNA, detection means, one or more containers for collecting and or holding the biological sample, and an instruction for its use.

Another preferred embodiment is a kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a therapy in a patient with a disease, the kit comprising one or more substrates for detecting MYG activity, detection means, one or more containers for collecting and or holding the biological sample, and an instruction for its use.

In a preferred embodiement of the invention the MYG can be useful in the diagnosis of for example cardiovascular diseases, Congestive Heart Failure, and metabolic diseases.

Examples

Example 1

Expression Analysis

Total cellular RNA was isolated from cells or tissues by TRIZOL-Reagent protocol according to the manufacturer's specifications (Molecular Research Center, Inc., Cincinatti, Ohio). Total RNA was treated with DNAse I to remove genomic DNA contamination according to the manufacturer's protocol (Invitrogen Corporation, Carlsbad, California) .

For relative quantitation of the mRNA distribution of MYG, total RNA from each tissue was first reverse transcribed. 1 mg of total RNA was reverse transcribed using 6,25 mM Oligo(dT)15 primer, 0.5 mM each of dATP, dCTP, dGTP and dTTP (Promega Corporation, Madison, Wisconsin), RNaseQut (Invitrogen, Groningen, Netherlands) in a final volume of 52,8 ml. The first strand synthesis is done by the ImProm-II System according to the manufacturer's protocol (Promega Corporation, Madison, Wisconsin). The reaction was incubated at 42°C for 60 minutes and cooled on ice. The volume was adjusted to 200 ml with water.

For relative quantitation of the distribution of MYG mRNA in cells and tissues the Applied Bioscience 7900HT Sequence Detection system was used according to the manufacturer's specifications and protocols. PCR reactions were set up to quantitate MYG and the housekeeping gene L32. Forward and reverse primers and probes for MYG were designed using the Applied Bioscience ABI Primer ExpressTM software and were synthesized by Eurogentec (Belgium). The MYG forward primer sequence was: Primer1 (SEQ ID NO: 3). The MYG reverse primer sequence was Primer2 (SEQ ID NO: 4). Probe1 (SEQ ID NO: 5), labelled with FAM (carboxyfluorescein succinimidyl ester) as the reporter dye and TAMRA (carboxytetramethylrhodamine) as the quencher, is used as a probe for MYG. The following reagents were prepared in a total of 20 ml : 1x qPCR-MasterMix (Eurogentec; Belgium) and Probe1 (SEQ ID NO: 5), MYG forward and reverse primers each at 200 nM, 200 nM MYG FAM/TAMRA-labelled probe, and 5 ml of template cDNA. Thermal cycling parameters were 10 min at 95°C, followed by 40 cycles of melting at 95°C for 15 sec and annealing/extending at 60°C for 1 min. The CT (threshold cycle) value is

calculated as described in the "Quantitative determination of nucleic acids" section. Relative expression of MYG was calculated using the normalized expression values through the house keeping gene L32.

Example 2

Production of MYG Specific Antibodies

Two approaches are utilized to raise antibodies to MYG, and each approach is useful for generating either polyclonal or monoclonal antibodies. In one approach, denatured protein from reverse phase HPLC separation is obtained in quantities up to 75 mg. This denatured protein is used to immunize mice or rabbits using standard protocols; about 100 μg are adequate for immunization of a mouse, while up to 1 mg might be used to immunize a rabbit. For identifying mouse hybridomas, the denatured protein is radioiodinated and used to screen potential murine B-cell hybridomas for those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg is sufficient for labeling and screening of several thousand clones.

In the second approach, the amino acid sequence of an appropriate MYG domain, as deduced from translation of the cDNA, is analyzed to determine regions of high antigenicity. Oligopeptides comprising appropriate hydrophilic regions are synthesized and used in suitable immunization protocols to raise antibodies. The optimal amino acid sequences for immunization are usually at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the protein is in its natural conformation.

Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH; Sigma, St. Louis, MO) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester, MBS. If necessary, a cysteine is introduced at the N-terminus of the peptide to permit coupling to KLH. Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% bovine serum albumin, reacting

with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas are prepared and screened using standard techniques. Hybridomas of interest are detected by screening with labeled MYG to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto, CA) are coated during incubation with affinity purified, specific rabbit anti-mouse (or suitable antispecies 1 g) antibodies at 10 mg/ml. The coated wells are blocked with 1% bovine serum albumin, (BSA), washed and incubated with supernatants from hybridomas. After washing the wells are incubated with labeled MYG at 1 mg/ml. Supernatants with specific antibodies bind more labeled MYG than is detectable in the background. Then clones producing specific antibodies are expanded and subjected to two cycles of cloning at limiting dilution. Cloned hybridomas are injected into pristane-treated mice to produce ascites, and monoclonal antibody is purified from mouse ascitic fluid by affinity chromatography on Protein A. Monoclonal antibodies with affinities of at least 10^8 M^{-1}, preferably 10^9 to 10^{10} M^{-1} or stronger, are typically made by standard procedures.

Example 3

Diagnostic Test Using MYG Specific Antibodies

Particular MYG antibodies are useful for investigating signal transduction and the diagnosis of infectious or hereditary conditions which are characterized by differences in the amount or distribution of MYG or downstream products of an active signaling cascade.

Diagnostic tests for MYG include methods utilizing antibody and a label to detect MYG in human body fluids, membranes, cells, tissues or extracts of such. The polypeptides and antibodies of the present invention are used with or without modification. Frequently, the polypeptides and antibodies are labeled by joining them, either covalently or noncovalently, with a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes,

substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, chromogenic agents, magnetic particles and the like.

A variety of protocols for measuring soluble or membrane-bound MYG, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on MYG is preferred, but a competitive binding assay may be employed.

Example 4

Identification of Other Members of the Signal Transduction Complex

Labeled MYG is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, MYG is covalently coupled to a chromatography column. Cell-free extract derived from synovial cells or putative target cells is passed over the column, and molecules with appropriate affinity bind to MYG. MYG-complex is recovered from the column, and the MYG-binding ligand disassociated and subjected to N-terminal protein sequencing. The amino acid sequence information is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

In an alternate method, antibodies are raised against MYG, specifically monoclonal antibodies. The monoclonal antibodies are screened to identify those which inhibit the binding of labeled MYG. These monoclonal antibodies are then used therapeutically.

Example 5

Microarray experiments

Total RNA extracted from cardiac tissue and was purified using an affinity resin column (RNeasy; Qiagen, Hilden, Germany), quantified by spectrophotometry (absorbance 260 nm), and the quality of RNA was assessed by microfluidics electrophoretical separation with a Bioanalyzer (Agilent Technologies, Palo Alto, USA). Purified total RNA (1 μg) was converted to cDNA using the Superscript Choice cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA), incorporating a T7-(dT)24 primer. Double-stranded cDNA was then purified by affinity resin column (Clean up Kit, Qiagen, Hilden, Germany) with ethanol extraction. Purified cDNA was used as a template for in vitro transcription reaction for the synthesis of biotinylated cRNA using an Enzo BioArray

HighYield RNA transcription labeling kit (Affymetrix, Santa Clara, CA), and further purified using an affinity resin column (Clean up Kit, Qiagen, Hilden, Germany). After purification, in vitro cRNA was fragmented in buffer containing magnesium at 95°C for 35 min. Fragmented cRNA was hybridized onto the Affymetrix GeneChip Rat Genome 230 2.0 Array. Briefly, 15 µg fragmented cRNA was added along with control cRNA (BioB, BioC, and BioD), herring sperm DNA (10 mg/ml), 10% DMSO, and acetylated BSA (50 mg/ml) to the hybridization buffer. The hybridization mixture was heated at 99°C for 5 min, incubated at 45°C for 5 min, centrifuged for 5 min at 13.000 rpm, and injected into the microarray. After hybridization at 45°C for 16 h rotating at 60 rpm, the array was washed and stained with the Affymetrix Fluidics Protocols-antibody amplification for Eukaryotic Targets, and scanned using an Affymetrix microarray scanner (GeneChip Scanner 3000 7G system) at 570 nm.

Example 6

Treatment of animals with PPARa modulators

For the experiments 7 to 8 weeks old male Spraque Dawley rats with body weights between 200 and 220 g were used. The animals were housed at 22°C with a 12 h light/dark cycle with free access to tap water and a standard pellet diet. PPARa modulators were administrated either by an intraperitoneal application or via drinking water. In both cases, concentrations of modulators were 0,15 mg/kg, 0,5 mg/kg, 1,5 mg/kg and 4 mg/kg, respectively. Animals were treated for 10 days. Thereby, the intraperitoneal application was done once daily. For each concentration, 5 animals were randomly allocated into suitable groups. As control, animals received drinking water only. Body weights were recorded at the beginning and at the end of the study.

Example 7

Tissue sampling

At the end of the study, animals were humanely killed by Isofluran inhalation. Blood samples were taken by heart punctuation using EDTA-S-Monovette blood collection systems. Hearts, liver, and fatty tissue was excised and weighed. Organs were freezed in liquid nitrogen immediately after excise. Oral mucosa probes were taken by swabbing the buccal mucosa with a sterile spattel. The probes were freezed in liquid nitrogen.

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Claims

1. A method of use of MYG as a biomarker for a PPAR modulator treatment comprising :

(a) obtaining a biological sample from a mammal,

(b) measuring the level of MYG in the biological sample,

(c) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(d) diagnosing a PPAR modulator treatment based upon the MYG level of the biological sample in comparison to the control sample.

2. A method of use of MYG as a biomarker for a PPAR modulator treatment comprising :

(a) measuring the level of MYG in a biological sample taken from a mammal,

(b) comparing the level of MYG in the biological sample with the level of MYG in a control sample, and

(c) diagnosing a PPAR modulator treatment based upon the MYG level of the biological sample in comparison to the control sample.

3. A method of claims 1 and 2 wherein the PPAR modulator treatment is a PPARa modulator treatment.

4. A method of claims 1 and 2 wherein the biological sample is comprised in a group of samples consisting of a blood sample, a plasma sample, a serum sample, a tissue sample, an oral mucosa sample, a saliva sample, an interstitial fluid sample or an urine sample.

5. A method of claims 1 and 2 wherein the level of MYG is determined by determining the level of MYG polynucleotide.

6. A method of claims 1 and 2 wherein the level of MYG is determined by determining the level of MYG polypeptide.

7. A method of claims 1 and 2 wherein the level of MYG is determined by determining the level of MYG activity.

8. A method of claims 1 and 2 wherein the mammal is a human.

9. A method of claims 1 and 2 wherein the level of MYG of the biological sample is elevated compared to the control sample.

10. A method of claims 1 and 2 wherein the use of MYG is combined with the use of one or more biomarkers.

11. A method of claims 1 and 2 wherein the use of MYG is combined with the use of one or more biomarkers which are comprised in a group of biomarkers consisting of MYH7, TNNT2, CKM, MYBPC3, TBX15, H19, SMPX, LDHB, PDK4, PYGM, CPT1A, MTE1, CTE1, UCP3 and FABP3.

12. A method of claims claims 1 and 2 wherein the use of MYG is combined with the use of one or more biomarkers which are comprised in a group of biomarkers consisting of BNP, ANP, Troponin, CRP, Myoglobin, CK-MB or metabolites.

13. A method of claims 1 and 2 wherein the use of MYG is combined with the use of one or more clinical biomarkers which are comprised in a group of biomarkers consisting of blood pressure, heart rate, pulmonary artery pressure, or systemic vascular resistance.

14. A method of claims 1 and 2 wherein the use of MYG is combined with the use of one or more diagnostic imaging methods which are comprised in a group of methods consisting of PET (Positron Emission Tomography), CT (Computed Tomography), ultrasonic, SPECT (Single Photon Emission Computed Tomography), Echocardiography, or Impedance Cardiography.

15. A kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a therapy in a patient with a disease, the kit comprising one ore more antibodies which specifically binds MYG, one or more containers for collecting and or holding the biological sample.

16. A kit for identifying an individual risk for developing a disease, for predicting a disease or an adverse outcome in a patient diagnosed with a disease, or for guiding a therapy in a patient with a disease, the kit comprising one or more probes or primers for detecting MYG mRNA, one or more containers for collecting and or holding the biological sample.

17. Use of epithelial tissue for the identification of biomarkers for guiding a therapy with nuclear hormone receptor modulators.

18. A method of claim 17 wherein the epithelial tissue is oral mucosa.

19. A method of claim 17 and claim 18 wherein a nuclear hormone receptor is a PPAR.

20. A method of claim 17 and claim 18 wherein a nuclear hormone receptor is a PPARa.

21. Use of transcriptional expression analysis for the identification of biomarkers for guiding a therapy with nuclear hormone receptor modulators.

22. Use of a biomarker for guiding a therapy with nuclear hormone receptor modulators, wherein the level of the biomarker is determined by transcriptional expression analysis.

23. A method of claim 21 and claim 22 wherein a nuclear hormone receptor is a PPAR.

24. A method of claim 21 and claim 22 wherein a nuclear hormone receptor is a PPARa.

Figures

Fig. 1

SEQ ID NO: 1

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Fig. 2

SEQ ID NO: 2

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Fig. 3

SEQ ID NO: 3

ACGCCACCAAGCACAAGA

Fig. 4

SEQ ID NO: 4

TCTTCAGGACTTGGATGATGACT

Fig. 5

SEQ ID NO: 5

CCCGGTCAAGTACCTGGAGTTTATCTCA

Fig. 6

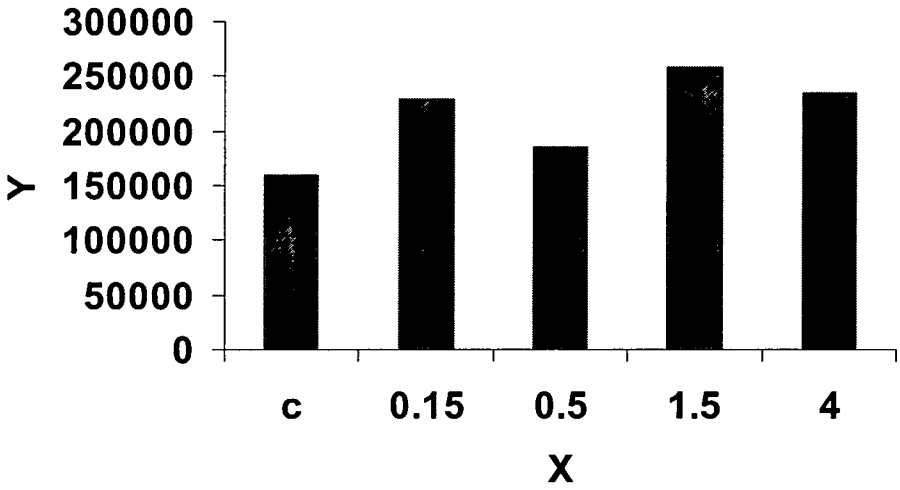

Fig. 7

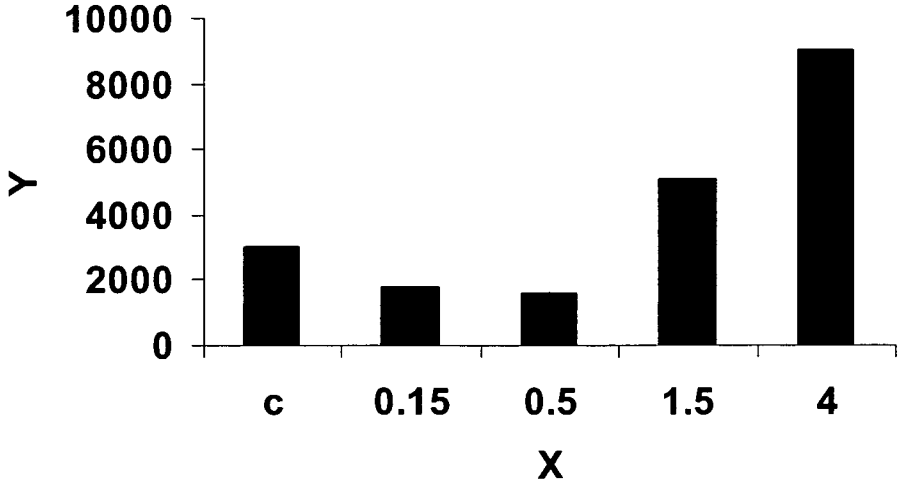
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Fig. 8

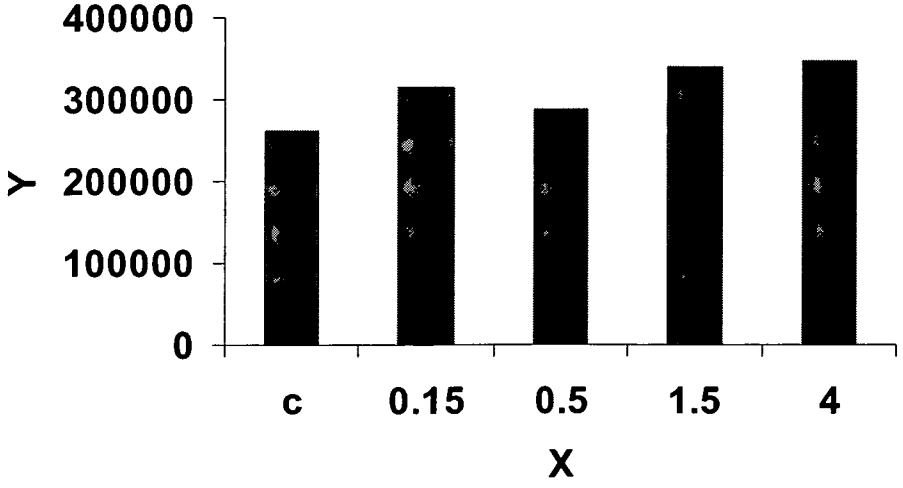
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Fig. 9

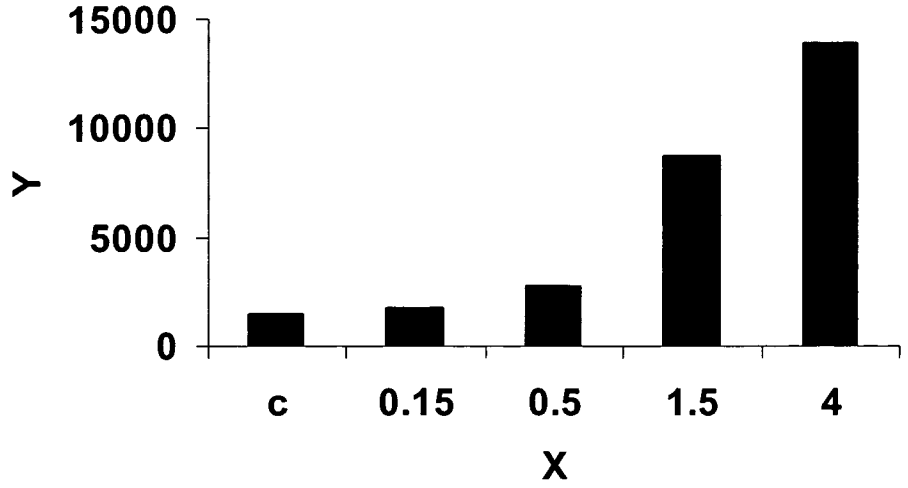

Fig. 10

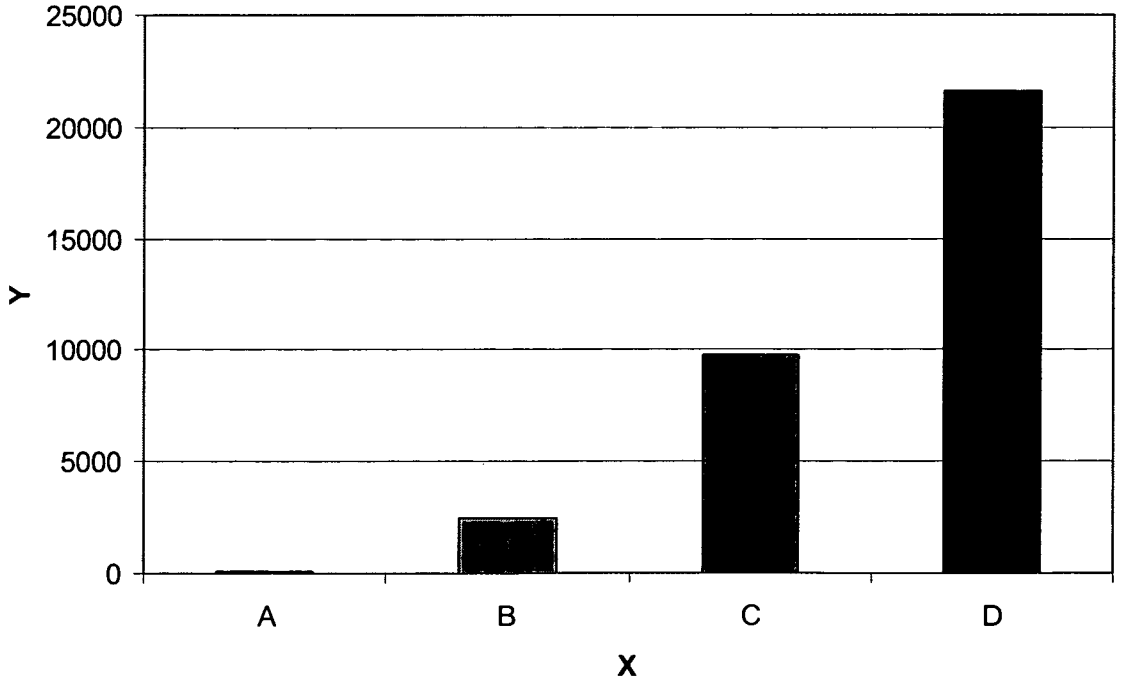

Fig. 11

SEQ ID NO: 6

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CGAGATGAAGGCATCTGAGGACTTAAAGAAGCATGGTGCCACTGTGCTCACCGCCCTG
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Fig. 12

SEQ ID NO: 7

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/001190

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/32 C12Q1/68

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

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, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WAY J M ET AL: "Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene expression in multiple insulin-sensitive tissues" 20010301; 20010300, vol. 142, no. 3, 1 March 2001 (2001-03-01), pages 1269-1277, XP002477799 table 2	1-4

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

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

Date of the actual completion of the international search	Date of mailing of the international search report
6 May 2008	25/06/2008
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Rosin, Oliver

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/001190

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PIERNO S ET AL: "Effects of chronic treatment with statins and fenofibrate on rat skeletal muscle: a biochemical, histological and electrophysiological study." BRITISH JOURNAL OF PHARMACOLOGY DEC 2006, vol. 149, no. 7, December 2006 (2006-12), pages 909-919, XP002479294 ISSN: 0007-1188 table 2	1-6
X	LEDL M ET AL: "Acute myopathy in a type 2 diabetic patient on combination therapy with metformin, fenofibrate and rosiglitazone" DIABETOLOGIA ; CLINICAL AND EXPERIMENTAL DIABETES AND METABOLISM, SPRINGER-VERLAG, BE, vol. 48, no. 10, 1 October 2005 (2005-10-01), pages 1996-1998, XP019322345 ISSN: 1432-0428 abstract	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/001190

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see annex

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-16

   Methods and kits using MYG as a marker.

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2. claims: 17-24

   Uses involving biomarkers for guiding a therapy with nuclear hormone receptor modulators.