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(54) Title: DIAGNOSTIC AND THERAPEUTIC COMPOSITIONS AND METHODS FOR AFFECTING TUMOR GROWTH USING OXYGEN MIMETIC AGENTS

(57) Abstract: The present invention relates generally to the use of oxygen mimetic compounds or agents to limit the growth of hypoxic tissue, such as solid tumors, through the delivery of cytotoxic agents, and/or a reduction in the amount of released pro-angiogenic factors responsible for new blood vessel formation.



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(A) TITLE

**DIAGNOSTIC AND THERAPEUTIC COMPOSITIONS AND
METHODS FOR AFFECTING TUMOR GROWTH USING
OXYGEN MIMETIC AGENTS**

6

(B) CROSS-REFERENCE TO RELATED APPLICATIONS

Not Applicable

(C) FEDERAL SPONSORSHIP

11

Not Applicable

(D) BACKGROUND OF THE INVENTION

(D1) FIELD OF THE INVENTION

The present invention is directed to delivering an agent with oxygen mimetic properties to hypoxic tissue such as tumor cells and affecting the growth of the hypoxic tissue. The agents of the present invention selectively target and incorporate into hypoxic tissue, and by inherent properties of the agent itself or through the cytotoxic action of agents conjugated to the oxygen mimetic agent, accomplish growth inhibition of the hypoxic tissue and/or cell killing. The diagnostic compositions and methods include oxygen mimetic agents conjugated to radionuclides, such as Technetium-99, Iodine-123, or Iodine-131, to provide a means of imaging the cancerous tissue. Comparison of the amounts of hypoxic tissue before and after various cancer therapies provides a measure of treatment efficacy.

26

(D2) DESCRIPTION OF RELATED ART

Anti-tumor therapy has, in general, consisted of radiotherapy, chemotherapy and immunotherapy. Radiotherapy can involve direct exposure of the cancerous tissue to radiation delivered by a targeting agent to the tumor site with minimal exposure of

1 normal tissue to radiation damage or radiation delivered to general areas of the body
with consequent increased exposure of normal tissue to radiation. Chemotherapy
involves delivery of cytotoxic agents to the tumor site with the aim of preferentially
destroying cancerous tissue over normal tissue. The literature is replete with examples
of side effects to both radiation therapy and chemotherapy. Immunotherapy has been
6 studied extensively over the past several years with varied results, depending on the
approach taken. Unfortunately, it is generally the case that tumor specific antibodies
will not in and of themselves exert sufficient anti-tumor effects to make them useful in
cancer therapy. In contrast with their efficacy in lymphomas, immunotoxins have
proven to be relatively ineffective in the treatment of solid tumors such as carcinomas.
11 The principal reason for this is that solid tumors are generally impermeable to
antibody-sized molecules: specific uptake values of less than 0.001% of the injected
dose/g of tumor are not uncommon in human studies. Furthermore, antibodies that
enter the tumor mass do not distribute evenly for several reasons. Firstly, the dense
packing of tumor cells and fibrous tumor stromas present a formidable physical barrier
16 to macro-molecular transport and combined with the absence of lymphatic drainage
create an elevated interstitial pressure in the tumor core which reduces extravasation
and fluid convection. Secondly, the distribution of blood vessels in most tumors is
disorganized and heterogeneous, so some tumor cells are separated from extravasating
antibody by large diffusion distances. Thirdly, all of the antibody entering the tumor
21 may become absorbed in perivascular regions by the first tumor cells encountered,
leaving none to reach tumor cells at more distant sites.

Hypoxia defines a metabolic state in which the concentrations of oxygen in
tissue are lower than required to sustain normal cellular metabolism yet are not totally
lacking oxygen, as would be found in a state of anoxia. Tissues are hypoxic if molecular
26 oxygen (O_2) levels are below normal, but not at zero (anoxia). There are substantial
fluctuations in O_2 levels among and within various tissues under normal perfusion, so
that there is no universal base-line criterion for hypoxia. For example, radiobiological
hypoxia is most pronounced at O_2 levels below 1000 ppm (0.1%; <0.1 mm Hg pO_2)
but in other tissues, metabolic effects may be apparent at O_2 concentrations just below

1 the venous blood concentration (<30 mm Hg pO_2). Hypoxia may develop in tissues because of transient capillary occlusion, vascular or arterial damage, or inadequate angiogenesis.

Tumor hypoxia occurs through several mechanisms. The most readily apparent mechanism is rapid, uncontrolled growth of the tumor tissue such that the existing
6 nutritional stores and blood supply (oxygen) are insufficient to support cellular processes.

Oxygen deficiency is associated with a number of physiological and pathological conditions. The concentration of oxygen in healthy cells varies substantially from tissue to tissue, with lowest concentrations occurring in cells farthest from capillaries in
11 the liver. In disease, hypoxia may occur as a result of aggressive tissue proliferation together with inadequate angiogenesis, a common condition in solid tumors (cancer). Hypoxia may also occur as a result of ischemia (decreased blood supply) because of vascular rupture (stroke), vascular blockage (heart attack) or vascular or arterial disease (diabetes). Cells that are hypoxic but alive exhibit a number of unique properties,
16 including increased resistance to radiation, and up-regulation of biological factors that favor or promote re-vascularization.

Low levels of oxygen and insufficient blood supply cause the tumor cells to release agents that, directly or indirectly, promote the formation of new blood vessels (angiogenesis). Released agents that promote angiogenesis include Hypoxia-Inducible
21 Factor alpha (HIF-1), Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), angiogenin, Hepatocyte Growth Factor (HGF), Insulin-like Growth Factors, Interleukin-8 (IL-8), Platelet Activating Factor (PAF), Platelet-Derived Endothelial Growth Factor (PDEGF), Platelet-Derived Growth Factor, Transforming Growth Factor- α (TGF- α), Transforming Growth Factor- β (TGF- β), Tumor Necrosis
26 Factor- α (TNF- α). VEGF, also known as Vascular Permeability Factor (VPF) is likely the most potent pro-angiogenic molecule of the above list (Brown et al, EXS, 79:233-269, 1997).

In response to lower levels of oxygen, tumor cells undergo a "stress response" and display lower radiation sensitivity. This oxygen effect in clinical radiotherapy has

1 proven to be a significant obstacle to effective treatment. Under conditions of low
oxygen levels, cell phenotypes are expressed which favor tumor progression through
mechanisms involving resistance to therapy in addition to increased angiogenesis.

Molecular oxygen contributes to the lethal effects of low linear-energy-transfer
(LET) ionizing radiation, and is therefore a vital component of successful radiation

6 therapy of cancer using therapeutic x-rays. Oxygen-deficient cells require
approximately three times more radiation for a lethal effect than for fully oxygenated
cells. This 'oxygen effect' is due to the reactivity of oxygen with molecules that have
unpaired electrons (free radicals) and to the reactivity of singlet oxygen and other
oxygen radical species. Reaction between oxygen and free radicals results in the
11 formation of peroxy species which can inhibit cellular repair mechanisms, and which
can react with other molecules such as DNA to inactivate them. Radiation therapy
generates high concentrations of short-lived reactive species such as solvated electrons,
hydrogen radicals and hydroxy radicals. In the presence of molecular oxygen there is
also a high probability that they will interact to produce oxygen radical anions
16 (superoxide), peroxy radicals and hydroperoxy radicals, which have longer half-lives and
which react with tissue macromolecules. These reactions result in the production of
repair-resistant 'chemical adducts' which contribute to cell (tumor) death. Certain
classes of molecules will similarly form repair resistant adducts in the absence of
oxygen; these compounds are called radiosensitizers. Nitroimidazoles are among the
21 most effective and least toxic radiosensitizers developed to date.

Exposure of cancerous tissue to high levels of oxygen reverses the resistance of
the tumor to radiation therapy. In the presence of normal to high levels of oxygen,
cancer cells initiate a rapid growth phase, essential for efficient killing by the radio-
therapeutic agent. Under such circumstances the cancer cells divide rapidly and release
26 cancer-specific molecules into the blood stream, detectable using standard assay
techniques.

Targeting of hypoxic tissue such as solid tumors has been accomplished with a
variety of compounds, which selectively incorporate into the hypoxic cells. One such
example is iodoazomycin arabinoside (IAZA) (Groshar et al, Journal of Nuclear

1 Medicine, 34:885-888, 1993). This molecule has been shown to selectively incorporate into hypoxic cells of solid tumors such as small-cell lung carcinoma, non-small-cell lung carcinoma, glioblastoma, and cancers of the Head and Neck.

Previous studies have used radiolabeled IAZA as a diagnostic probe to image hypoxic tumors. ¹²³I-IAZA was well tolerated in individuals with various malignancies
6 when used as a tool to image hypoxic tissue (Urtasun et al, British Journal of Cancer, Supplement, 27:S209-S212, 1996). The doses used in imaging are much lower than would be used in therapy.

Some studies have shown superior therapeutic efficacy using tumor directed internalizing antibodies labeled with Auger-electron emitters such as ¹²⁵Iodine (¹²⁵I) in
11 comparison to beta-emitters such as ¹³¹I (Behr et al, International Journal of Cancer, 76:738-748, 1998). Higher doses of the Auger-electron emitters are tolerated by the recipient resulting in improved therapeutic outcome. The lower toxicity of the Auger-emitters may be due to the short path length of the low-energy electrons (micrometers) versus the longer path length of the beta-emitters (millimeters). Although Auger-
16 emitters are better tolerated (i.e. shorter path length of the low-energy electrons), treatment efficacy is greatly improved if the agents carrying the Auger-emitters are internalized by the tumor cell leading to improved targeting of the tumor cell DNA. Targeting of hypoxic tissue with Auger-electron labeled oxygen mimetics such as ¹²⁵I-IAZA selectively damages the DNA of the hypoxic tissue in turn preventing release of
21 pro-angiogenic factors such as HIF-1 and VEGF.

However, use of ¹³¹I-conjugated oxygen mimetic (i.e. ¹³¹I-IAZA), due to its slightly longer radiation path length, in some cases provides better therapeutic efficacy by destroying not only the hypoxic tissue responsible for the uptake of the agent, but also tissue that is immediately adjacent to the cancer tissue. This tissue is most often
26 cancerous tissue that is slowly progressing to a hypoxic state.

The efficacy of treatment varies between the different cancers, between the various therapies, between individuals receiving the same therapy, and even within the neoplasm itself with some cancer cells more resistant to a given therapy than others. Thus, often times patients receive a therapy that is ineffective in treating the

1 malignancy, yet the patient still suffers the side-effects of the therapy. These side effects
are not trivial and contribute significantly to the morbidity of the cancer patient
including hair loss, loss of taste, dry mouth, extreme nausea, cachexia, and damage to
the bone marrow. It is therefore of the utmost importance to choose the most
appropriate therapy and to monitor the efficacy of that therapy in a timely manner in
6 order to minimize unnecessary patient discomfort.

Current methods for monitoring cancer therapy include Magnetic Resonance
Imaging (MRI), radiographic analysis (X-ray), CAT scans and manual palpation.
Although these methods are readily available, they rely on detecting a measurable
reduction in tumor size and therefore require the physician to wait for a significant
11 period of time post therapy before attempting assessment. Should the patient be
receiving a therapy that is ineffective against that particular neoplasm, they are destined
to suffer the side effects of the therapy until the physician determines that the treatment
is not working, which can be as long as three to four weeks.

Rapidly growing neoplasms become hypoxic due to the cancerous tissue
16 overgrowing the existing blood and oxygen supply. The current invention encompasses
methods for rapidly determining the effectiveness of cancer therapy through the use of
oxygen mimetic agents conjugated to radionuclides.

(E) SUMMARY OF THE INVENTION

21 The present invention relates to delivery of oxygen mimetic molecules, such as
IAZA to hypoxic tissue, such as a tumor mass, as a means to selectively reduce cell
growth and/or kill the target tissue. The present invention also relates to the use of
oxygen mimetic molecules bound to radionuclides as a means of monitoring the efficacy
of various cancer therapies.

26 A hypoxic state in tumor tissue leads to enhanced resistance to radio- and
chemotherapeutic agents, in addition to initiation of new blood vessel growth through
the release of various agents that promote angiogenesis. Delivery of an agent that
specifically targets hypoxic tissue and also has oxygen mimetic properties is most
desirable.

1 Addition of the oxygen mimetic molecule to the hypoxic tissue (e.g. tumor) interferes with the natural compensatory mechanism for hypoxic stress (i.e. the calling of new blood vessels, angiogenesis), in turn limiting further tumor growth. For example release of agents such as HIF-1 and/or VEGF are reduced thereby limiting the formation of new blood vessels feeding the tumor.

6 The oxygen mimetic molecule can be delivered in a conjugated or un-conjugated form. Preferred agents to which the oxygen mimetic is conjugated include cytotoxic agents, radioactive agents and oligonucleotides.

 Addition of a radio-conjugated oxygen mimetic (e.g. ^{125}I -IAZA, ^{131}I -IAZA) to hypoxic tissue accomplishes several tasks: I) the hypoxic tissue of the tumor is targeted preferentially, II) the radiosensitivity of the tumor tissue is increased due to the oxygen mimetic properties of the agent, III) the Auger-emitting ^{125}I conjugate selectively targets the hypoxic cells, which have internalized the agent, IV) the ^{131}I conjugate destroys hypoxic tissue and marginally hypoxic tissue through the "crossfire" effect of the ^{131}I isotope, V) release of agents promoting blood vessel growth (e.g. VEGF) are reduced.

16 Typical vascularized tumors are the solid tumors, particularly carcinomas, which require a vascular component for the provision of oxygen and nutrients. Exemplary solid tumors to which the present invention is directed include, but are not limited to, carcinomas of the lung, breast, ovary, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, prostate, thyroid, squamous cell carcinomas, adenocarcinomas, small cell carcinomas, melanomas, gliomas, neuroblastomas, and the like.

(F) DESCRIPTION OF DRAWINGS

Not applicable

(G) DETAILED DESCRIPTION OF THE INVENTION

 The present invention provides compositions and methods for delivering agents with oxygen mimetic properties to hypoxic tissue, such as tumor cells, thereby affecting the growth of the hypoxic tissue. In preferred embodiments of the invention, the

1 agents with oxygen mimetic properties are nitroimidazole or nitroimidazole-like compounds, and they are allowed to incorporate in the hypoxic tissue, thus inducing an altered state. In accordance with the present invention, the altered state leads to a mediation in the amount of released pro-angiogenic factors. As used herein, mediation refers to an increase, a decrease, or combinations thereof.

6 In accordance with one embodiment of the present invention, there is provided a method for measuring the effectiveness of therapy intended to kill neoplastic tissue or cells in a mammal, comprising the steps of obtaining molecules with oxygen mimetic properties, which bind preferentially to hypoxic cancerous tissue, the oxygen mimetic agent being labeled; contacting the labeled oxygen mimetic with tissue of a mammal
11 that has received therapy to kill neoplastic cells *in vivo*; and determining the effectiveness of therapy by measuring the binding of the labeled oxygen mimetic agent to the remaining hypoxic cancerous tissue.

In a preferred embodiment of the invention, the oxygen mimetic agent binds specifically to hypoxic cancer tissue or cells, and the contacting step preferably
16 comprises administering the labeled oxygen mimetic to a mammal *in vivo*. Preferred labels include radionuclides, magnetic resonance enhancing agents, and radiopaque materials, and preferred methods for measuring binding or incorporation of labeled oxygen mimetic to hypoxic tissue include imaging techniques including scintigraphic, magnetic resonance and radiographic imaging.

21 The present invention provides compositions and methods for monitoring the efficacy of cancer therapy by delivering labeled agents with oxygen mimetic properties to hypoxic tissue, such as tumor cells, pre- and post-treatment, thereby providing a measure of tumor tissue reduction based on the binding or incorporation of the oxygen mimetic agent into the remaining hypoxic neoplastic tissue.

26 The present invention also provides compositions and methods for measuring hypoxia *in vivo*, preferably hypoxia related to and/or associated with cancer, by delivering labeled agents with oxygen mimetic properties to hypoxic tissue, such as tumor cells, pre- and post-treatment, thereby providing a measure of localized hypoxia based on the binding or incorporation of the oxygen mimetic agent into the remaining

1 hypoxic tissue (e.g., neoplastic tissue).

The oxygen mimetic agent is constructed such that the agent is preferentially incorporated into hypoxic tissue. In a preferred embodiment of the invention, the agent would preferentially incorporate into tumor cells, in vivo. Examples of oxygen mimetic agents suitable for radiolabeling include, but are not limited to: IAZA,

6 iodoazomycin galactoside (IAZG), iodoazomycin pyranoside (IAZP),
fluoroiodoazomycin pyranoside (FIAZP), IAZGlu (a glucoside), 1-(4-iodo-4-
deoxylyxopyranosyl)-2-nitroimidazole (IAZLP), iodoazomycin riboside (IAZR),
iodovinylmisonidazole (IVM), 2-nitroimidazolyl-1-(iodo-4-hydroxyphenyl) ethanols
(NIHE's), 2-(2-nitroimidazolyl)iodohydroxyacetophenones (NIHA's), [2-(4-
11 iodophenoxy)ethyl]-2-nitroimidazole (IPENI), 1-(2-hydroxy-3-methoxy)-4(5)-iodo-5(4)-
nitroimidazole (I-nitroMHPI's), iodometronidazole, 4-bromomisonidazole (Br-MISO),
and misonidazole (MISO). An exemplary oxygen mimetic would be iodoazomycin
arabinoside (IAZA).

In a further preferred embodiment of the invention, the oxygen mimetic agent
16 would be radiolabeled with Auger electron emitters. Exemplary Auger-emitters
include, but are not limited to: ¹²⁵Iodine, ¹²³Iodine.

In a further preferred embodiment of the invention, the oxygen mimetic agent
would be radio labeled with ¹³¹Iodine.

For imaging purposes any of the well know medical radionuclides can be used.
21 Suitable radionuclides include ¹³¹Iodine, ¹²³Iodine, ¹²⁵Iodine, ¹¹¹Indium, ¹¹³Indium,
⁶⁷Gallium, ⁹⁹Technetium or other suitable gamma-emitters. Radiopaque materials also
may be used to label the oxygen mimetic agent. Suitable radiopaque materials include,
but are not limited to iodine compounds, barium compounds, gallium compounds, and
thallium compounds. Specific examples of radiopaque materials include barium,
26 diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide,
iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic
acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosumetic acid, iotasul, iotetric
acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine,
metrizamide, metrizoate, propylidone, and thallous chloride.

1 Materials that can be detected by or that enhance the effects of magnetic resonance imaging equipment also may be conjugated to the oxygen mimetic agent of the present invention. Suitable magnetic resonance-enhancing compounds include, but are not limited to, gadolinium, copper, iron, and chromium.

 In an embodiment of the invention, alpha-emitting and beta-emitting
6 radionuclides may be used. Such compounds include, but are not limited to, I-131, Y-90, Cu-67, Au-198, and P-32.

 As used herein, "administering" refers to any action that results in exposing or contacting a composition containing a binding agent with a pre-determined cell, cells, or tissue, typically mammalian. Administering may be conducted *in vivo*, *in vitro*, or *ex vivo*.
11 For example, a composition may be administered by injection or through an endoscope or catheter. Administering also includes the direct application to cells of a composition according to the present invention. For example, during the course of surgery, the vasculature of tumor or hyperplastic tissue may be exposed. In accordance with an embodiment of the invention, the exposed cells or vasculature may be exposed
16 directly to a composition of the present invention, e.g., by washing or irrigating the surgical site, and/or the cells.

 The composition may also include pharmaceutically acceptable carriers. Pharmaceutically acceptable carriers include but are not limited to saline, sterile water, phosphate buffered saline, and the like. Other buffering agents, dispersing agents, and
21 inert non-toxic substances suitable for delivery to a patient may be included in the compositions of the present invention. The compositions may be solutions suitable for administration, and are typically sterile and free of undesirable particulate matter. The compositions may be sterilized by conventional sterilization techniques.

 For example, the therapeutic or diagnostic agent may be introduced into the
26 patient by an intravenous, subcutaneous, intraperitoneal, intrathecal, intravesical, intradermal, intramuscular, or intralymphatic route. The composition may be in solution, tablet, aerosol, or multi-phase formulation forms. Liposomes, long-circulating liposomes, immunoliposomes, biodegradable microspheres, micelles, or the like may also be used as a carrier, vehicle, or delivery system. The clinician may compare the

1 responses associated with these different routes in determining the most effective route
of administration. The invention should not be limited to any particular method of
introducing the binding agent into the patient.

Administration may be once, more than once, and over a prolonged period. As
the compositions of this invention may be used for patients in a serious disease state,
6 i.e., life threatening or potentially life-threatening, excesses of the binding agent may be
administered if desirable. Actual methods and protocols for administering
pharmaceutical compositions, including dilution techniques for injections of the present
compositions, are well known or will be apparent to one skilled in the art. Some of
these methods and protocols are described in Remington's Pharmaceutical Science,
11 Mack Publishing Co. (1982).

The composition may be administered in combination with other agents or
regimens to effect tumor arrest, regression or killing. The composition may be
administered in the presence of agents that enhance the uptake of the composition such
as permeating agents.

16 One skilled in the art will also recognize that liposomes, nanospheres, micelles,
or microspheres may be used to administer a composition, and that such administration
may result in a therapeutically desirable benefit.

Definitions:

21 As used herein, "oxygen mimetic" refers generally to the group of molecules
known as nitroimidazoles, which undergo reversible 1-electron reductions in hypoxic
tissue. One or more of these reduced products can react chemically with cellular
components to form non-diffusible adducts.

As used herein, hypoxic tissue refers to any tissue, including cancerous tissue
26 that contains lower levels of oxygen (e.g., < 10 mm Hg) than normally found in that
tissue.

As used herein, adjunct therapy refers to any treatment used in combination
with, or as follow-up to, an existing therapeutic regimen. As an example, Head and
Neck tumors treated with an existing chemotherapeutic regimen such as cisplatin,

- 1 cyclophosphamide and doxorubicin, as an example, could be concomitantly or treated
in follow-up with the oxygen mimetic therapy of the present invention.

EXAMPLES

Example 1.

- 6 Using a modification of the method published by Sakaguchi et al (Journal of
Medical Chemistry, 26:20-24, 1983) azomycin arabinoside was prepared by coupling
azomycin to a 1-bromo-protected sugar. Azomycin arabinoside was iodinated using the
triprophine/iodine method of Jette et al (Radiation Research, 105:169-179, 1986).

- Azomycin arabinoside was synthesized by coupling the corresponding protected
11 1-bromo sugar to azomycin using a modification of the published procedure. Although
the α -bromo anomer was used to produce the β -nucleoside, and contrary to earlier
reports of β -nucleoside formation, the α -nucleoside has been confirmed to be the major
product of the coupling and, when C5'-iodinated, to be the product referred to in the
literature as IAZA.

- 16 1- α -D-(5-Iodo-5-deoxy-arabinofuranosyl)-2-nitroimidazole (IAZA) was initially
radiolabeled by exchange from Na*I in DMF, but for patient studies the pivalic acid
melt was used because of greater radiochemical yield ($\sim 90\%$) and higher chemical and
radiochemical purity (both $> 92\%$).

- Conformational differences between IAZR and IAZA, resulting from the change
21 in configuration at C-1' (from β to α) and C-2' (from 'ribo - down' in IAZR to 'arabino
- up' in IAZA) introduced substantial physicochemical changes. The P of IAZA (4.98)
is double that for IAZR, but the *in vitro* rate of hypoxia-dependent adduct formation
was identical to IAZR rates, and radiosensitization and cytotoxicity properties were
similar between the two nucleosides.

26

Example 2.

Growth of tumor cells such as EMT-6 (mouse fibrosarcoma), LnCAP (human
prostate cancer) and MCF-7 (human breast carcinoma) under hypoxic conditions
demonstrated selective uptake of oxygen mimetic molecules such as IAZA, ^{131}I -IAZA,

1 and ^{125}I -IAZA.

Example 3.

Patients with various solid tumors were imaged with ^{123}I -IAZA to identify hypoxic tissue in the tumor mass. Of 23 Small Cell Lung Carcinomas imaged, 15
6 demonstrated uptake of the ^{123}I -IAZA in the tumor tissue. Of 6 glioblastomas imaged, 2 demonstrated uptake of ^{123}I -IAZA in the tumor tissue. Of 20 Head and Neck cancers imaged, 10 demonstrated uptake of the ^{123}I -IAZA in the tumor tissue. Of 4
Astrocytomas imaged, 1 demonstrated uptake of the ^{123}I -IAZA in the tumor tissue. Of
1 Rhabdomyosarcomas imaged, 1 demonstrated uptake of the ^{123}I -IAZA in the tumor
11 tissue.

Example 4.

^{131}I -IAZA is used to image various tumors in human subjects and demonstrates selective incorporation in these hypoxic tissues. Imaging studies will be performed in 5
16 subjects with tumors demonstrating defined areas of hypoxia.

^{131}I -IAZA is administered by slow intravenous injection; in previous studies no side effects to the injection have been noted; 200-300 MBq will be administered and the patient monitored after injection with measurement of pulse and blood pressure and observation. Planar whole body images will be acquired immediately after injection
21 and at 4, 24, 48, 72 and 96 hours after injection. Each set of images will take approximately 30 minutes. No SPECT images will be required for this protocol. Images will be acquired on a Marconi Axis gamma camera interfaced to an Odyssey computer. A high energy collimator will be used for all images and all images acquired to time.

26 Prior to imaging, and at each daily imaging time point, blood samples will be collected for CBC and SMA12 to confirm the lack of adverse events after injection of the radiopharmaceutical labeled with Iodine-131. No changes were observed after ^{123}I -IAZA injection.

Dosimetry calculations will be performed using the MIRD formalism and

1 patterns of uptake and distribution reviewed from the derived images. The key data
derived from this study will be (a) retention of ^{131}I -IAZA in tumor and (b) whole body
dosimetry calculations. A successful outcome will be demonstration of prolonged
uptake and retention, low whole body dosimetry and adequate tumor dosimetry.

6 Example 5.

An oxygen mimetic agent such as that produced in Example 1 (IAZA) can be
labeled with I-125, I-131 or I-123 by standard procedures as outlined by Weibe et al (US
patent 5401490).

11 Example 6.

^{131}I -IAZA is used to image solid tumors such as tumors of the Head and Neck,
prostate tumors, small cell lung carcinomas, and the like, prior to initiating cancer
therapy. Radiographic analysis using the labeled oxygen mimetic provides a baseline
reading of the location and extent of hypoxic neoplastic tissue. The subject is then
16 exposed to the anti-tumor therapy and after waiting an appropriate period of time for
the therapy to have taken effect, as would be known to those skilled in the art, the
tumor is again imaged to measure the incorporation of the ^{131}I -IAZA into the tumor
tissue. Reduction in ^{131}I -IAZA-incorporation is indicative of a desirable therapeutic
regimen. An increase in the area of ^{131}I -IAZA-incorporation may prompt higher dosage
21 of the therapeutic agent or abandoning of the current therapy in favor of a more
promising therapy. In this way, therapy can be adjusted on an individual basis to
maximize tumor killing while minimizing undesirable treatment side effects.

Example 7.

26 IAZA has been studied in a variety of experimental pathologies. In a
photodynamic therapy (PDT) rat prostate tumor model (Copenhagen x Fisher rats
bearing both Dunning R3327R), ^{123}I IAZA uptake in regions of PDT-induced hypoxia
showed an inverse correlation with $^{99\text{m}}\text{Tc}$ -HMPAO uptake. This provided strong
evidence for hypoxia-selective binding of ^{123}I IAZA. These conclusions were recently

1 confirmed in autoradiographic studies of a rat model of cerebral occlusion, in which
dual radionuclide autoradiography clearly depicted uptake of $^{125}\text{IAZA}$ in ischemic areas
and (mutually exclusively) uptake of $^{99\text{m}}\text{Tc-HMPAO}$ in well-perfused regions.
Preliminary imaging studies in a surgical model of canine myocardial ischemia showed
uptake of $^{123}\text{IAZA}$ in *ex vivo* images but not in *in vivo* images taken 4 h after dosing.

6 Example 8.

In vitro binding rates for azomycin derivatives, for example, Miso [Chapman, et
al., *Cancer Res.*, 43:1523-1528 (1983)], increase as a function of their concentration in the
culture medium. The azomycin nucleosides demonstrate this property over a similar
11 concentration range (20-1000 μM).

This concentration dependency has also been demonstrated *in vivo* in murine
EMT-6 and RIF-1 models. In these studies, $^{125}\text{IAZA}$ binding was directly proportional
to the intraperitoneal dose of $^{125}\text{IAZA}$ at concentrations of 0.5, 5 and 50 μM , indicating
concentration-independent access of the tracer to the hypoxic regions of the tumor and
16 confirming dose-dependent binding. It has also been shown that large iv doses of
 $^{125}\text{IAZA}$, given after iv $^{125}\text{IAZA}$, produce a drastic 'wash-out' effect, lowering blood levels
dramatically without affecting tumor uptake to produce tumor:blood ratios of 8.7 at 4
h (compared to 6.3 in controls) in the murine EMT-6 model. Although the impact of
dose on uptake in hypoxic tissue has not been systematically investigated in patients,
21 radiopharmacokinetic and radiotracer kinetics in human volunteers who received iv
doses of $^{123}\text{IAZA}$ ranging from 0.1 to 10 mg show no discernible difference in plasma
clearance and whole body elimination over this 1000-fold concentration range.

Example 9.

26 $^{125}\text{IAZR}$ was obtained by exchange from Na^{125}I in DMF at 84° for 3 h
(radiochemical yield 85%). IAZR was approximately 5 times more lipophilic than Miso
($P = 2.1$ vs. 0.43, respectively), both of which are considerably more lipophilic than the
non-iodinated AZR ($P = 0.04$). Introduction of iodine also increased protein binding
(from $<1\%$ for Miso and AZR, to 20% for IAZR); radiosensitizer efficacy was

1 increased by 2-3 times over AZR (5-10 times over Miso), and cytotoxicity was about 25
times greater than Miso. In graded hypoxia studies, the rate of adduct formation by
IAZR was 2.5-3 times greater than for Miso at concentrations from 10- 100 μ M.

Example 10.

6 1-(6-Iodo-6-deoxy- β -D-galactopyranosyl)-2-nitroimidazole (IAZG) was designed
to have a lower *P* than IAZA. This was accomplished by using a hexose rather than the
pentose sugars of IAZR and IAZA. The non-iodinated nucleoside (AZG) was prepared
by coupling the blocked α -bromogalactose with azomycin, and IAZG was prepared by
iodination with triphenylphosphine and iodine, using standard work-up procedures.
11 IAZG was reported to have a *P* of 0.57 and hypoxia-dependent binding rates somewhat
lower than for IAZA and Miso in EMT-6 cells *in vitro*.

¹²⁵IAZG (exchange labeling) was initially found to be inferior to IAZA,
providing lower tumor uptake and somewhat slower blood clearance after injection
into mice bearing implanted EMT-6 tumors. More recent data also ascribe an *in vitro*
16 binding rate to IAZG that is lower than that for IAZA, thereby providing independent
confirmation of the earlier findings.

Example 11.

21 1-(4-Iodo-4-deoxy-L-xylopyranosyl)-2-nitroimidazole (IAZP) was synthesized by
coupling the protected arabinopyranosyl bromide to azomycin, followed by deblocking
and iodination using triphenylphosphine and iodine, with the appropriate work-up.
IAZP differs from IAZA in that the sugar is in the pyranosyl (5-carbons and oxygen in
the ring) form rather than the furanosyl form, so that both compounds have the C2' &
C3' 'arabino' configuration, but significantly, the iodo substituent is on C4', a
26 secondary alkyl iodide in IAZP, rather than as a primary alkyl iodide. This alteration
was introduced in order to stabilize the nucleoside against deiodination, without greatly
affecting other properties. The configuration of this nucleoside at C' was originally
assigned as β -, but recent findings have shown, based on NMR and x-ray
crystallography, respectively, that the configuration of this nucleoside is the α - form.

- 1 Schneider also reports the recovery of the D-enantiomer. The *P* values reported for IAZP, however, are much lower than for IAZA.

Example 12.

- Recently developed 2-nitroimidazole nucleosides with lower *P* values than IAZA include the glucoside, the 2'-I-arabinoside and the glucuronide. The glucoside (IAZGlu), a hexose glycoside like IAZG, is slightly more lipophilic than IAZG (*P* = 1.07 vs. 0.57 in this report) but has among the lowest binding rates and provided the lowest tumor:blood ratio. The other new nucleoside, 1-(4-iodo-4-deoxylyxopyranosyl)-2-nitroimidazole (IAZLP) is slightly less lipophilic than IAZGlu, intermediate in binding rate and produced the lowest of the reported tumor:blood ratios in a tumor model.

Example 13.

- The radioiodinated 2-nitroimidazoles (azomycin derivatives) comprise the main body of the literature that deals with agents for scintigraphic detection of tissue hypoxia. It is clear from the literature that ring-halogenated nitroimidazoles (radioiodine or radiobromine) are not chemically or metabolically labile (Br-Miso; 4-I-5-nitroMHPI) and/or have low electron affinity (Iodometronidazole; 4-I-5-nitroMHPI). An advantage of the side-chain-halogenated azomycin derivatives is their relatively narrow range of E^1_7 's, within the range for oxygen-reversible reductions to occur in hypoxic tissues. The molecular design then becomes primarily a matter of adjusting (non-reductive) metabolic and pharmacokinetic properties. Compounds reported to date fall into two general categories *viz.*, misonidazole congeners and azomycin glycosides.

1 (H) CLAIMS

We claim:

1. A method of treating hypoxic tissue comprising administering a nitroimidazole or nitroimidazole-like agent with oxygen mimetic properties, allowing the agent to incorporate into the hypoxic tissue, and inducing an altered state of the hypoxic
6 cells leading to a mediation in the amount of released pro-angiogenic factors.
2. The method of claim 1, wherein the mammal is a human.
3. The method of claim 2, wherein the nitroimidazole or nitroimidazole-like agents are
11 selected from, but are not limited to, iodoazomycin arabinoside (IAZA), iodoazomycin galactoside (IAZG), iodoazomycin pyranoside (IAZP), fluoroiodoazomycin pyranoside (FIAZP), IAZGlu (a glucoside), 1-(4-iodo-4-deoxylyxopyranosyl)-2-nitroimidazole (IAZLP), iodoazomycin riboside (IAZR), iodovinylmisonidazole (IVM), 2-nitroimidazolyl-1-(iodo-4-hydroxyphenyl) ethanols
16 (NIHE's), 2-(2-nitroimidazolyl)iodohydroxyacetophenones (NIHA's), [2-(4-iodophenoxy)ethyl]-2-nitroimidazole (IPENI), 1-(2-hydroxy-3-methoxy)-4(5)-iodo-5(4)-nitroimidazole (I-nitroMHPI's), iodometronidazole, 4-bromomisonidazole (Br-MISO), and misonidazole (MISO).
- 21 4. The method of claim 2, wherein the pro-angiogenic factors include, but are not limited to, Hypoxia-Induced Factor alpha (HIF-1), Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), angiogenin, Hepatocyte Growth Factor (HGF), Insulin-like Growth Factors, Interleukin-8 (IL-8), Platelet Activating Factor (PAF), Platelet-Derived Endothelial Growth Factor (PDEGF), Platelet-
26 Derived Growth Factor (PDGF), Transforming Growth Factor- α (TGF- α), Transforming Growth Factor- β (TGF- β), Tumor Necrosis Factor- α (TNF- α).
5. The method of claim 1, wherein mediation in the release of pro-angiogenic molecules comprises reducing the formation of new blood vessels feeding the

- 1 hypoxic tissue.
6. The method of claim 2, wherein the agent is conjugated to a radioisotope.
7. The method of claim 6, wherein the agent is conjugated to an Auger electron-
- 6 emitting isotope.
8. The method of claim 6, wherein the agent is conjugated to a beta particle emitting isotope.
- 11 9. The method of claim 8 wherein the isotope is ¹³¹I.
10. The method of claim 7, wherein incorporation of the conjugate into the hypoxic tissue further limits release of proangiogenic factors through damage of cellular molecules required for the production of said proangiogenic factors.
- 16 11. The method of claim 10, wherein hypoxic tissues are selectively affected over normal tissues due to preferential incorporation of the conjugate into hypoxic tissue and the subsequent low energy effects of the Auger electron emission from the radionuclide.
- 21 12. The method of claim 10, wherein hypoxic tissues are selectively affected over normal tissues due to preferential incorporation of the conjugate into hypoxic tissue and the subsequent effects of the beta particle emission from the radionuclide.
- 26 13. The method of claim 2, wherein the nitroimidazole or nitroimidazole-like agent is conjugated to an oligonucleotide.
14. The method of claim 13, wherein nitroimidazole or nitroimidazole-like agents are selected from, but are not limited to, IAZA, iodoazomycin galactoside (IAZG),

- 1 iodoazomycin pyranoside (IAZP), fluoroiodoazomycin pyranoside (FIAZP),
IAZGlu (a glucoside), 1-(4-iodo-4-deoxylyxopyranosyl)-2-nitroimidazole (IAZLP),
iodoazomycin riboside (IAZR), iodovinylmisonidazole (IVM), 2-nitroimidazolyl)-1-
(iodo-4-hydroxyphenyl)ethanols (NIHE's), 2-(2-
nitroimidazolyl)iodohydroxyacetophenones (NIHA's), [2-(4-iodophenoxy)ethyl]-2-
6 nitroimidazole (IPENI), 1-(2-hydroxy-3-methoxy)-4(5)-iodo-5(4)-nitroimidazole (I-
nitroMHPI's), iodometronidazole, 4-bromomisonidazole (Br-MISO), and
misonidazole (MISO).
15. The method of claim 2, wherein the nitroimidazole or nitroimidazole-like agent
11 is conjugated to a cytotoxic agent.
16. The method of claim 2, wherein administration of the nitroimidazole or
nitroimidazole-like agent is performed as an adjunct to other methods of treating
solid tumors.
- 16 17. The method of claim 6, wherein administration of the nitroimidazole or
nitroimidazole-like agent is performed as an adjunct to other methods of treating
solid tumors.
- 21 18. A composition for treating hypoxic tissue in vivo comprising a nitroimidazole or
nitroimidazole-like agents, said agents are one or more agents selected from the
group consisting of IAZA, iodoazomycin galactoside (IAZG), iodoazomycin
pyranoside (IAZP), fluoroiodoazomycin pyranoside (FIAZP), IAZGlu (a glucoside),
1-(4-iodo-4-deoxylyxopyranosyl)-2-nitroimidazole (IAZLP), iodoazomycin riboside
26 (IAZR), iodovinylmisonidazole (IVM), 2-nitroimidazolyl)-1-(iodo-4-
hydroxyphenyl)ethanols (NIHE's), 2-(2-nitroimidazolyl)iodohydroxyacetophenones
(NIHA's), [2-(4-iodophenoxy)ethyl]-2-nitroimidazole (IPENI), 1-(2-hydroxy-3-
methoxy)-4(5)-iodo-5(4)-nitroimidazole (I-nitroMHPI's), iodometronidazole, 4-
bromomisonidazole (Br-MISO), and misonidazole (MISO); and a pharmaceutically

1 acceptable carrier.

19. A method for measuring the effectiveness of therapy intended to kill tumor cells in vivo, in a mammal, comprising the steps of: obtaining a nitroimidazole or nitroimidazole-like agent with oxygen mimetic properties, said agent being labeled; contacting said labeled agent with hypoxic tissue of a mammal that has received therapy to kill malignant cells in vivo; and determining the effectiveness of said therapy by measuring the incorporation of said labeled agent in said hypoxic tissue.

20. The method of claim 19 wherein said label is a radionuclide.

21. The method of claim 20, wherein said radionuclide is selected from the group consisting of TC-99, I-123, I-131, In-111 and Ga-67.

22. The method of claim 21 where the agent with oxygen mimetic properties include, but are not limited to, iodoazomycin arabinoside (IAZA), iodoazomycin galactoside (IAZG), iodoazomycin pyranoside (IAZP), fluoroiodoazomycin pyranoside (FIAZP), IAZGlu (a glucoside), 1-(4-iodo-4-deoxylyxopyranosyl)-2-nitroimidazole (IAZLP), iodoazomycin riboside (IAZR), iodovinylmisonidazole (IVM), 2-nitroimidazolyl-1-(iodo-4-hydroxyphenyl) ethanols (NIHE's), 2-(2-nitroimidazolyl)iodohydroxyacetophenones (NIHA's), [2-(4-iodophenoxy)ethyl]-2-nitroimidazole (IPENI), 1-(2-hydroxy-3-methoxy)-4(5)-iodo-5(4)-nitroimidazole (I-nitroMHPI's), iodometronidazole, 4-bromomisonidazole (Br-MISO), and misonidazole (MISO).

23. The method of claim 2, wherein said label is a radiopaque material.

24. The method of claim 2, wherein said label is a magnetic resonance enhancing agent.

25. The method of claim 2, wherein the incorporation of said labeled agent into said

- 1 hypoxic tissue is measured by imaging said label in vivo.
26. The method of claim 10, wherein said imaging is scintigraphic imaging.
27. The method of claim 10, wherein said imaging is radiographic imaging.
- 6
28. A method for measuring hypoxia *in vivo* comprising obtaining a nitroimidazole or nitroimidazole-like agent with oxygen mimetic properties, said agent being labeled; contacting said labeled agent with hypoxic tissue in vivo; and measuring the incorporation of said labeled agent in said hypoxic tissue.
- 11
29. A method of treating hypoxic tissue comprising administering a first radiolabeled nitroimidazole or nitroimidazole-like agent with oxygen mimetic properties and determining the presence of hypoxic tissue; treating any hypoxic tissue with a second nitroimidazole or nitroimidazole-like agent and allowing the agent to
- 16 incorporate into the hypoxic tissue; and administering a third radiolabeled nitroimidazole or nitroimidazole-like agent to determine the presence of hypoxic tissue.
30. The method of claim 29 wherein the first, second, or third nitroimidazole, or
- 21 combinations thereof, is IAZA.
31. The method of claim 29 wherein the second nitroimidazole is radiolabeled.
- 26