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(71) Applicants (for all designated States except US): **THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPT. OF HEALTH AND HUMAN SERVICES** [US/US]; National Institutes of Health, Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, MD 20852-7660 (US). **LABORATOIRE HRA PHARMA** [FR/FR]; 15, rue Béranger, F-75003 Paris (FR). **ERASMUS UNIVERSITY MEDICAL CENTER ROTTERDAM** [NL/NL]; 'sGravendijkwal 230, NL-3015 CE Rotterdam (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **NIEMAN, Lynnette, K.** [US/US]; 8220 Hamilton Spring Ct., Bethesda, MD 20817 (US). **ULMANN, André** [FR/FR]; 23, rue Pascal, F-75005 Paris (FR). **FEELDERS, Richard** [NL/NL]; Breitnersingel 29, NL-3055 BA Rotterdam (NL).

(74) Agent: **SIEGEL, Susan, Alpert**; Klarquist Sparkman, LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, OR 97204 (US).

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(54) Title: COMPOSITIONS FOR AND METHODS OF TREATMENT AND ENHANCED DETECTION OF NON-PITUITARY TUMORS

(57) Abstract: Methods of detecting adrenocorticotropin-secreting non-pituitary tumor are disclosed. The methods include administering an inhibitor of glucocorticoid receptor activity, such as mifepristone. Administering the inhibitor of glucocorticoid receptor activity increases the expression of somatostatin receptors in the adrenocorticotropin-secreting non-pituitary tumor. After administration of the inhibitor of glucocorticoid receptor activity, the subject is further administered an agent that specifically binds a somatostatin receptor, such as somatostatin receptor 2. The agent bound to the somatostatin receptor is detected, which detects the adrenocorticotropin-secreting non-pituitary tumor in the subject. Methods of treating or inhibiting, or both, an adrenocorticotropin-secreting non-pituitary tumor in a subject are disclosed. The methods include administering to the subject and the inhibitor of glucocorticoid receptor activity. After administration of the inhibitor of glucocorticoid receptor activity, the subject is administered a cytotoxic agent that specifically binds the somatostatin receptor.



COMPOSITIONS FOR AND METHODS OF TREATMENT AND ENHANCED DETECTION OF NON-PITUITARY TUMORS

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This claims the benefit of U.S. Provisional Application No. 61/533,664, filed
September 12, 2011, which is incorporated by reference herein.

FIELD OF THE DISCLOSURE

- 10 This disclosure relates to the field of cancer and specifically to methods of
imaging and treating adrenocorticotrophic hormone secreting tumors.

PARTIES TO JOINT RESEARCH AGREEMENT

- 15 This invention was made under Public Health Service Cooperative Research
and Development Agreement (PHS-CRADA) No. 02241, between the National
Institutes of Health National Cancer Institute and Laboratoire HRA Pharma Corp.

BACKGROUND

- 20 Cushing syndrome is an endocrine disorder caused by excessive production
of glucocorticoids. In 10%-20% of patients this syndrome results from ectopic
production of adrenocorticotrophic hormone (ACTH) and very rarely by ectopic
corticotropin-releasing hormone (CRH) production. Small cell carcinoma of the
lung, islet cell tumor of the pancreas, medullary thyroid cancer, pheochromocytoma,
and foregut carcinoid tumors are the most common sources of ectopic ACTH
production. The mean age at diagnosis in patients with ectopic ACTH production is
25 40 years and the male-to-female ratio is almost 1:1.

- 30 However, in about 50% of subjects with Cushing syndrome, the ectopic
source of ACTH production cannot be found despite aggressive, extensive, repeated
and expensive testing. These subjects are at high risk of death due to sepsis,
hypertension, gastrointestinal pathology, and metastatic dissemination. More than
50% of patients with ectopic ACTH production cannot be treated surgically or fail
medical therapy and therefore, they finally undergo adrenalectomy with life-long
hormone replacement therapy. However, if the source of ectopic ACTH production

is found before the tumor metastasizes, resection is usually curative. Thus, the ability to image primary or metastatic lesions, or both, has proved to be the key to successful treatment. Conventional imaging is generally performed with X-ray computed tomography (CT) and magnetic resonance imaging (MRI) of the chest and abdomen. ¹²³I-metaiodobenzylguanidine (MIBG) is taken up by adrenal tissue and pheochromocytomas and may be useful in identifying this source of ectopic ACTH secretion; however, other modalities, including somatostatin receptor scintigraphy, also are potentially more useful in the localization of these tumors.

Somatostatin receptor scintigraphy using [¹¹¹In-diethylene triamine penta-acetic acid (DTPA)-D-Phe]-pentetreotide (OCTREOSCAN™, OCT), an analog of somatostatin, can identify gastroenteropancreatic tumors, carcinoids, and medullary thyroid carcinomas, all of which express somatostatin receptors; however, results with somatostatin receptor scintigraphy have been disappointing when the conventional dose of 6 mCi of [¹¹¹In-DTPA-D-Phe]-pentetreotide has been used. The ability of scintigraphic studies to detect lesions depends not only on the size of the lesion, but also on the amount of radioactivity in that lesion. Thus, a higher dose of radiopharmaceutical may lead to higher concentrations in a lesion and better visualization by scintigraphy. Indeed, in thyroid cancer, it is known that higher doses of radioactive ¹³¹I can detect more lesions than lower doses. Furthermore, investigators using high doses of [¹¹¹In-DTPA-D-Phe]-pentetreotide for therapy (180 mCi) have observed more lesions on patients' post therapy scans as compared to their diagnostic scans; however, a significant drawback of higher dose administration of radiopharmaceutical is an increased exposure of the subject to radioactivity, which can lead to the development of unintended disorders.

SUMMARY OF THE DISCLOSURE

It is disclosed herein that increasing the concentration of imaging agent at the tumor site—by increasing the expression of somatostatin receptors in the adrenocorticotropin-secreting non-pituitary tumor—without increasing the dose of the imaging agent, improves rates of tumor identification in patients with functional hypercortisolism. Disclosed are methods of detecting adrenocorticotropin-secreting non-pituitary tumors. The disclosed methods include administering an inhibitor of

glucocorticoid receptor activity, such as, but not limited to, mifepristone. Administering the inhibitor of glucocorticoid receptor activity increases the expression of somatostatin receptors in the adrenocorticotropin-secreting non-pituitary tumor. After administration of the inhibitor of glucocorticoid receptor activity, the subject is further administered an agent that specifically binds a somatostatin receptor, such as somatostatin receptor 2. The agent bound to the somatostatin receptor is detected, which detects the adrenocorticotropin-secreting non-pituitary tumor in the subject. In specific examples, the non-pituitary tumor is a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary thyroid carcinoma. In some examples, the agent that specifically binds the somatostatin receptor is somatostatin, a somatostatin analog, or an antibody that specifically binds the somatostatin receptor. In specific examples, a somatostatin analog is [¹¹¹In-DTPA-D-Phe]-pentetreotide.

Also disclosed are methods of treating or inhibiting, or both, an adrenocorticotropin-secreting non-pituitary tumor in a subject. The methods include administering to the subject an inhibitor of glucocorticoid receptor activity. After administration of the inhibitor of glucocorticoid receptor activity, the subject is administered a cytotoxic agent that specifically binds the somatostatin receptor. In some examples, a subject is selected for treatment that has or is diagnosed with an adrenocorticotropin-secreting non-pituitary tumor.

The foregoing and other features and advantages of the disclosure will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1E are a set of digital images of [¹¹¹In-DTPA⁰]octreotide and CT imaging results in Patient 1 before (**FIGS. 1A-1B**) and after 6 months of therapy with mifepristone (**FIGS. 1C-1E**). Before therapy was initiated, CT scan (**FIG. 1A**) shows a small round nodule in the right upper lung (white arrow), which is not

visible at the [^{111}In -DTPA ^{0}]octreotide scan (**FIG. 1B**). After 6 months of therapy, the CT scan shows the same lesion (white arrow) within the upper lobe of the right lung (**FIG. 1C**). At that time a repeat [^{111}In -DTPA ^{0}]octreotide scan shows pathological uptake at the site of the lesions (**FIG. 1D** and **FIG. 1E**; black arrows).

- 5 This result demonstrates that treatment of a subject with mifepristone enhances the detection of ACTH secreting tumors.

FIGS. 2A-2D are a set of digital images of [^{111}In -DTPA ^{0}]octreotide and CT imaging results in Patient 2 before (**FIGS. 2A-2B**) and after 12 months of therapy with mifepristone (**FIGS. 2C-2D**). Before therapy was initiated, CT scan (**FIG. 2A**) shows three contiguous nodules in the right middle lung, which were originally not appreciated (white arrow), and were not visible at the [^{111}In -DTPA ^{0}]octreotide scan (**FIG. 2B**). After 12 months of therapy, the CT scan shows the same contiguous nodules in the right middle lung (**FIG. 2C**). At that time a repeat [^{111}In -DTPA ^{0}]octreotide scan shows pathological uptake at the site of these nodules (**FIG. 2D**, black arrow).

FIG. 3 is a bar graph showing somatostatin and dopamine receptor mRNA subtype expression. Values represent the mean $\pm$ Standard Error of the Mean (SEM) of two duplicate measurements. Expression levels are normalized against the housekeeping gene hprt.

20 **FIG. 4** is a bar graph showing inhibition of ACTH release by cultured carcinoid tumor cells of this patient after 96 hr. Cells were cultured in the absence (control, CT) or presence of 10 nM octreotide (OCT), 10 nM cabergoline (CAB) or their combination (OCT+CAB). At the end of the incubation time, media were collected and ACTH levels were determined. All experimental conditions were performed in quadruplicate. Values represent percent change $\pm$ SEM relative to control (= untreated cells).

FIGS. 5A-5E are digital images of immunohistochemistry for the sst₂ and D₂ receptor in the primary carcinoid tissue of a patient. All images were taken at a magnification of 100x. **FIG. 5A** is a digital image of hematoxylin and eosin stain. **FIG. 5B** is a digital image of a negative control (omission of the primary sst₂ antibody). **FIG. 5C** is a digital image of a staining with a sst₂ polyclonal antibody. **FIG. 5D** is a digital image of a staining with a sst₂ antibody after neutralization with

an immunizing sst₂ receptor peptide. **FIG. 5E** is a digital image of a staining with a D₂ monoclonal antibody.

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

5 I. Introduction

Ectopic adrenocorticotropin (ACTH) secretion (EAS) by a non-pituitary tumor is an uncommon cause of ACTH-dependent Cushing's syndrome. It is most frequently caused by either a bronchial carcinoid or a small-cell lung carcinoma, which accounts for approximately 50% of all cases. Thymic carcinoids, gastro-
10 entero-pancreatic neuro-endocrine tumors, and medullary thyroid carcinoma are also known to cause this syndrome. The overall prognosis of the patient is largely determined by the nature of the underlying malignancy and the tumor stage at the time of diagnosis. Thus, early detection of these tumors is an important step in early treatment and favorable outcome of subjects diagnosed with such tumors.

15 Surgical removal of the tumor is the primary treatment of EAS. However in some patients the tumor cannot be identified by routine imaging procedures, including ultrasound, X-ray computed tomography (CT) and Magnetic resonance imaging (MRI). The fact that a significant proportion of these tumors express somatostatin receptors (sst) enables the use of sst-scintigraphy with ¹¹¹In-Pentetreotide (OCTREOSCAN™) and ¹¹¹In-DTPA-D-Phe]-pentetreotide to localize the tumors.
20 The expression of sst also has therapeutic purposes because a significant proportion of EAS patients responds to treatment with traditional somatostatin analogues such as octreotide.

Unfortunately, OCTREOSCAN™ does not detect all EAS tumors. In one
25 large series, OCTREOSCAN™ imaging had a sensitivity of only 49 %. In addition, an important subset of EAS patients does not show any clinical or biochemical response to traditional somatostatin analogues such as octreotide that mainly target somatostatin receptor subtype 2 (sst₂).

Many tumors express up to five different receptors for somatostatin
30 (somatostatin receptors 1-5 (sst₁₋₅)) on their surface. This expression enables somatostatin and its analogs (such as synthetic analogs) to bind to the surface of the tumor cells. When a compound such as somatostatin or an analog, is conjugated to a

label or cytotoxin, for example with a radioactive label or cytotoxin, cells to which the compound binds can be imaged, for example to detect a tumor cell, or killed, for example to treat the tumor and, thus, treat the cancer; however, some tumors do not express high levels of somatostatin receptors, such as somatostatin receptor 2 (sst₂).

5 Thus, the treatment of and imaging of such tumors is less successful. There is a high variability in the expression of sst₂ among differing tumor types. Thus, methods of increasing the expression of somatostatin receptors, and in particular sst₂, would lead to better treatment outcomes, for example, by increasing the effectiveness of treatment with cytotoxic somatostatin analogs, and earlier detection of tumors, for
10 example, by imaging tumors at an earlier stage in the disease.

As disclosed herein, high cortisol levels, is seen in subjects suffering from Cushing's syndrome, cause a decrease in the expression level of sst₂, which is the primary binding site (along with sst₅) for somatostatin analogs, such as [¹¹¹In-DTPA-D-Phe]-pentetreotide. [¹¹¹In-DTPA-D-Phe]-pentetreotide is a somatostatin
15 analog used to image sst₂ expressing tumors. As disclosed herein, treatment of a subject with an anti-corticoid agent (such as an antagonist of the glucocorticoid receptor; for example, mifepristone) reduces the biological action of cortisol, which in turn increases the expression of sst₂ in tumors. Increased expression of sst₂ can be utilized for imaging tumors with a labeled somatostatin analog or for treatment of a
20 sst₂-expressing tumor, for example with a cytotoxic somatostatin analog. Thus, disclosed herein are methods of enhancing the detection of a tumor by suppression of cortisol activity. Also disclosed herein are methods of treating a tumor with a suppressor of corticoid activity in combination with a cytotoxic somatostatin analog or increasing the effectiveness of a treatment of a tumor with a cytotoxic
25 somatostatin analog using a suppressor of cortisol activity, or both.

II. *Summary of Terms*

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in
30 Benjamin Lewin, *Genes IX*, published by Jones and Bartlet, 2008 (ISBN 0763752223); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0632021829); and Robert A.

Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 9780471185710).

The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. The term “comprises” means “includes.”

- 5 Similarly, including “A” or “B” refers to including “A,” “B,” and “A and B.” In case of conflict, the present specification, including explanations of terms, will control.

To facilitate review of the various embodiments of this disclosure, the following explanations of terms are provided:

- Administration:** The introduction of a composition into a subject by a
10 chosen route. For example, if the chosen route is intravenous, the composition, such as a suppressor of glucocorticoid receptor activity, (for example a glucocorticoid receptor antagonist), is administered by introducing the composition into a vein of the subject. In some examples, a suppressor of glucocorticoid receptor activity is administered to a subject, for example a subject with cancer, such as a solid tumor.

- 15 **Adrenocorticotrophic hormone (ACTH), or corticotropin:** A polypeptide tropic hormone typically produced and secreted by the anterior pituitary gland. In some examples it is produced and secreted by non-pituitary tumors. Its principal effects are increased production and release of corticosteroids and cortisol from the adrenal cortex.

- 20 **Agent:** Any substance or any combination of substances that is useful for achieving an end or result, for example, a substance useful for increasing the expression of ssr_2 in tumor in a subject. Agents include effector molecules and detectable markers. In some embodiments, the agent is a detectable marker, chemotherapeutic agent, or toxin. The skilled artisan will understand that particular
25 agents may be useful to achieve more than one result; for example, an agent may be useful as both a detectable marker and an anti-tumor agent.

- Animal:** Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term “subject” includes both human and veterinary
30 subjects. In some examples, a subject is a subject suffering from cancer, such as a solid tumor.

Antibody: A polypeptide ligand comprising at least a light chain or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen or a fragment thereof, for example an epitope on a glucocorticoid receptor or a somatostatin receptor, such as somatostatin receptor 2 (sst₂). Antibodies can include a heavy and a light chain, each of which has a variable region, termed the variable heavy (V_H) region and the variable light (V_L) region. Together, the V_H region and the V_L region are responsible for binding the antigen recognized by the antibody.

The term antibody includes intact immunoglobulins and the variants and portions of them well known in the art, such as Fab' fragments, F(ab')₂ fragments, single chain Fv proteins ("scFv"), and disulfide stabilized Fv proteins ("dsFv"). A scFv protein is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an immunoglobulin are bound by a linker, while in dsFvs, the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. The term also includes genetically engineered forms such as chimeric antibodies (for example, humanized antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, *Pierce Catalog and Handbook*, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

Typically, a naturally occurring immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region and a variable region, (the regions are also known as "domains"). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs". The extent of the framework region and CDRs have been defined (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human

Services, 1991, which is hereby incorporated by reference). The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. An antibody that binds an antigen of interest has a specific V_H region and the V_L region sequence, and thus specific CDR sequences. Antibodies with different specificities (due to different combining sites for different antigens) have different CDRs. Although it is the CDRs that vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. These positions within the CDRs are called specificity determining residues (SDRs).

References to " V_H " or " VH " refer to the variable region of an immunoglobulin heavy chain, including that of an Fv, scFv, dsFv or Fab. References to " V_L " or " VL " refer to the variable region of an immunoglobulin light chain, including that of an Fv, scFv, dsFv or Fab.

A "monoclonal antibody" is an antibody produced by a single clone of B-lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected, or a progeny thereof. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. Monoclonal antibodies include humanized monoclonal antibodies.

Antigen: A compound, composition, or substance that can stimulate the production of antibodies or a T-cell response in an animal, including compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous

immunogens. The term "antigen" includes all related antigenic epitopes. "Epitope" or "antigenic determinant" refers to a site on an antigen to which B and/or T-cells respond. In one embodiment, T-cells respond to the epitope, when the epitope is presented in conjunction with an MHC molecule. Epitopes can be formed both from
5 contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5, about 9, or about 8-10 amino acids in a unique
10 spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance.

An antigen can be a tissue-specific antigen, or a disease-specific antigen. These terms are not exclusive, as a tissue-specific antigen can also be a disease
15 specific antigen. A tissue-specific antigen is expressed in a limited number of tissues, such as a single tissue. Specific non-limiting examples of a disease-specific antigen are an antigen whose expression correlates with, or is predictive of, tumor formation, for example a somatostatin receptor, such as sst₂.

Chemotherapeutic agents: Any chemical agent with therapeutic usefulness
20 in the treatment of diseases characterized by abnormal cell growth. Such diseases include tumors, neoplasms, and cancer as well as diseases characterized by hyperplastic growth such as psoriasis. In one embodiment, a chemotherapeutic agent is a radioactive compound, such as radiolabeled somatostatin or a somatostatin analog. One of ordinary skill in the art can readily identify a chemotherapeutic agent
25 of use (see for example, Slapak and Kufe, *Principles of Cancer Therapy*, Chapter 86 in Harrison's Principles of Internal Medicine, 14th edition; Perry *et al.*, Chemotherapy, Ch. 17 in Abeloff, *Clinical Oncology 2nd ed.*, 2000 Churchill Livingstone, Inc; Baltzer and Berkery. (eds): *Oncology Pocket Guide to Chemotherapy*, 2nd ed. St. Louis, Mosby-Year Book, 1995; Fischer Knobf, and
30 Durivage (eds): *The Cancer Chemotherapy Handbook*, 4th ed. St. Louis, Mosby-Year Book, 1993). Combination chemotherapy is the administration of more than one agent to treat cancer.

Conditions sufficient to detect: Any environment that permits the desired activity, for example, that permits an antibody to bind an antigen, such as sst₂ or a peptide, such as a somatostatin analog, for example a labeled somatostatin analog that binds sst₂, and the interaction to be detected. For example, such conditions can
5 include a detection means such as imaging equipment.

Cortisol or hydrocortisone (CAS Registration No: 50-23-7): A specific type of steroid hormone, or glucocorticoid, produced by the adrenal gland.

Detectable label: A detectable molecule (also known as a label) that is conjugated directly or indirectly to a second molecule, such as somatostatin or a
10 somatostatin analog, to facilitate detection of the second molecule. For example, the detectable marker can be capable of detection by diagnostic imaging techniques (such as CT scans, MRIs, ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include
15 fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). Various methods of labeling polypeptides are known in the art and may be used.

Detecting: To identify the existence, presence, or fact of something, for example a receptor on the surface of a cell, such as a tumor cell, for example the sst₂
20 receptor on the surface of a tumor cell. General methods of detecting are known to the skilled artisan and may be supplemented with the protocols and reagents disclosed herein.

Diagnostic: Identifying the presence or nature of a pathologic condition, such as, but not limited to cancer, such as a solid tumor. Diagnostic methods differ
25 in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of true positives). The "specificity" of a diagnostic assay is 1 minus the false positive rate, where the false positive rate is defined as the proportion of those without the disease who test positive. While a particular diagnostic method may not provide a definitive
30 diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis. "Prognostic" is the probability of development (for example severity) of a pathologic condition, such as cancer, or metastasis.

Effective amount or Therapeutically effective amount: The amount of agent that is sufficient to bring about the desired effect, for example, the amount of suppressor of glucocorticoid receptor activity to reduce or inhibit the activity of the glucocorticoid receptor. In another example, the amount of a chemotherapeutic agent, that is sufficient to prevent, treat (including prophylaxis), reduce and/or ameliorate the symptoms and/or underlying causes of any of a disorder or disease, for example to prevent, inhibit, or treat cancer, or both, such as solid tumor. In some embodiments, an "effective amount" is sufficient to reduce or eliminate a symptom of a disease. An amount sufficient to achieve a desired biological effect, for example an amount that is effective to decrease the size (*e.g.*, volume), side effects and/or metastasis of cancer. In particular examples, it is an amount effective to decrease the size of a solid tumor, for example by at least 30%, 40%, 50%, 70%, 80%, 90%, 95%, 99% or even 100% (complete elimination of the tumor).

Effector molecule: A molecule intended to have or produce a desired effect; for example, a desired effect on a cell to which the effector molecule is targeted. Effector molecules include such molecules as polypeptides, radioisotopes and small molecules. Non-limiting examples of effector molecules include toxins and chemotherapeutic agents. In some embodiments, an effector molecule, such as a cytotoxic molecule, is linked to a somatostatin or a somatostatin analog.

Inhibiting or treating a disease: Inhibiting the full development of a disease or condition, for example, cancer, such as solid tumor. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. The term "ameliorating," with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, such a metastasis, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology.

Inhibitor of glucocorticoid receptor activity: An agent that decreases the activity of the glucocorticoid receptor, for example by inhibiting the activation or inhibition of downstream targets of the glucocorticoid receptor. In some examples, an inhibitor of glucocorticoid receptor activity is a glucocorticoid receptor antagonist. In some examples, an inhibitor of glucocorticoid receptor activity is a glucocorticoid antagonist.

Glucocorticoids (GC) are a class of steroid hormones that bind to the glucocorticoid receptor (GR), which is present in almost every vertebrate animal cell. GCs cause their effects by binding to the glucocorticoid receptor (GR). Glucocorticoids are distinguished from mineralocorticoids and sex steroids by their specific receptors, target cells, or effects. Cortisol (or hydrocortisone) is the most important human glucocorticoid. It is essential for life, and it regulates or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions.

Glucocorticoid receptor (GR, or GCR): A cellular receptor to which cortisol and other glucocorticoids bind. Glucocorticoid receptor is also known as NR3C1 (nuclear receptor subfamily 3, group C, member 1). When glucocorticoids bind to the GR, its primary mechanism of action is the regulation of gene transcription. The unbound receptor resides in the cytosol of the cell. After the receptor is bound to glucocorticoid, the receptor-glucocorticoid complex can take either of two paths. The activated GR complex up-regulates the expression of anti-inflammatory proteins in the nucleus or represses the expression of pro-inflammatory proteins in the cytosol, for examples by preventing the translocation of other transcription factors from the cytosol into the nucleus. In humans, the GR protein is encoded by *NR3C1* gene which is located on chromosome 5 (5q31).

Glucocorticoid receptor antagonist: Compounds that inhibit the activity of the glucocorticoid receptor, for example by inhibiting the binding of glucocorticoids, such as cortisol to the glucocorticoid receptor. Examples of glucocorticoid receptor antagonist include RU486 (also known as RU38486 and mifepristone), cyproterone and RU40555.

Lanreotide (CAS Registry Number: 108736-35-2) A long-acting analogue of somatostatin.

Mifepristone: A cortisol and progesterone analog with anti-glucocorticoid and antiprogesterin activity. Mifepristone, also known as RU486 and RU38486, is an antagonist of the glucocorticoid receptor (GR). The agent can effectively reverse the clinical and glucocorticoid-dependent biochemical features of Cushing's syndrome.

5 **Octreotide (CAS Registry Number: 83150-76-9):** An octapeptide somatostatin analogue sold under the trade name SANDOSTATIN® by Novartis Pharmaceuticals. Octreotide itself is an eight amino acid cyclic peptide, biologically-active analogue of the native somatostatin and has a longer plasma half-life than somatostatin. Octreotide is used in nuclear medicine imaging by labeling with
10 indium (In)-111. When covalently linked to ethylene-triamine-pentaacetic acid (DTPA) it becomes pentetreotide (OCTREOSCAN™). Octoreotide has been radiolabelled with gallium-68 enabling imaging with positron emission tomography (PET) which provides high resolution and sensitivity. Octreotide can also be labeled
15 receptor radionuclide therapy (PART) for the treatment of tumors expressing somatostatin-receptors.

Pentetreotide or [¹¹¹In-DTPA-D-Phe]-pentetreotide (CAS Registry Number: 138661-02-6): A DTPA conjugate of octreotide, which is a long-acting analog of the human hormone, somatostatin. Indium (In)-111 pentetreotide binds to
20 somatostatin receptors on cell surfaces throughout the body. Within an hour of injection, most of the dose of ¹¹¹In- pentetreotide distributes from plasma to extravascular body tissues and concentrates in tumors containing a high density of somatostatin receptors. After background clearance, visualization of somatostatin receptor-rich tissue is achieved. In addition to somatostatin receptor-rich tumors, the
25 normal pituitary gland, thyroid gland, liver, spleen and urinary bladder also are visualized in most patients, as is the bowel, to a lesser extent.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 19th Edition (1995), describes
30 compositions and formulations suitable for pharmaceutical delivery of the fusion proteins herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol, or the like, as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Polypeptide: A polymer in which the monomers are amino acid residues that are joined together through amide bonds. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred. The terms "polypeptide" or "protein" as used herein is intended to encompass any amino acid sequence and include modified sequences such as glycoproteins. The term "polypeptide" is specifically intended to cover naturally occurring proteins, as well as those that are recombinantly or synthetically produced.

Sequence identity: The similarity between two nucleic acid sequences, or two amino acid sequences, is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman *Adv. Appl. Math.* 2: 482-489, 1981; Needleman & Wunsch *J. Mol. Biol.* 48: 443-453, 1970; Pearson & Lipman *Proc. Natl. Acad. Sci. USA* 85: 2444-2448, 1988; Higgins & Sharp *Gene* 73: 237-244, 1988; Higgins & Sharp *CABIOS* 5: 151-153, 1989; Corpet *et al. Nucl. Acids Res.* 16: 10881-10890, 1988; Huang *et al. Comput. Appl. Biosci.* 8: 155-165, 1992; and Pearson *et al. Meth. Mol. Bio.* 24, 307-31, 1994. Altschul *et al. (J. Mol. Biol.* 215: 403-410, 1990), presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* *J. Mol. Biol.* 215: 403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx.

Somatostatin: A peptide hormone that regulates the endocrine system and affects neurotransmission and cell proliferation via interaction with G-protein-coupled somatostatin receptors. Somatostatin has two active forms produced by alternative cleavage of a single preproprotein: one of 14 amino acids, the other of 28 amino acids. Exemplary amino acid and nucleic acid sequences of human somatostatin can be found on GENBANK® at accession numbers NP_001039 and NM_001048, respectively, which are specifically incorporated herein by reference in their entirety as available September 1, 2011.

Somatostatin analog: An agent that mimics the *in vivo* activity of somatostatin, for example binding to somatostatin receptors, such as somatostatin receptor 2 (sst₂).

Somatostatin receptor: A family of G protein-coupled receptors that bind somatostatin or its analogs. There are 5 somatostatin receptors, sst₁₋₅. Exemplary amino acid and nucleic acid sequences of human somatostatin receptor 1 (sst₁) can be found on GENBANK® at accession numbers NP_001040 and NM_001049, respectively, which are specifically incorporated herein by reference in their entirety as available September 1, 2011. Exemplary amino acid and nucleic acid sequences of human somatostatin receptor 2 (sst₂) can be found on GENBANK® at accession numbers NP_001041 and 1 NM_001050.2, respectively, which are specifically incorporated herein by reference in their entirety as available September 1, 2011. Exemplary amino acid and nucleic acid sequences of human somatostatin receptor 3 (sst₃) can be found on GENBANK® at accession numbers NP_001042 and NM_001051, respectively, which are specifically incorporated herein by reference in their entirety as available September 1, 2011. Exemplary amino acid and nucleic acid sequences of human somatostatin receptor 4 (sst₄) can be found on GENBANK® at accession numbers NP_001043 and NM_001052, respectively, which are specifically incorporated herein by reference in their entirety as available September 1, 2011.

Exemplary amino acid and nucleic acid sequences of human somatostatin receptor 5 (sst₅) can be found on GENBANK® at accession numbers NP_001044.1 and NM_001053.3, respectively, which are specifically incorporated herein by reference in their entirety as available September 1, 2011.

5 **Toxin:** An effector molecule that induces cytotoxicity when it contacts a cell. Specific, non-limiting examples of toxins include, but are not limited to, abrin, ricin, *Pseudomonas* exotoxin (PE, such as PE35, PE37, PE38, and PE40), diphtheria toxin (DT), botulinum toxin, saporin, restrictocin or gelonin, or modified toxins thereof, or other toxic agents that directly or indirectly inhibit cell growth or kill cells. For
10 example, PE and DT are highly toxic compounds that typically bring about death through liver toxicity. PE and DT, however, can be modified into a form for use as an immunotoxin by removing the native targeting component of the toxin (such as the domain Ia of PE and the B chain of DT) and replacing it with a different targeting moiety, such as an agent that specifically binds a sst₂ receptor, for example a
15 somatostatin analog.

Tumor or cancer: The product of neoplasia is a neoplasm (a tumor or cancer), which is an abnormal growth of tissue that results from excessive cell division. A tumor that does not metastasize is referred to as “benign.” A tumor that invades the surrounding tissue and/or can metastasize is referred to as “malignant.” Neoplasia is
20 one example of a proliferative disorder. A solid tumor is an abnormal mass of tissue that usually does not contain cysts or liquid areas. A solid tumor is a cancer of body tissues other than blood, bone marrow, or the lymphatic system, thus for the purposes of this disclosure cancers of the blood, such as leukemias and lymphomas are not solid tumors.

25 Examples of solid cancers, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer (such as adenocarcinoma), lung cancers, gynecological cancers (such
30 as, cancers of the uterus (*e.g.*, endometrial carcinoma), cervix (*e.g.*, cervical carcinoma, pre-tumor cervical dysplasia), ovaries (*e.g.*, ovarian carcinoma, serous cystadenocarcinoma, mucinous cystadenocarcinoma, endometrioid tumors,

celioblastoma, clear cell carcinoma, unclassified carcinoma, granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (*e.g.*, squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (*e.g.*, clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma), embryonal rhabdomyosarcoma, and fallopian tubes (*e.g.*, carcinoma)),
5 prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma,
10 bronchogenic carcinoma, renal cell carcinoma, liver cancer, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, and CNS tumors (such as a glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma and
15 retinoblastoma), and skin cancer (such as melanoma and non-melanoma). In specific examples a tumor is a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary thyroid carcinoma.

20 Suitable methods and materials for the practice or testing of this disclosure are described below. Such methods and materials are illustrative only and are not intended to be limiting. Other methods and materials similar or equivalent to those described herein can be used. For example, conventional methods well known in the art to which a disclosed invention pertains are described in various general and more
25 specific references, including, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Press, 2001; Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates, 1992 (and Supplements to 2000); Ausubel *et al.*, *Short Protocols in
30 Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, 4th ed., Wiley & Sons, 1999; Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1990; and Harlow and

Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1999. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

5 **III. Overview of Several Embodiments**

A Methods of Enhancing the Detection of Tumors

 Methods are provided for enhancing the detection of a tumor in a subject, such as a subject suspected of having an ectopic adrenocorticotropin (ACTH) secreting (EAS) tumor. EAS tumors are non-pituitary tumors. In some examples an
10 EAS tumor is a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary thyroid carcinoma. The methods include administering to the subject an inhibitor of glucocorticoid receptor (GR) activity. In some examples, the
15 inhibitor of GR activity is a small molecule inhibitor of GR activity. In some examples, the inhibitor of GR activity is a GR antagonist, such as mifepristone, cyproterone, RU40555, RU38486, or a combination thereof. In specific examples, the GR antagonist is mifepristone. Thus, in some examples, the subject is administered an effective amount of mifepristone. In some examples, the inhibitor of
20 GR activity is administered over a period of time at a specified dose. Exemplary doses and periods of administration are given in Section C.

 Administration of the inhibitor of GR activity results in an increase in the expression, such as the surface expression, of somatostatin receptors, for example somatostatin receptor 2 (sst₂). In specific examples, administration of the GR
25 antagonist to the subject increases the expression of sst₂ on the ectopic adrenocorticotropin-secreting tumor. The increase in the expression of the somatostatin receptors, such as sst₂, on the surface of tumor cells serves as a target for agents that specifically bind somatostatin receptors, such as sst₂. This increase in expression of somatostatin receptors, such as sst₂, can be used to enhance the
30 detection of a tumor in a subject using an agent that specifically binds to the somatostatin receptors, such as sst₂. In other words, after administration of the inhibitor of GR activity, the number of somatostatin receptors present on the surface

of the tumor cell is increased relative to the cells of other tissues. Thus, when agents that bind somatostatin receptors are administered to the subject, these agents will concentrate on, or in (for example due to endocytosis), the cells expressing the somatostatin receptors. In this way, the site of tumor, and/or size or other parameter, can be detected by detecting the agent that binds to the somatostatin receptor, for example using a labeled agent capable of detection that bind to the somatostatin receptor, such as sst₂. In some examples, a subject is selected for treatment with the inhibitor of GR activity who is suspected of having an ectopic adrenocorticotropin (ACTH) secretion (EAS) by a non-pituitary tumor, such as a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary thyroid carcinoma.

In some embodiments, after the subject is administered the antagonist of the GR, the subject is further administered an agent that specifically binds somatostatin receptors, such as sst₂. In some examples, the agent is somatostatin or a somatostatin analog, which can be tagged, for example with a detectable label. Examples of somatostatin analogs are readily known to those of ordinary skill in the art and are commercially available, for example from Novartis. In some examples, the somatostatin analog is octreotide, lanreotide, or pentetreotide. In specific examples, the agent that specifically binds a somatostatin receptor is [¹¹¹In-DTPA-D-Phe]-pentetreotide. In some examples, the agent is an antibody that specifically binds the somatostatin receptor.

The detectable labeled somatostatin, somatostatin analog or antibody can then be detected for example using a means of imaging the subject, thereby detecting a tumor in the subject. In some examples of the disclosed methods, an agent that specifically binds somatostatin receptors is conjugated to a detectable marker. Suitable detectable markers are known to the skilled artisan and can include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds. For example, the detectable marker can be capable of detection by diagnostic imaging techniques (such as CT scans, MRIs, ultrasound, fiberoptic examination, laparoscopic examination and scintigraphy). A non-limiting

exemplary a magnetic agent is gadolinium ⁶⁸gallium, and non-limiting exemplary radioactive labels include ¹¹¹In, ¹²⁵I, ¹³¹I, ³⁵S or ³H.

After the administration of the inhibitor of GR activity, it takes some period of time before the expression of the somatostatin receptor is expressed on the surface of the tumor cell. Thus in some examples, the agent that specifically binds to the somatostatin receptor, such as sst₂, is administered after the inhibitor of GR activity, which can be separated by hours, days, weeks or even months. For example, the agent that specifically bind the somatostatin receptor is administered about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12, hours, about 13 hours, about 14, hours , about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22, hours, about 23 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 2 months, about 3 months, about 4 months, about 6 months, or about 1 year after administration of the inhibitor of GR activity, for example, about 1-2 hours, about 1-5 hours, about 3-7 hours, about 5-10 hours, about 7-20 hours, about 10 hour to about 1 day, about 15 hours to about 2 days, about 1 day to about 5 days, about 3 days to about 10 days, about 5 days to about 15 days, about 10 days to about 3 weeks, about 2 weeks to about 5 weeks, about 3 weeks to about 7 weeks, about 5 weeks to about 2 months, about 1 month to about 3 months, about 2 months to about 6 months, or about 3 months to about 1 year after administration of the inhibitor of GR activity. In some examples, the chemotherapeutic agent that specifically binds somatostatin receptors is administered over a period of time at a specified dose. Exemplary doses and periods of administration are given in Section C.

In some embodiments, an effective amount of an agent that specifically binds somatostatin receptors is administered to the subject for a sufficient amount of time for the agent to form a complex with the somatostatin receptors, which can then be detected. Detection of the complex in the subject determines the presence of cells,

such as tumor cells in the subject, that express somatostatin receptors, which detects tumor cells in the subject. Non-limiting examples of detection include radiolocalization, radioimaging, magnetic resonance imaging (such as using an agent that specifically binds somatostatin receptor conjugated to an iron oxide), positron emission tomography (such as using an ¹¹¹indium-labeled agent that specifically binds somatostatin receptor). In several examples, the disclosed method detects EAS non-pituitary tumors in a subject, such as a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary thyroid carcinoma. In some examples, the presence of the tumor is detected after treatment, such as surgical resection and/or chemotherapy to determine if the treatment was successful, for examples as measured by a reduction in tumor volume and/or disappearance of the tumor.

15

B. Methods of Treatment

This disclosure also provides for methods of treating and/or inhibiting a tumor in a subject, such as an ectopic adrenocorticotropin (ACTH) secreting (EAS) non-pituitary tumor. Such methods treat or inhibit the cancer in a subject (such as reduce the volume or size of the tumor, or reduce metastasis of the tumor, for example by at least 20%, at least 40%, at least 50%, at least 75%, at least 80%, at least 90% or at least 95%, relative to the absence of the treatment). In some examples an EAS non-pituitary tumor is a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary thyroid carcinoma. In particular examples, the method includes selecting a subject who has or is suspected of having an EAS non-pituitary tumor, for example selecting a subject who has or is suspected of having a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary thyroid carcinoma EAS non-pituitary tumor, for example using

30

the methods described above.

The methods include administering to the subject an effective amount of an inhibitor of glucocorticoid receptor (GR) activity to increase the expression of a somatostatin receptor, such as somatostatin receptor 2 (sst₂). In some examples, the subject is administered a small molecule inhibitor of GR activity, such as one or more of mifepristone, cyproterone, RU40555, RU38486, or a combination thereof. In specific examples, the subject is administered an effective amount of mifepristone. In some examples, the inhibitor of GR activity is administered over a period of time at a specified dose. Exemplary doses and periods of administration are given in Section C.

After the subject is administered the antagonist of the GR and the number of somatostatin receptors present on EAS tumor cells increases, the subject is further administered an chemotherapeutic agent that specifically binds somatostatin receptors, such as sst₂. In some examples, the chemotherapeutic agent is somatostatin or a somatostatin analog, which can be tagged, for example with a cytotoxin, for example a radioactive compound or protein based toxin. Examples of somatostatin analogs are readily known to those of ordinary skill in the art and are commercially available. In some examples, the subject is somatostatin analog is octreotide, lanreotide, or pentetreotide. In some examples, the chemotherapeutic agent is an antibody that specifically binds the somatostatin receptor. In some examples, the chemotherapeutic agent that specifically binds somatostatin receptors is administered over a period of time at a specified dose. Exemplary doses and periods of administration are given in Section C.

After the administration of the inhibitor of GR activity, it takes some period of time before the expression of the somatostatin receptor is expressed on the surface of the tumor cell. In some examples the amount or presence of or amount of somatostatin receptors present on the tumor is determined prior to administration of the chemotherapeutic agent that specifically binds to the somatostatin receptor.

In some examples, the chemotherapeutic agent that specifically binds to the somatostatin receptor, such as sst₂, is administered after the inhibitor of GR activity, which can be separated by hours, days, weeks or even months. For example, the agent that specifically binds the somatostatin receptor is administered about 1 hour,

about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12, hours, about 13 hours, about 14, hours , about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22, hours, 5 about 23 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 2 months, about 3 months, 10 about 4 months, about 6 months, or about 1 year after administration of the inhibitor of GR activity, for example, about 1-2 hours, about 1-5 hours, about 3-7 hours, about 5-10 hours, about 7-20 hours, about 10 hour to about 1 day, about 15 hours to about 2 days, about 1 day to about 5 days, about 3 days to about 10 days, about 5 days to about 15 days, about 10 days to about 3 weeks, about 2 weeks to about 5 weeks, 15 about 3 weeks to about 7 weeks, about 5 weeks to about 2 months, about 1 month to about 3 months, about 2 months to about 6 months, or about 3 months to about 1 year after administration of the inhibitor of GR activity. In some examples, the subject is also treated surgically, for example to remove the tumor, or a significant portion of it. In some examples, additional chemotherapeutic agents are administered 20 to the subject.

C. Therapeutic Compositions

Therapeutic and/or diagnostic compositions can be administered *in vivo* to a cell or subject. Generally, it is desirable to prepare the compositions as 25 pharmaceutical compositions appropriate for the intended application. Accordingly, methods for making a medicament or pharmaceutical composition containing an agent that specifically binds a somatostatin receptor or inhibits GR activity in the above methods are included herein. Typically, preparation of a pharmaceutical composition (medicament) entails preparing a pharmaceutical composition that is 30 essentially free of pyrogens, as well as any other impurities that could be harmful to humans or animals. Typically, the pharmaceutical composition contains appropriate salts and buffers to render the components of the composition stable and allow for

uptake by target cells and/or binding to the surface of target cells, such as tumor cells.

Therapeutic and/or diagnostic compositions can be provided as parenteral compositions, such as for injection or infusion. Such compositions are formulated generally by mixing a disclosed therapeutic agent at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, for example one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. In addition, a therapeutic and/or diagnostic composition can be suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 3.0 to about 8.0, preferably at a pH of about 3.5 to about 7.4, 3.5 to 6.0, or 3.5 to about 5.0. Useful buffers include sodium citrate-citric acid and sodium phosphate-phosphoric acid, and sodium acetate/acetic acid buffers. The active ingredient, optionally together with excipients, can also be in the form of a lyophilisate and can be made into a solution prior to parenteral administration by the addition of suitable solvents. Solutions such as those that are used, for example, for parenteral administration can also be used as infusion solutions.

The nature of the carrier will depend on the particular mode of administration being employed. For example, parenteral formulations usually contain injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (such as powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch or magnesium stearate. In addition, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any

conventional media or agent is incompatible with the active ingredient, its use in the pharmaceutical compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions. For example, certain therapeutic and/or diagnostic compositions can include agents in water, mixed with a suitable
5 surfactant, such as hydroxypropylcellulose. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

10 Administration of therapeutic and/or diagnostic compositions can be by any common route as long as the target tissue is available via that route. This includes oral, nasal, ocular, buccal, or other mucosal (such as rectal or vaginal) or topical administration. Alternatively, administration will be by orthotopic, intradermal subcutaneous, intramuscular, intraperitoneal, or intravenous injection routes.

15 The therapeutic and/or diagnostic compositions can also be administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the
20 composition may contain about 100 mg of human serum albumin per milliliter of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like may be used. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyl oleate.
25 Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the pharmaceutical composition are
30 adjusted according to well-known parameters.

Additional formulations are suitable for oral administration. Oral formulations can include excipients such as, pharmaceutical grades of mannitol,

lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions (medicaments) typically take the form of solutions, suspensions, aerosols or powders. Exemplary formulations can be found in U.S. Patent publication No. 20020031527. When the route is topical, the form
5 may be a cream, ointment, salve or spray.

An appropriate effective amount can be readily determined by one of skill in the art. Such an amount will fall in a relatively broad range that can be determined through routine trials, for example within a range of about 10 µg to about 1000 mg. However, doses above and below this range may also be found effective. The
10 therapeutic and/or diagnostic compositions can be administered to the subject in a single bolus delivery, via continuous delivery (for example, continuous transdermal, mucosal or intravenous delivery) over an extended time period, or in a repeated administration protocol (for example, by an hourly, daily or weekly, repeated administration protocol). The effective dosage of the therapeutic and/or diagnostic
15 composition can be provided as repeated doses within a prolonged prophylaxis or treatment regimen that will yield clinically significant results, for example to alleviate one or more symptoms or detectable conditions associated with a targeted disease or condition as set forth herein or in an amount sufficient to image a tumor.

The appropriate dose will vary depending on the characteristics of the
20 subject, for example, whether the subject is a human or non-human, the age, weight, and other health considerations pertaining to the condition or status of the subject, the mode, route of administration, and number of doses, time and route of administration, other drugs or treatments being administered concurrently, as well as the specific pharmacology of the therapeutic and/or diagnostic composition for
25 eliciting the desired activity or biological response in the subject.

An effective amount is also one in which any toxic or detrimental side effects of the compound and/or other biologically active agent is outweighed in clinical terms by therapeutically beneficial effects. A non-limiting range for a therapeutically effective amount of an inhibitor of GR activity or an agent that specifically binds
30 somatostatin receptors within the methods and formulations of the disclosure is about 0.0001 µg/kg body weight to about 10 mg/kg body weight per dose, such as about 0.0001 µg/kg body weight to about 0.001 µg/kg body weight per dose, about

0.001 $\mu\text{g/kg}$ body weight to about 0.01 $\mu\text{g/kg}$ body weight per dose, about 0.01 $\mu\text{g/kg}$ body weight to about 0.1 $\mu\text{g/kg}$ body weight per dose, about 0.1 $\mu\text{g/kg}$ body weight to about 10 $\mu\text{g/kg}$ body weight per dose, about 1 $\mu\text{g/kg}$ body weight to about 100 $\mu\text{g/kg}$ body weight per dose, about 100 $\mu\text{g/kg}$ body weight to about 500 $\mu\text{g/kg}$ body weight per dose, about 500 $\mu\text{g/kg}$ body weight per dose to about 1000 $\mu\text{g/kg}$ body weight per dose, or about 1.0 mg/kg body weight per dose to about 10 mg/kg body weight per dose. Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the subject. In one embodiment, the dosage is administered once as a bolus, but in another embodiment can be applied periodically until a therapeutic result is achieved.

In some examples, an inhibitor of GR activity, such as mifepristone is administered at a concentration of about 1 mg to about 1000 mg total daily dose (for example in one dose, two doses, three doses, for doses, or even continuously, for example using a pump), for example a subject is administered a daily dose of about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg, such as from about 1 mg to about 10 mg, about 5 mg to about 20 mg, about 10 mg to about 75 mg, about 50 mg to about 100 mg, about 75 mg to about 150 mg, about 100 mg to about 200 mg, about 150 mg to about 300 mg, about 200 mg to about 500 mg, about 250 mg to about 750 mg, or about 500 mg to about 1000 mg.

In some examples, the subject is treated with the inhibitor of GR activity for a prolonged period of time, for example a prolonged period of time prior to administration of the agent that specifically binds a somatostatin receptor. In some examples, the agent that inhibits the activity of the GR receptor is administered over a period of about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21

hours, about 22, hours, about 23 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, 5 about 21 days, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 2 months, about 3 months, about 4 months, about 6 months, or about 1 year after administration of the inhibitor of GR activity, for example, about 1-2 hours, about 1-5 hours, about 3-7 hours, about 5-10 hours, about 7-20 hours, about 10 hour to about 1 day, about 15 hours to about 2 days, about 1 day to about 5 days, about 3 days to 10 about 10 days, about 5 days to about 15 days, about 10 days to about 3 weeks, about 2 weeks to about 5 weeks, about 3 weeks to about 7 weeks, about 5 weeks to about 2 months, about 1 month to about 3 months, about 2 months to about 6 months, or about 3 months to about 1 year.

In some examples, an agent that specifically binds a somatostatin receptor is 15 administered at a concentration of about 1 mg to about 1000 mg total daily dose (for example in one dose, two doses, three doses, for doses, or even continuously, for example using a pump), for example a subject is administered a daily dose of about 1 mg, about 5 mg, about 10 mg, about 15, mg, about 20 mg, about 30 mg, about 40, mg about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 20 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, or about 1000 mg, such as from about 1 mg to about 10 mg, about 5 mg to about 20 mg, about 10 mg, to about 75 mg, about 50 mg to about 100 mg, about 75 mg to about 150 mg, about 100 mg, to 25 about 200 mg, about 150 mg to about 300 mg, about 200 mg to about 500 mg, about 250 mg to about 750 mg or about 500 mg to about 1000 mg. In some examples, the subject is administered an agent that specifically a somatostatin receptor for a prolonged period of time. In some examples, the agent that specifically a somatostatin receptor is administered over a period of about 1 hour, about 2 hours, 30 about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12, hours, about 13 hours, about 14, hours , about 15 hours, about 16 hours, about 17 hours, about 18

hours, about 19 hours, about 20 hours, about 21 hours, about 22, hours, about 23 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 2 months, about 3 months, about 4 months, about 6 months, or about 1 year after administration of the inhibitor of GR activity, for example, about 1-2 hours, about 1-5 hours, about 3-7 hours, about 5-10 hours, about 7-20 hours, about 10 hour to about 1 day, about 15 hours to about 2 days, about 1 day to about 5 days, about 3 days to about 10 days, about 5 days to about 15 days, about 10 days to about 3 weeks, about 2 weeks to about 5 weeks, about 3 weeks to about 7 weeks, about 5 weeks to about 2 months, about 1 month to about 3 months, about 2 months to about 6 months, or about 3 months to about 1 year.

The therapeutic and/or diagnostic compositions can be delivered by way of a pump (see Langer, *supra*; Sefton, *Crit. Rev. Biomed. Eng.* 14:201, 1987; Buchwald *et al.*, *Surgery* 88:507, 1980; Saudek *et al.*, *New Engl. J. Med.* 321:574, 1989) or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution can also be employed. One factor in selecting an appropriate dose is the result obtained, as measured by the methods disclosed here, as are deemed appropriate by the practitioner. Other controlled release systems are discussed in Langer (*Science* 249:1527-33, 1990).

In one example, a pump is implanted (for example see U.S. Patent Nos. 6,436,091; 5,939,380; and 5,993,414). Implantable drug infusion devices are used to provide patients with a constant and long-term dosage or infusion of a therapeutic agent. Such device can be categorized as either active or passive.

Active drug or programmable infusion devices feature a pump or a metering system to deliver the agent into the patient's system. An example of such an active infusion device currently available is the Medtronic SYNCHROMED™ programmable pump. Passive infusion devices, in contrast, do not feature a pump, but rather rely upon a pressurized drug reservoir to deliver the agent of interest. An example of such a device includes the Medtronic ISOMED™.

In particular examples, therapeutic compositions including a disclosed therapeutic agent are administered by sustained-release systems. Suitable examples of sustained-release systems include suitable polymeric materials (such as, semi-permeable polymer matrices in the form of shaped articles, for example films, or
5 microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt). Sustained-release compositions can be administered orally, parenterally, intracisternally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), or as an oral or nasal spray.
10 Sustained-release matrices include polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman *et al.*, *Biopolymers* 22: 547-556, 1983, poly(2-hydroxyethyl methacrylate)); (Langer *et al.*, *J. Biomed. Mater. Res.* 15: 167-277, 1981; Langer, *Chem. Tech.* 12:98-105, 1982, ethylene vinyl acetate (Langer *et al.*, *Id.*) or poly-D-(-)-3-hydroxybutyric acid
15 (EP 133,988).

Polymers can be used for ion-controlled release. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accts. Chem. Res.* 26: 537-542, 1993). For example, the block copolymer, polaxamer 407 exists as a viscous yet mobile liquid at low temperatures
20 but forms a semisolid gel at body temperature. It has shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston *et al.*, *Pharm. Res.* 9: 425-434, 1992; and Pec, *J. Parenter. Sci. Tech.* 44: 58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema *et al.*, *Int. J. Pharm.* 112: 215-244, 1994). In yet another aspect, liposomes are used for controlled release as
25 as well as drug targeting of the lipid-capsulated drug (Betageri *et al.*, *Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA, 1993). Numerous additional systems for controlled delivery of therapeutic proteins are known (for example, U.S. Patent No. 5,055,303; U.S. Patent No. 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; and U.S. Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent
30 No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent

No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342; and U.S. Patent No. 5,534,496).

An agent that specifically binds sst₂ (such as a somatostatin analog or an antibody) can be conjugated to an agent, such as an effector molecule or detectable marker, using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. Conjugates include, but are not limited to, molecules in which there is a covalent linkage of an effector molecule or a detectable marker to an agent that specifically binds sst₂. One of skill in the art will appreciate that various effector molecules and detectable markers can be used, including (but not limited to) chemotherapeutic agents, anti-angiogenic agents, toxins, radioactive agents such as ¹²⁵I, ³²P, ¹⁴C, ³H and ³⁵S and other labels, target moieties and ligands, *etc.*

The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect. Thus, for example, the effector molecule can be a cytotoxin that is used to bring about the death of a particular target cell (such as a tumor cell).

Effector molecules and detectable markers can be linked to an agent of interest using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. The procedure for attaching an effector molecule or detectable marker to a peptide, such as somatostatin, a somatostatin analog or an antibody that binds a somatostatin receptor, varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine (-NH₂) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group. Alternatively, the peptide is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules such as those available from Pierce Chemical Company, Rockford, IL. The linker can be any molecule used to join the antibody to the effector molecule or detectable marker. The linker is capable of forming covalent bonds to both the peptide and to the effector molecule or detectable marker. Suitable linkers are well known to those of skill in the art and include, but are not limited to,

straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the peptide and the effector molecule or detectable marker are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In some circumstances, it is desirable to free the effector molecule or detectable marker from the peptide when the conjugate has reached its target site. Therefore, in these circumstances, conjugates will include linkages that are cleavable in the vicinity of the target site. Cleavage of the linker to release the effector molecule or detectable marker from the antibody may be prompted by enzymatic activity or conditions to which the conjugate is subjected either inside the target cell or in the vicinity of the target site. When the target site is a tumor, a linker which is cleavable under conditions present at the tumor site (for example, when exposed to tumor-associated enzymes or acidic pH) may be used.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, labels (such as enzymes or fluorescent molecules), drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide. For example, the antibodies and peptides can be conjugated with small molecular weight drugs such as Monomethyl Auristatin E (MMAE), Monomethyl Auristatin F (MMAF), mayansine, mayansine derivatives, including the derivative of maytansine known as DM-1, or other chemotherapeutic agents to make an antibody- or peptide-drug conjugate (ADC).

Toxins can be employed with an agent that specifically binds a somatostatin receptor. Exemplary toxins include *Pseudomonas* exotoxin (PE), ricin, abrin, diphtheria toxin and subunits thereof, ribotoxin, ribonuclease, saporin, and calicheamicin, as well as botulinum toxins A through F. These toxins are well known in the art and many are readily available from commercial sources (for example, Sigma Chemical Company, St. Louis, MO). Contemplated toxins also include variants of the toxins (see, for example, see, U.S. Patent Nos. 5,079,163 and 4,689,401).

Saporin is a toxin derived from *Saponaria officinalis* that disrupts protein synthesis by inactivating the 60S portion of the ribosomal complex (Stirpe *et al.*, *Bio/Technology*, 10:405-412, 1992). However, the toxin has no mechanism for specific entry into cells, and therefore requires conjugation to an agent that
5 specifically binds to a somatostatin receptor, such as somatostatin, a somatostatin analog or an antibody that specifically binds somatostatin receptors, that recognizes a cell-surface protein, such as a somatostatin receptor, that is internalized in order to be efficiently taken up by cells.

Diphtheria toxin is isolated from *Corynebacterium diphtheriae*. Typically,
10 diphtheria toxin for use in immunotoxins is mutated to reduce or to eliminate non-specific toxicity. A mutant known as CRM107, which has full enzymatic activity but markedly reduced non-specific toxicity, has been known since the 1970's (Laird and Groman, *J. Virol.* 19: 220-227, 1976), and has been used in human clinical trials. See, U.S. Patent No. 5,792,458 and U.S. Patent No. 5,208,021.

15 Ricin is the lectin RCA60 from *Ricinus communis* (Castor bean). For examples of ricin, see, U.S. Patent No. 5,079,163 and U.S. Patent No. 4,689,401. *Ricinus communis* agglutinin (RCA) occurs in two forms designated RCA₆₀ and RCA₁₂₀ according to their molecular weights of approximately 65 and 120 kD, respectively (Nicholson & Blaustein, *J. Biochim. Biophys. Acta* 266: 543-547,
20 1972). The A chain is responsible for inactivating protein synthesis and killing cells. The B chain binds ricin to cell-surface galactose residues and facilitates transport of the A chain into the cytosol (Olsnes *et al.*, *Nature* 249: 627-631, 1974 and U.S. Patent No. 3,060,165).

Ribonucleases have also been conjugated to targeting molecules for use as
25 immunotoxins (see Suzuki *et al.*, *Nat. Biotech.* 17: 265-270, 1999). Exemplary ribotoxins such as α -sarcin and restrictocin are discussed in, for example Rathore *et al.*, *Gene* 190: 31-35, 1997; and Goyal and Batra, *Biochem.* 345 Pt 2: 247-254, 2000. Calicheamicins were first isolated from *Micromonospora echinospora* and are members of the enediyne antitumor antibiotic family that cause double strand breaks
30 in DNA that lead to apoptosis (see, for example Lee *et al.*, *J. Antibiot.* 42: 1070-1087, 1989). The drug is the toxic moiety of an immunotoxin in clinical trials (see, for example, Gillespie *et al.*, *Ann. Oncol.* 11: 735-741, 2000).

Abrin includes toxic lectins from *Abrus precatorius*. The toxic principles, abrin a, b, c, and d, have a molecular weight of from about 63 and 67 kD and are composed of two disulfide-linked polypeptide chains A and B. The A chain inhibits protein synthesis; the B chain (abrin-b) binds to D-galactose residues (see, Funatsu
5 *et al.*, *Agr. Biol. Chem.* 52: 1095-1097, 1988; and Olsnes, *Method Enzymol.* 50: 330-335, 1978).

In one embodiment, the toxin is *Pseudomonas* exotoxin (PE) (U.S. Patent No. 5,602,095). As used herein, PE includes full-length native (naturally occurring) PE or a PE that has been modified. Such modifications can include, but are not
10 limited to, elimination of domain Ia, various amino acid deletions in domains Ib, II and III, single amino acid substitutions and the addition of one or more sequences at the carboxyl terminus (for example, see Siegall *et al.*, *J. Biol. Chem.* 264: 14256-14261, 1989).

PE employed with the provided agents, such as antibodies, can include the
15 native sequence, cytotoxic fragments of the native sequence, and conservatively modified variants of native PE and its cytotoxic fragments. Cytotoxic fragments of PE include those which are cytotoxic with or without subsequent proteolytic or other processing in the target cell. Cytotoxic fragments of PE include PE40, PE38, and PE35. For additional description of PE and variants thereof, see for example, U.S.
20 Patent Nos. 4,892,827; 5,512,658; 5,602,095; 5,608,039; 5,821,238; and 5,854,044; PCT Publication No. WO 99/51643; Pai *et al.*, *Proc. Natl. Acad. Sci. USA*, 88: 3358-3362, 1991; Kondo *et al.*, *J. Biol. Chem.*, 263:9470-9475, 1988; Pastan *et al.*, *Biochim. Biophys. Acta*, 1333:C1-C6, 1997. In some examples, the PE is PE38.

Also contemplated herein are protease-resistant PE variants and PE variants
25 with reduced immunogenicity, such as, but not limited to PE-LR, PE-6X, PE-8X, PE-LR/6X and PE-LR/8X (see, for example, Weldon *et al.*, *Blood* 113: 3792-3800, 2009; Onda *et al.*, *Proc. Natl. Acad. Sci. USA*, 105: 11311-11316, 2008; and PCT Publication Nos. WO 2007/016150, WO 2009/032954 and WO 2011/032022, which are herein incorporated by reference).

30 In some examples, the PE is a variant that is resistant to lysosomal degradation, such as PE-LR (SEQ ID NO: 45; Weldon *et al.*, *Blood* 113: 3792-3800, 2009; PCT Publication No. WO 2009/032954). In other examples, the PE is a

variant designated PE-LR/6X (SEQ ID NO: 46; PCT Publication No. WO 2011/032022). In other examples, the PE variant is PE with reducing immunogenicity, such as a PE including an amino acid sequence set forth as SEQ ID NO: 47. In other examples, the PE is a variant designated PE-LR/8M (PCT
5 Publication No. WO 2011/032022).

An agent that specifically binds to a somatostatin receptor, such as sst₂, can also be conjugated with a detectable marker; for example, a detectable marker capable of detection by a diagnostic imaging technique (such as a CT scan, MRI, ultrasound, fiber-optic examination, and laparoscopic examination). Specific, non-
10 limiting examples of detectable markers include radioactive isotopes and heavy metals or compounds (for example, super-paramagnetic iron oxide nanocrystals for detection by MRI). An agent that specifically binds somatostatin receptors, such as sst₂, can be conjugated with a paramagnetic agent, such as gadolinium. Paramagnetic agents such as super-paramagnetic iron oxide are also of use as labels.
15 An agent that specifically binds somatostatin receptors, such as sst₂, can also be conjugated with lanthanides (such as europium and dysprosium), and manganese. An agent that specifically binds somatostatin receptors, such as sst₂, can also be conjugated with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to
20 detect cells expressing somatostatin receptors, such as sst₂, by x-ray, emission spectra, or other diagnostic techniques. Further, the radiolabel may be used therapeutically as a toxin for treatment of tumors in a subject. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: ³H, ¹⁴C, ¹⁵N, ³⁵S, ⁹⁰Y, ⁹⁹Tc, ¹¹¹In, ¹²⁵I, ¹³¹I. Means of detecting such
25 detectable markers are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters.

Kits

The disclosure also provides kits that include one or more inhibitors of the
30 glucocorticoid receptor activity and one or more agents that specifically bind somatostatin receptors, such as sst₂, which can be tagged, for example with a detectable label or a cytotoxic agent, or both. The inhibitors of the glucocorticoid

receptor activity and the agent that specifically binds somatostatin receptors can be in the same or in separate containers. In some examples, the inhibitors of the glucocorticoid receptor activity and the agent that specifically binds somatostatin receptors are lyophilized, and reconstituted before administration to a subject. Kits
 5 can optionally include other agents, such as pharmaceutically-acceptable carriers, other chemotherapeutic agents, instructions, and the like.

EXAMPLES

Example 1

10 A female 40-year old patient was referred to Erasmus medical center by her primary physician with a clinical suspicion of Cushing's syndrome. The patient had complained of severe fatigue that was progressive over the past few months, combined with easy bruising, muscle weakness, and mild alopecia. She reported a weight gain of approximately 8 kg. She did not take any medication except for oral
 15 contraceptives. Previous medical history was unremarkable except for an extra-uterine pregnancy 15 years ago and a more recent episode of trigeminal neuralgia. A physical examination indicated that she had a Cushing-like appearance with marked facial and central obesity, combined with proximal muscle atrophy and weakness. Routine laboratory parameters were normal, including serum potassium levels.
 20 Endocrinological laboratory evaluation revealed mildly elevated serum cortisol levels without diurnal variation, insufficient overnight suppression of serum cortisol after administration of 1-mg oral dexamethasone, and elevated 24-h urinary free cortisol (UFC) levels (Table 1).

25 **Table 1. Laboratory parameters at time of diagnosis (Patient 1)**

	Value	Ref range
Serum cortisol (nmol/l)		
09.00 hr	792	200-800
17.00 hr	924	
22.00 hr	979	
24.00 hr	866	
Serum ACTH (pmol/l)		
09.00 hr	7.4	<11
17.00 hr	11.0	

22.00 hr	9.9	
24.00 hr	9.9	
1 mg dex. suppression test		
cortisol 09.00 hr (nmol/l)	646	< 50
High-dose dex. suppression test (48 h 2mg/day)		
cortisol baseline (nmol/l)	530	
cortisol post-dex (nmol/l)	108	< 50

Plasma ACTH levels were within the high-normal range. High-dose dexamethasone (8 mg), was followed by more than 50% suppression of serum cortisol. A gadolinium-enhanced MRI of the pituitary did not reveal abnormalities. Bilateral inferior petrosal sinus sampling showed a central-to-peripheral ACTH gradient below 2.0 before and below 3.0 after CRH administration, consistent with an ectopic cause of the ACTH secretion. An abdominal MRI was normal. A round nodule, 4 mm in diameter, in the right upper lung was initially reported as a non-specific nodule on chest CT (FIG. 1A). Somatostatin receptor scintigraphy with [¹¹¹In-DTPA⁰]octreotide (OCTREOSCAN™) did not show pathological uptake (FIG. 1B), nor did additional DOPA-PET or 5-hydroxytryptamine (HTP)-PET scans. The patient was treated with the glucocorticoid receptor antagonist mifepristone (Laboratoire HRA-Pharma, Paris, France) at a dose of 400 to 600 mg/day, which clearly improved her condition with 23 kg weight loss and disappearance of Cushingoid features. At follow-up, six months after initiating mifepristone treatment, a repeat chest CT showed the same nodule in the right upper lung without signs of growth (4 mm in diameter, FIG. 1C). A repeat OCTREOSCAN™ showed a positive uptake at the site of this nodule (FIG. 1D and 1E). Subsequently, the patient underwent resection of the right upper lobe of the lung, which revealed a small tumor of 5 mm in diameter. Immediately after resection, the fresh carcinoid tissue was obtained for further analysis *in vitro*. Pathological examination showed a neuroendocrine tumor with that stained ACTH, synaptophysin and chromogranin A on IHC. The patient recovered without any major complications. She was treated with hydrocortisone substitution, which was gradually tapered. At present, 10 months after surgery, she is doing well without any evidence of recurrent

hypercortisolism.

Surgical tissue and cell isolation: After resection, the fresh carcinoid tissue was placed in 4°C Minimal Essential Medium (MEM) with Earle's salts, supplemented with 10% fetal calf serum (FCS), L-glutamine (2 mmol/l), penicillin (10^5 U/l) and fungizone (0.25 mg/l). The tissue was dispersed with dispase 10^3 U/l (Roche, Almere, the Netherlands) + collagenase 2 mg/ml (Sigma Aldrich, Zwijndrecht, the Netherlands) at 37°C for 1 h to obtain a single cell population. Viable carcinoid cells were counted in a standard haematocytometer. A 3×10^5 aliquot of the cells was used for qPCR analysis. The remaining cells were cultured in 48-well plates (Corning, Cambridge, USA) at a density of 40,000 cells/well for 4-6 days at 37 °C in a humidified incubator in 5% CO₂. At that time, culture medium was refreshed and 96 h incubations were started with octreotide 10^{-8} M (Novartis, Basel, Switzerland), cabergoline 10^{-8} M (Pfizer, Capelle a/d IJssel, the Netherlands), or their combination. At the end of the incubation period, cultured media were collected and stored at – 20°C for hormone analysis after addition of aprotinin (4×10^5 IU/ml medium; Bayer, Mijdrecht, the Netherlands) to prevent ACTH degradation. All experimental conditions were performed in quadruplicate.

Quantitative PCR: All samples were assayed in duplicate and values were normalized against the expression of the housekeeping gene hprt. Dilution curves were constructed to calculate PCR efficiencies for every primer-probe set. Estimated copy numbers were calculated using the comparative threshold method with efficiency correction.

25

Immunohistochemistry: The expression of sst₂ and D₂ in the carcinoid tissue was assessed by cutting cryostat sections (5µm) and performing immunohistochemistry. The polyclonal anti-sst₂ antibody (Gramsch laboratories, Schwabshausen, Germany) was used at a dilution of 1:2000 and the monoclonal anti-D₂ antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA) was used at a dilution of 1:400. The secondary antibody was Poly-AP-Goat anti Mouse/Rabbit IgG from PowerVision+

30

(ImmunoVision Technologies Co, Brisbane, CA, USA), followed by incubation in New Fuchsin solution.

Statistics: All data were analysed with GRAPHPAD® Prism software (San Diego, CA, USA). Data on hormone release are expressed as mean $\pm$ SEM. All experiments were run in quadruplicate. Overall differences between treatment groups were determined by ANOVA. In case of significant differences found by ANOVA, a multiple comparison between groups was performed with a Newman-Keuls test. *P*-values less than 0.05 were considered statistically significant.

10

In vitro results: The somatostatin and dopamine receptor mRNA subtype expression pattern in the carcinoid tumor cells is depicted in Figure 3. Sst₂ was highly expressed in this tumor (0.18 copies/hprt), while sst₁, sst₃, and sst₅ expression was low to very low (0.03, 0.01 and 0.03 copies/hprt, respectively). D₂ mRNA was also highly expressed in this tumor (2.13 copies/hprt).

15

In the cultured tumor cells of this patient, octreotide (OCT, -25%) and cabergoline (CAB, -25%) at 10 nM concentration both decreased ACTH release after 96 h, although these inhibitions were not significant at the 0.05 significance level (Figure 4). The combination of OCT and CAB was less efficacious (-20%) than either agent alone.

20

By immunohistochemistry, the tumor tissue was clearly positive for sst₂ with a membranous staining pattern, which was not present when the primary sst₂ antibody was pre-incubated with an immunizing sst₂ receptor peptide or with omission of the primary sst₂ antibody (Figure 5A-D). D₂ was also strongly expressed by this tumor (Figure 5E).

25

Example 2

A 56-year old man presented to NIH with long-standing signs and symptoms of Cushing's syndrome. Initial diagnostic evaluation had been compatible with ectopic ACTH secretion, but no primary tumor had been found after extensive imaging. Treatment regimens with ketoconazole and metyrapone were unsuccessful

30

or withdrawn due to severe side effects. He was controlled on aminoglutethimide. Twelve years after his first symptoms, he was targeted for possible treatment with mifepristone, as aminoglutethimide had been withdrawn from the market. He discontinued medical treatment six weeks before admission so that the differential
5 diagnosis could be re-evaluated. At that time, physical features were mildly Cushingoid and his mood was depressed. Urine cortisol excretion was approximately 80-times the upper reference range, plasma ACTH was five-times the upper reference range, and plasma cortisol was elevated without ultradian variation. After obtaining informed consent, inferior petrosal sinus sampling, CRH testing, and
10 8mg dexamethasone suppression tests were consistent with an ectopic source of ACTH. Imaging of the neck, chest and abdomen with CT and MRI did not reveal discrete abnormalities (FIG. 2A), and somatostatin receptor scintigraphy with [¹¹¹In-DTPA⁰]octreotide (OCTREOSCAN™) did not show pathological uptake (FIG. 2B). 18F-DOPA PET scan showed a small focus in the right lung that was not confirmed
15 by any other imaging modality. The patient was treated with mifepristone, initially at a dose of 400 mg/day, which was then titrated to 600 or 800 mg daily. He had cyclic variation in cortisol production and on occasion the agent was discontinued for a few days because of signs of adrenal insufficiency. At follow-up, 12 months after initiating mifepristone treatment, he stated that he felt better and his
20 Cushingoid features had resolved. Repeat imaging showed three contiguous nodules in the right middle lung on OCTREOSCAN™, MRI and F-DOPA PET (FIG. 2D). These were seen in retrospect on the CT scan also (FIG. 2C). Subsequently, the patient underwent resection of the right middle lobe of the lung, which revealed three small masses, 7 to 10 mm in diameter. Pathological examination showed
25 neuroendocrine tumor with positive immunohistochemical staining for ACTH, synaptophysin, TTF1 and chromogranin A. After surgery, ACTH fell to the lower reference range and cortisol levels were subnormal. He was discharged on hydrocortisone and continues to do well 15 months after the procedure, with no evidence of recurrent hypercortisolism.

30

Example 3

Evaluation of Subject for Response to the Glucocorticoid Receptor Antagonist Mifepristone

5 Patients are evaluated at a clinical center on an inpatient ward. Each subject undergoes a complete medical history, physical examination, and chemical screening tests, including CHEM 20, complete blood count, plasma adrenocorticotrophic hormone (ACTH) levels, and urinary free cortisol. Patients also undergo measurement of diurnal cortisol in blood and saliva to confirm the
10 diagnosis of Cushing syndrome. This is followed by a Corticotropin-releasing hormone (CRH) stimulation test, inferior petrosal sinus sampling (IPSS) and 8 mg overnight dexamethasone suppression test. Patients undergo MRI before IPSS; those with a mass greater than 6 mm diameter may not need IPSS, if CRH and dexamethasone suppression tests are compatible with Cushing's disease. All of these
15 tests are performed as part of the standard evaluation of Cushing syndrome.

Patients whose results are consistent with Cushing disease will be referred for trans-sphenoidal surgery at another institution, and will not undergo the imaging studies described below. Patients whose evaluation indicates ectopic ACTH secretion (generally, a lack of response to CRH or dexamethasone, and a lack of
20 central to peripheral ACTH gradient on IPSS) will undergo standard, medically-indicated, imaging studies for localization of the tumor, including CT (neck, chest, abdomen, and pelvis), MRI (neck, abdomen, and pelvis), and 6 mCi standard OCTREOSCAN™ (L-OCT). Women will undergo a urine pregnancy test within two days before administration of any isotope. The chest MRI is obtained as a gated
25 study using a 3 Tesla magnet with T1 and T2 sequences. Increased magnetic field strength compared to a conventional 1.5T MRI will allow for stronger signal and, therefore, improved signal-to-noise ratio. Additional free breathing techniques will be employed to avoid breath-holding, which may be difficult for these patients due to possible volume overload. The combination of both higher signal and decreased
30 motion artifacts will significantly improve resolution and allow for better delineation of small lesions that may be responsible for ectopic ACTH production, and ultimately allow for accurate localization for surgical removal of such lesions.

The duration of standard imaging studies is about 10-15 minutes for either CT of the neck, chest, abdomen, or pelvis, and 1-2 hours for either MRI of the pituitary, chest, abdomen, or pelvis. Patients without active hypercortisolism in whom the standard OCTREOSCAN™ is negative will undergo H-OCT. If a tumor
5 is found, patients will undergo surgical resection, usually in a subsequent admission. A gated chest MRI can be obtained using a 3 Tesla magnet with T1 and T2 sequences. Patients with active hypercortisolism will receive the mifepristone-L-OCT combination as a research study (see below).

Octreoscan procedures: Patient preparation includes a cathartic (magnesium
10 citrate or other laxative) the night before 24-hour scan to minimize bowel activity, unless there is a clinical reason not to do so. Patients are also encouraged to hydrate well before and after administration of [¹¹¹In-DTPA-D-Phe]-pentetreotide to aid in renal elimination of unbound material.

The patient is injected intravenously with approximately 6mCi (L-OCT) or 18
15 mCi [¹¹¹In-DTPA-D-Phe]-pentetreotide (H-OCT) and imaged 4 and 24 h later. Whole-body, planar spots, and SPECT images are obtained as indicated. Additional delayed images may be obtained, if necessary. A SPECT-CT, which allows for co-registered images to help localize the site of a positive result. Octreotide imaging takes 1-3 h each day, depending on the clinical situation. Patients having problems
20 controlling their urine may need to use a Foley or condom catheter for approximately 24 h after the injection of Octreotide.

For the combined mifepristone study, mifepristone will be administered at a total daily dose of 600 mg (given as one 200 mg tablet tid, per os) starting 10 days before the second L-OCT (after the first is completed). It will be given in the
25 morning (before breakfast), middle of the day (either immediately before lunch for subjects who have late lunch or 2 h after lunch for subjects who have an early lunch), and evening (either immediately before dinner for subjects who have late dinner or 2 h after dinner for subjects who have an early dinner). Blood (8 mL) will be drawn on the morning of the second scan, and will be stored for possible
30 measurement of mifepristone. No other medical treatment for high cortisol will be given during this time. The L-OCT will be repeated.

Evaluation and treatment at the conclusion of imaging episodes:

The results of all imaging studies are reviewed at the end of each admission. Imaging results will be correlated. If the conventional imaging studies localize a possible tumor, the patient will undergo surgical resection, probably during a
5 subsequent admission. If the conventional imaging studies are equivocal, additional studies will be performed as clinically indicated. These studies may include venous sampling for ACTH measurement in chest or abdominal vessels, ultrasound examination, bone scan, or other tests. If the conventional imaging studies are negative, the patient will not undergo surgery. If Cushing syndrome recurs or if
10 patients are not cured by initial resection, patients are offered re-evaluation to localize any residual tumor or recurrence. In that case, all clinical, biochemical, and imaging tests may be repeated similar to the initial visit.

While this disclosure has been described with an emphasis upon particular embodiments, it will be obvious to those of ordinary skill in the art that variations of
15 the particular embodiments may be used, and it is intended that the disclosure may be practiced otherwise than as specifically described herein. Features, characteristics, compounds, chemical moieties, or examples described in conjunction with a particular aspect, embodiment, or example of the invention are to be understood to be applicable to any other aspect, embodiment, or example of the
20 invention. Accordingly, this disclosure includes all modifications encompassed within the spirit and scope of the disclosure as defined by the following claims.

We claim:

1. A method, comprising:
administering to the subject an inhibitor of glucocorticoid receptor;
5 administering an agent that specifically binds the somatostatin receptor; and
detecting the agent bound to the somatostatin receptor.
2. The method of claim 1, for use in a subject to detect an
adrenocorticotropin-secreting non-pituitary tumor.
10
3. The method of claim 2, wherein the non-pituitary tumor is a small
cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid
cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a
thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor or a medullary
15 thyroid carcinoma.
4. The method of any one of claims 1-3, wherein the inhibitor of
glucocorticoid receptor activity comprises a glucocorticoid receptor antagonist.
- 20 5. The method of claim 4, wherein the glucocorticoid receptor
antagonist comprises mifepristone, cyproterone, RU40555, or RU38486.
6. The method of claim 5, wherein the glucocorticoid receptor
antagonist is mifepristone.
25
7. The method of any one of claims 1-6, wherein the somatostatin
receptor is somatostatin receptor 2 (sst₂).
8. The method of any one of claims 1-7, further comprising selecting a
30 subject for administration of the inhibitor of glucocorticoid receptor activity,
wherein the subject is suspected of having an ectopic adrenocorticotropin-secreting
non-pituitary tumor.

9. The method of claim 8, wherein the ectopic adrenocorticotropin-secreting non-pituitary tumor is a small cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid cancer, a pheochromocytoma, a foregut
5 carcinoid tumor, a bronchial carcinoid, a thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor, or a medullary thyroid carcinoma.

10. The method of claim 8, wherein the subject has ectopic adrenocorticotropin secretion.

10

11. The method of claim 8, wherein the subject is diagnosed with Cushing's syndrome.

12. The method of any one of claims 1-11, wherein the agent that
15 specifically binds the somatostatin receptor is somatostatin, a somatostatin analog, or an antibody that specifically bind the somatostatin receptor.

13. The method of claim 12, wherein the agent that specifically binds the somatostatin receptor is labeled with a detectable label.

20

14. The method of claim 12 or 13, wherein the somatostatin analog comprises octreotide, lanreotide, pentetreotide, or a combination thereof.

15. The method of claim 14, wherein the somatostatin analog comprises
25 [¹¹¹In-DTPA-D-Phe]-pentetreotide.

16. The method of any one of claims 1-15, wherein detection comprises X-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, fiber-optic examination, laparoscopic examination, Positron emission tomography
30 (PET), scintigraphy, or a combination thereof.

17. The method of claim 16, wherein detection comprises scintigraphy.

18. A method of treating or inhibiting, or both, an adrenocorticotropin-secreting non-pituitary tumor in a subject, comprising:

5 administering to the subject an inhibitor of glucocorticoid receptor; and
administering to the subject a cytotoxic agent that specifically binds the
somatostatin receptor.

19. The method of claim 18, wherein the non-pituitary tumor is a small
10 cell carcinoma of the lung, an islet cell tumor of the pancreas, a medullary thyroid
cancer, a pheochromocytoma, a foregut carcinoid tumor, a bronchial carcinoid, a
thymic carcinoid, a gastro-entero-pancreatic neuro-endocrine tumor, or a medullary
thyroid carcinoma.

20. The method of any one of claims 18-19, wherein the inhibitor of
15 glucocorticoid receptor activity comprises a glucocorticoid receptor antagonist.

21. The method of claim 20, wherein the glucocorticoid receptor
antagonist comprises mifepristone, cyproterone, RU40555, or RU38486.

20

22. The method of claim 21, wherein the glucocorticoid receptor
antagonist is mifepristone.

23. The method of any one of claims 18-22, wherein the somatostatin
25 receptor is somatostatin receptor 2 (sst₂).

24. The method of any one of claims 18-23, wherein the cytotoxic agent
that specifically binds the somatostatin receptor is somatostatin, a somatostatin
analog, or an antibody that specifically bind the somatostatin receptor.

30

25. The method of any one of claims 18-24, wherein the somatostatin
analog comprises octreotide, lanreotide, pentetreotide, or a combination thereof.

26. The method of claim 25, wherein the somatostatin analog is conjugated to a cytotoxin.

5 27. The method of claim 26, wherein the cytotoxin comprises a radioactive compound.

28. The method of any one of claims 18-27, further comprising selecting a subject with an adrenocorticotropin-secreting non-pituitary tumor.

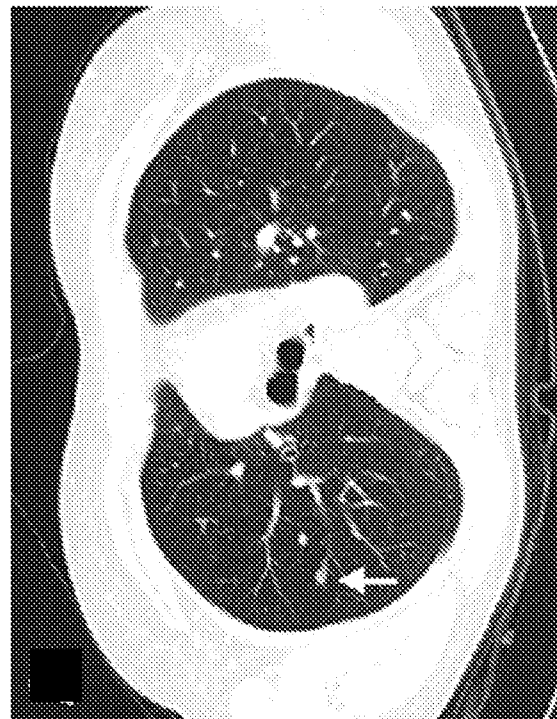


FIG. 1A

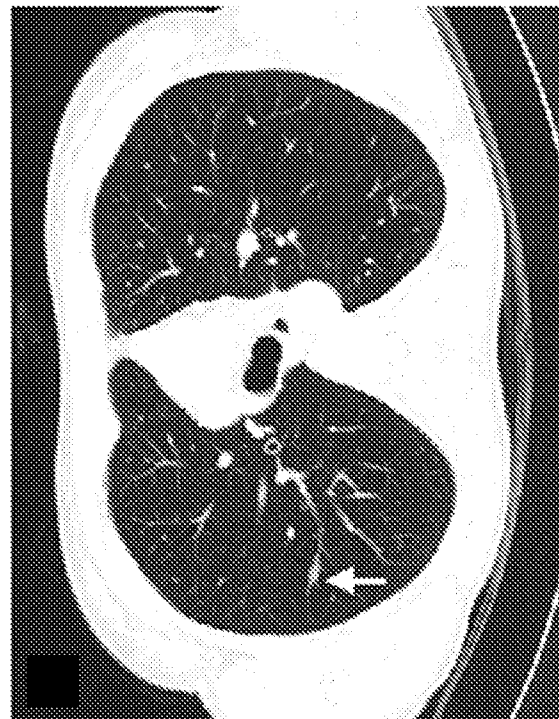


FIG. 1C

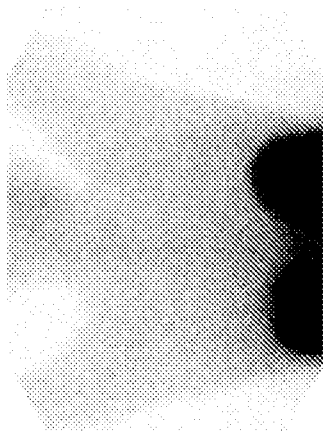


FIG. 1B

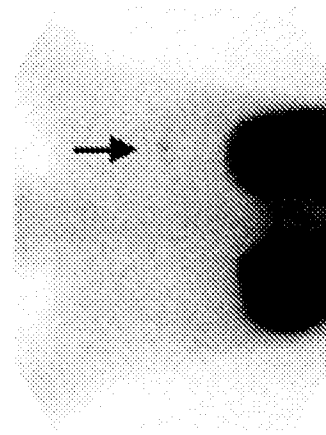


FIG. 1D

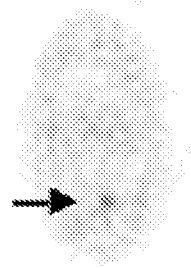


FIG. 1E

FIG. 2A

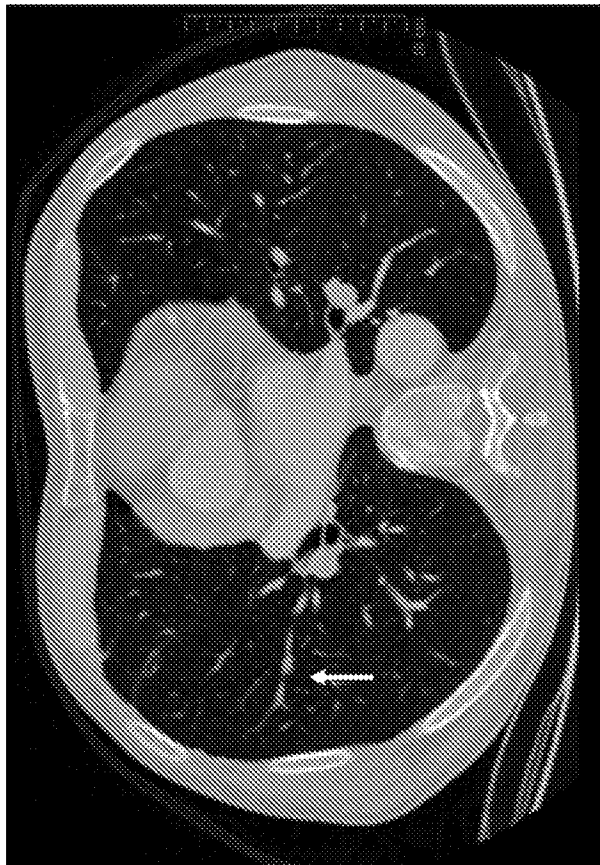


FIG. 2B

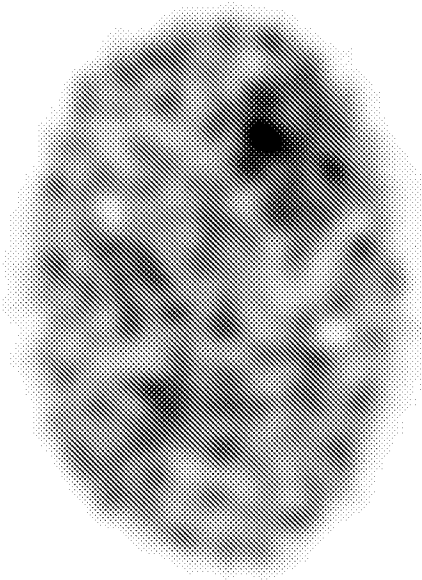


FIG. 2C

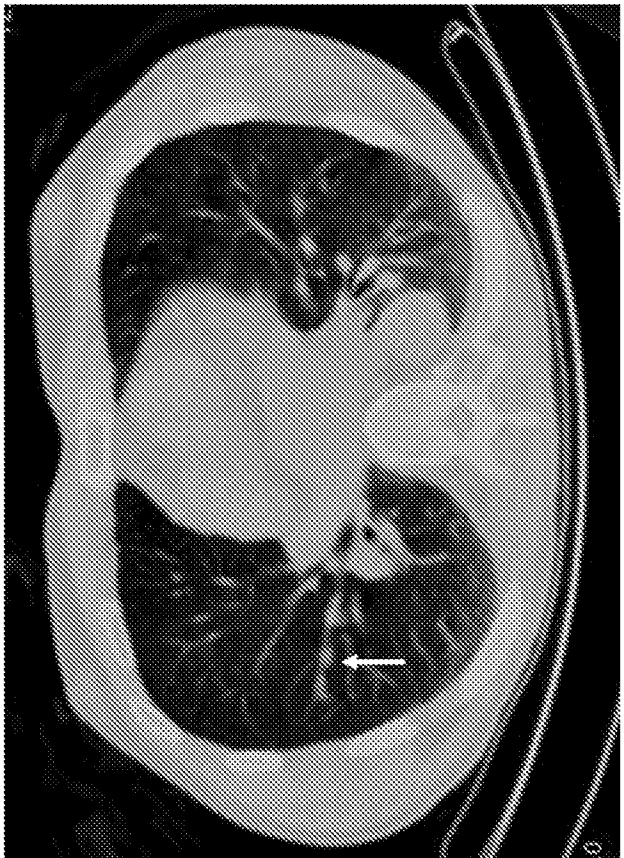
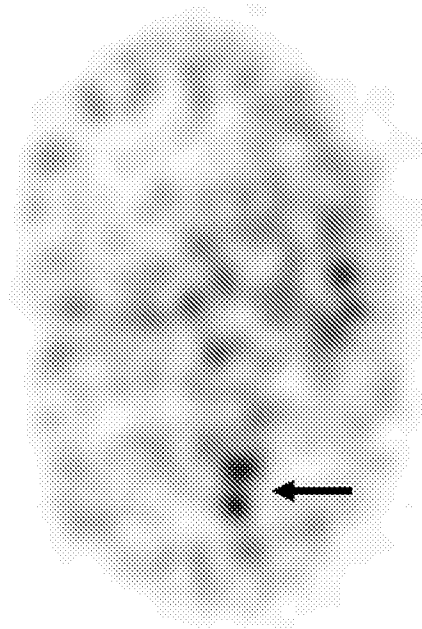


FIG. 2D



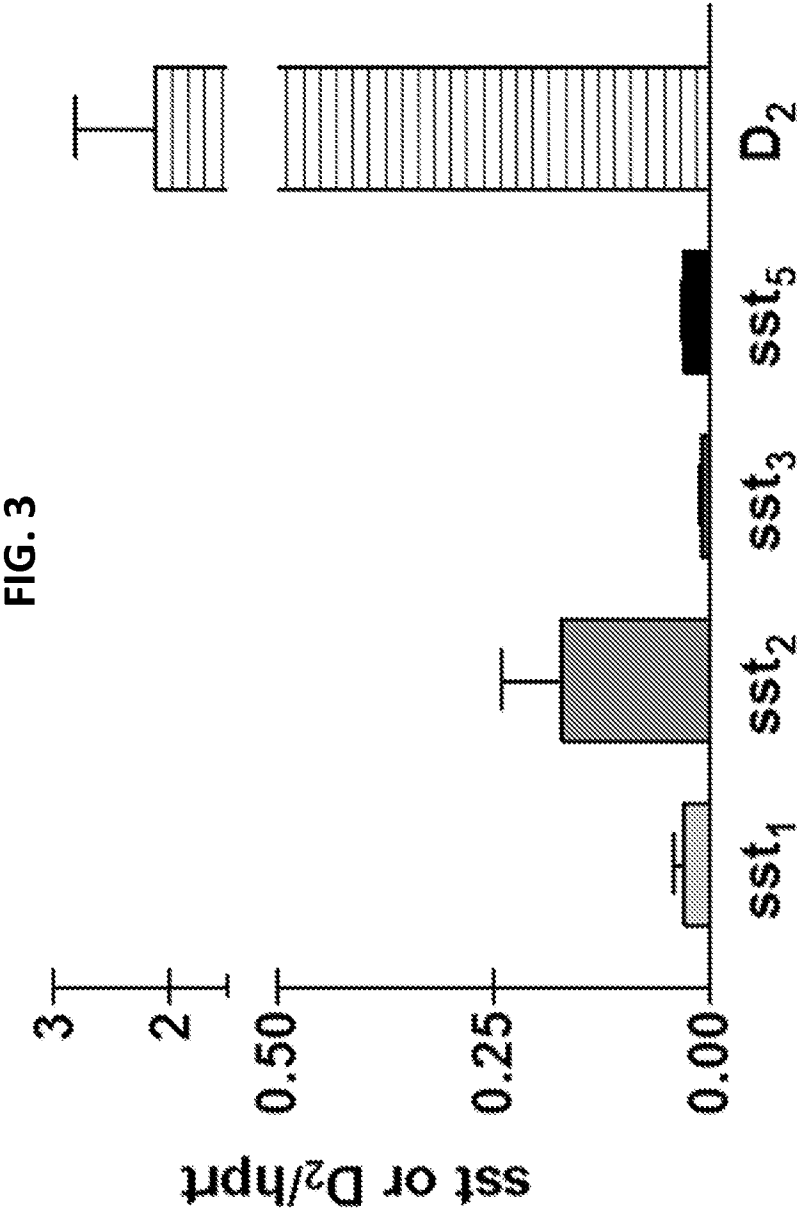


FIG. 4

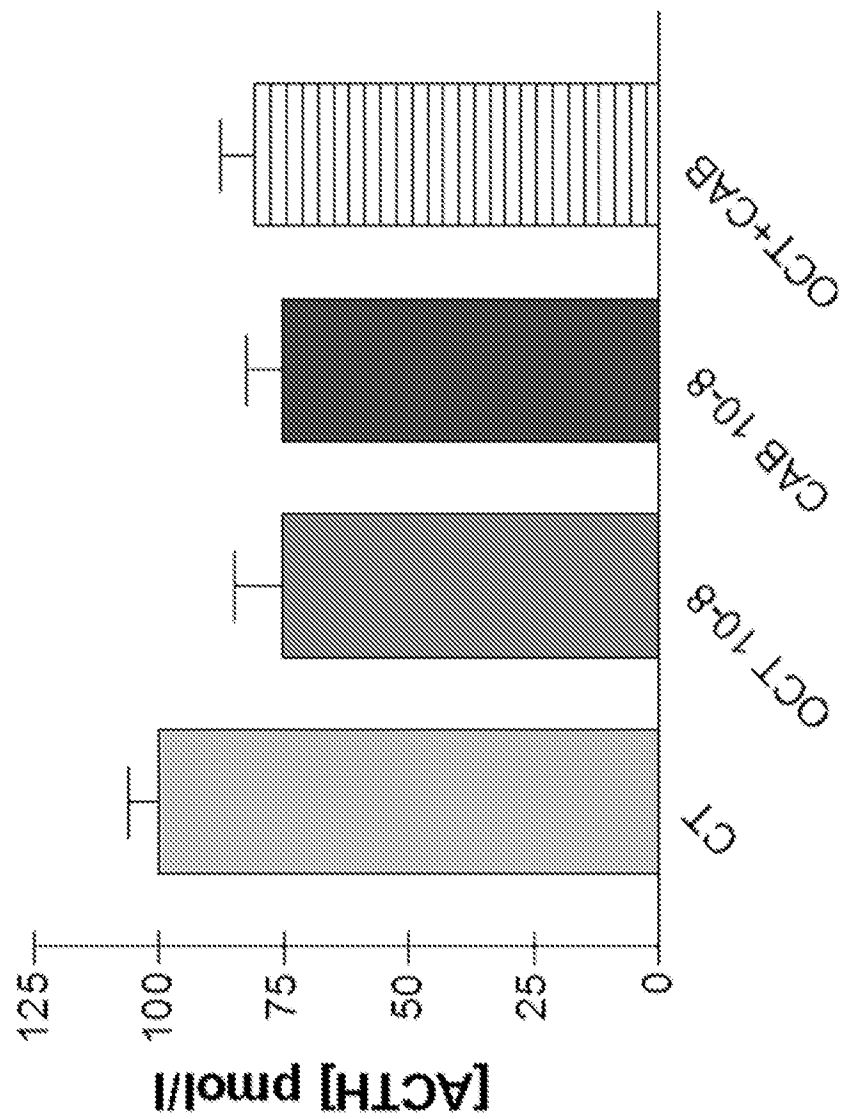


FIG. 5A

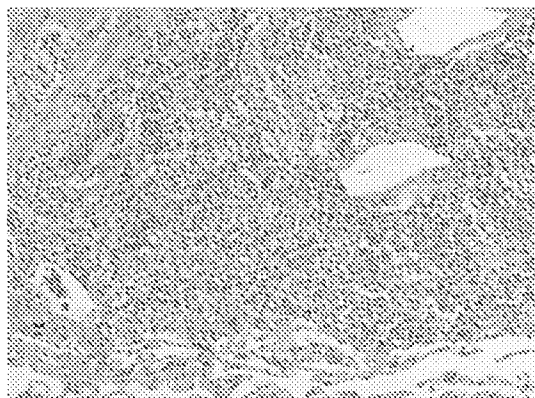


FIG. 5B



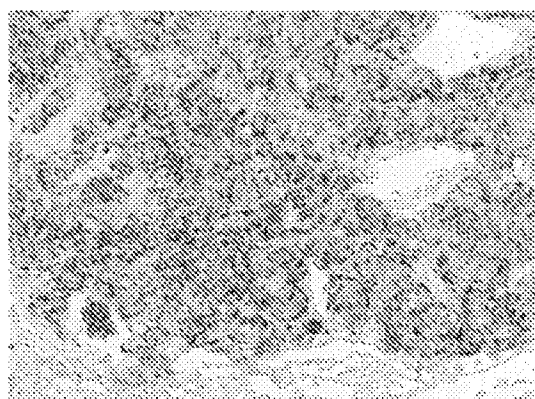
FIG. 5C



FIG. 5D



FIG. 5E



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/054666

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K51/08 A61P35/00 A61K45/06
ADD.

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)
A61K

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

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

EPO-Internal, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Christiaan de Bruin: "Somatostatin and dopamine receptors as molecular targets for the medical treatment of Cushing's disease", 1 July 2009 (2009-07-01), XP002689849, ISBN: 978-90-8559-545-8 Chapter 6, pages 107-118; Chapter 8, page 139; Chapter 9, paragraph bridging pages 150-151 ----- -/--	1-28



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"E" earlier application or patent but published on or after the international filing date

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"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

14 January 2013

Date of mailing of the international search report

23/01/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Birikaki, Lemonia

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/054666

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	F CASTINETTI ET AL: "Merits and pitfalls of mifepristone in Cushing's syndrome", EUROPEAN JOURNAL OF ENDOCRINOLOGY, vol. 160, no. 6, 1 June 2009 (2009-06-01), pages 1003-1010, XP55048968, ISSN: 0804-4643, DOI: 10.1530/EJE-09-0098 abstract pages 1003-1004, "Introduction" pages 1007-1009, "Discussion" -----	1-28
Y	S. TSAGARAKIS: "A Reappraisal of the Utility of Somatostatin Receptor Scintigraphy in Patients with Ectopic Adrenocorticotropin Cushing's Syndrome", JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM, vol. 88, no. 10, 1 October 2003 (2003-10-01), pages 4754-4758, XP55049072, ISSN: 0021-972X, DOI: 10.1210/jc.2003-030525 the whole document -----	1-28
Y	SANGUIN F ET AL: "Diagnostic and therapeutic challenge in the management of a patient with ectopic adrenocorticotropin secretion", JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION, vol. 33, no. 7, 2010, pages 507-508, XP008159100, ISSN: 0391-4097 the whole document -----	1-28
X,P	DE BRUIN C ET AL: "Mifepristone Effects on Tumor Somatostatin Receptor Expression in Two Patients with Cushing's Syndrome due to Ectopic Adrenocorticotropin Secretion", JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM, vol. 97, no. 2, February 2012 (2012-02), pages 455-462, XP008159158, DOI: 10.1210/JC.2011-1264 the whole document -----	1-28