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54. TÍTULO Monóxido de Carbono como un biomarcador y agente terapéutico

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71. SOLICITANTE

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3

10)

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud
- ☐ Comprobante de pago por reivindicación de prioridad
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona Jurídica
- ☐ Poderes, si fuera el caso
- ☐ Documento de cesión del inventor al solicitante o a su causante
- ☐ Declaraciones
- ☒ Copia de la solicitud Internacional. (Resumen, descripción, reivindicación, Dibujos).
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicación, Dibujos)
- ☒ Copia del examen preliminar efectuado por la IPEA
- ☐ De ser el caso, copia del contrato de acceso
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- ☐ De ser el caso, certificado de depósito del material biológico
- ☐ Arte final 12 x 12 y 6 x 6 cm
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- ☒ Otros:
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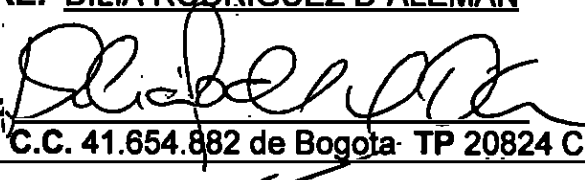
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12) Solicitud concesión de la patente,

NOMBRE: DILIA RODRÍGUEZ D'ALEMAN

FIRMA:



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WO 03/094932 A1

(54) Title: CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT

(57) Abstract: The present invention relates to the use of carbon monoxide (CO) as a biomarker and therapeutic agent of heart, lung, liver, spleen, brain, skin and kidney diseases and other conditions and disease states including, for example, asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary hypertension, secondary pulmonary hypertension, cancers, including lung, larynx and throat cancer, arthritis, wound healing, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and pulmonary vascular thrombotic diseases such as pulmonary embolism. CO may be used to provide anti-inflammatory relief in patients suffering from oxidative stress and other conditions especially including sepsis and septic shock. In addition, carbon monoxide may be used as a biomarker or therapeutic agent for reducing respiratory distress in lung transplant patients and to reduce or inhibit oxidative stress and inflammation in transplant patients.

CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT

TECHNICAL FIELD

The present invention relates to the use of carbon monoxide (CO) as a
5 biomarker and therapeutic agent of heart, lung, liver, spleen, brain, skin and kidney
diseases and other conditions and disease states including, for example, asthma,
emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis,
pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung
diseases including primary pulmonary hypertension, secondary pulmonary
10 hypertension, cancers, including lung, larynx and throat cancer, arthritis, wound
healing, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and
pulmonary vascular thrombotic diseases such as pulmonary embolism. CO may be
used to provide anti-inflammatory relief in patients suffering from oxidative stress and
other conditions especially including sepsis and septic shock. In addition, CO may be
15 used to store organs prior to transplantation. In addition, carbon monoxide may be used
as a biomarker or therapeutic agent for reducing respiratory distress in lung transplant
patients, to reduce or inhibit oxidative stress, inflammation or rejection of transplants in
transplant patients.

BACKGROUND

20 Heme oxygenase (HO) catalyzes the first and rate limiting step in the
degradation of heme to yield equimolar quantities of biliverdin IX α , carbon monoxide
(CO), and iron (Choi et al., Am. J. Respir. Cell Mol. Biol. 15: 9-19; and Maines, Annu.
Rev. Pharmacol. Toxicol. 37: 517-554). Three isoforms of HO exist; HO-1 is highly
inducible while HO-2 and HO-3 are constitutively expressed (Choi et al., supra,
25 Maines, supra and McCoubrey et al., E. J. Bioch. 247: 725-732). Although heme is the
major substrate of HO-1, a variety of non-heme agents including heavy metals,
cytokines, hormones, endotoxin and heat shock are also strong inducers of HO-1
expression (Choi et al., supra, Maines, supra and Tenhunen et al., J. Lab. Clin. Med.
75: 410-421). This diversity of HO-1 inducers has provided further support for the
30 speculation that HO-1, besides its role in heme degradation, may also play a vital
function in maintaining cellular homeostasis. Furthermore, HO-1 is highly induced by

a variety of agents causing oxidative stress including hydrogen peroxide, glutathione depletors, UV irradiation, endotoxin and hyperoxia (Choi, et al. supra, Maines, supra and Keyse, et al. Proc. Natl. Acad. Sci. USA . 86: 99-103). One interpretation of this finding is that HO-1 can serve as a key biological molecule in the adaptation and/or

5 defense against oxidative stress (Choi, et al., supra, Lee, et al., Proc Natl Acad Sci USA 93: 10393-10398; Otterbein et al., Am. J. J. Respir. Cell Mol .Biol. 13: 595-601; Poss et al., Proc. Natl. Acad. Sci. USA. 94: 10925-10930; Vile, et al., Proc. Natl. Acad. Sci. 91: 2607-2610; Abraham et al., Proc. Natl. Acad. Sci. USA. 92: 6798-6802; and Vile and Tyrrell, J. Biol. Chem. 268: 14678-14681. Our laboratory and others have shown

10 that induction of endogenous HO-1 provides protection both *in vivo* and *in vitro* against oxidative stress associated with hyperoxia and lipopolysaccharide-induced tissue injury (Lee et al., supra, Otterbein, et al., supra and Taylor et al., Am. J. Physiol. 18: L582-L591). We have also shown that exogenous administration of HO-1 via gene transfer can provide protection against oxidant tissue injury and elicit tolerance to hyperoxic

15 stress (Otterbein, et al., Am. J. Resp. Crit. Care Med. 157: A565 (Abstr)).

Carbon monoxide (CO) is a gaseous molecule with known toxicity and lethality to living organisms (Haldane, Biochem. J. 21: 1068- 1075; and Chance, et al., 1970, Ann. NY Acad Sci. 174: 193-204.). However, against this known paradigm of CO toxicity, there has been renewed interest in recent years in CO behaving as a regulatory

20 molecule in cellular and biological processes based on several key observations. Mammalian cells have the ability to generate endogenous CO primarily through the catalysis of heme by the enzyme heme oxygenase (HO) (Choi et al., supra and Maines, supra). The total cellular production of CO is generated primarily via heme degradation by HO (Marilena, Biochem. Mol. Med. 61: 136-142 and Verma, et al.,

25 1993, Science 259: 381-384). Moreover, CO, akin to the gaseous molecule nitric oxide, plays important roles in mediating neuronal transmission (Verma et al., supra and Xhuo, et al., Science 260: 1946-1950) and in the regulation of vasomotor tone (Morita, and Kourembanas, 1995, J. Clin. Invest. 96: 2676-2682.; Morita et al., 1995 Proc. Natl. Acad. Sci. USA 92: -1479; and Goda et al., 1998, J. Clin. Inv. 101: 604-12).

30 Other publication relating to the biological actions of CO include Pinsky et al., U.S. Patent No. 6,316,403, Sato et al., J. Immunol. 166: 4185-4194 (2001); Fujita et al., Nat. Med. 7(5): 598-604 (2001); Nachar et al., High Altitude Medicine & Biology 2:377-385 (2001); Vassalli et al., Crit. Care. Med. 29: 359-366 (2001); Otterbein et al., Am. J.

Physiol. Lung Cell Mol. Physiol. 276: L688-L694 (1999); Cardell et al., Brit. J. Pharmacol. 124: 1065-1068 (1998); Otterbein et al., Nat. Med. 6(4): 422-428 (2000); and Otterbein et al., Am. J. Physiol. Lung Cell Mol. Physiol, 279: L1029-L1037 (2000).

5 Septic shock and sepsis syndrome, resulting from excessive stimulation of immune cells, particularly monocytes and macrophages, remains one of the leading causes of death in hospitalized patients. Parillo, et al., Ann. Intern. Med. 113, 991-992 (1992). The pathophysiological alterations observed in sepsis are often not due to the infectious organism itself, but rather to the uncontrolled production of pro-
10 inflammatory cytokines and chemokines including TNF- α , IL-1, and MIP-1 that leads to leukocyte recruitment, capillary leak and ultimately participates in the lethality of sepsis. Beutler, et al., 232, 977-980 (1986); Netea, et al., Immunology 94, 340-344 (1998); and Wolpe, et al., J. Exp. Med. 167, 570-581 (1988). Lipopolysaccharide (LPS), a constituent of the gram negative bacterial cell wall, is the leading cause of
15 sepsis, and when administered experimentally to macrophages or mice, mimics the same inflammatory response. Following LPS administration, there is a rapid but transient increase in these pro-inflammatory mediators which are subsequently down-modulated by a battery of anti-inflammatory cytokines including interleukin-10 (IL-10) and interleukin-4 (IL-4), which inhibit the synthesis of the pro-inflammatory cytokines
20 and chemokines. J. Exp. Med. 177, 1205-1208 (1993). LPS initially binds to the CD14 and toll-like receptor (TLR) 2 (or 4) at the cell surface, (Yang, et al., Nature. 395 : 284-288 (1998) and Chow, et al., J. Biol. Chem. 274 : 10689-10692 (1999) and has then been shown to activate the mitogen activated protein (MAP) kinase pathways including p38, p42/p44 ERK and JNK (MAP) kinases. Liu, et al., J. Immunol. 153, 2642-2652
25 (1994); Hambleton, et al., Proc. Natl. Acad. Sci. USA. 93, 2274-2778 (1996); Han, et al., J. Biol. Chem. 268, 25009-25014 (1993); Han, et al., Science 265, 808-811 (1994); Sanghera, et al., J. Immunol. 156, 4457-4465 (1996), and Raingeaud, et al., J. Biol. Chem. 270, 7420-7426 (1995). The relationship between the activation of these signaling molecules, downstream cytokine expression, and physiologic function
30 represents an active line of investigation.

The United States Government has provided support for research which led to the present invention under one or more of NIH grant numbers HL60234, AI42365 and HL55330. Consequently, the government retains certain rights in the invention.

SUMMARY

10 15 20

The present invention relates to novel pharmaceutical compositions for delivering to patients suffering from the effects of oxidative stress, the compositions comprising effective concentrations of carbon monoxide in a gaseous mixture comprising oxygen and optionally, nitrogen gas (as well as other minor optional gaseous components). An additional aspect of the present invention is directed to a method for delaying the onset of, inhibiting or alleviating the effects of oxidative stress, the method comprising delivering a therapeutic gas comprising carbon monoxide in an amount and for a time effective to delay the onset of, inhibit or alleviate the effects of oxidative stress in the patient. It has unexpectedly been discovered that the delivery of a therapeutic gas comprising low concentrations (i.e., concentrations ranging from about 1 ppb (part per billion) to about 3,000 ppm (preferably above about 0.1 ppm within this range) of the gas, preferably about 1 ppm to about 2,800 ppm, more preferably about 25 ppm to about 750 ppm, even more preferably about 50 ppm to about 500 ppm, e.g., about 250 ppm) of carbon monoxide is an extremely effective method for delaying the onset of, inhibiting or reversing the effects of oxidative stress in a patient. This is an unexpected result. It is noted here that in the method of the present invention, an amount of carbon monoxide in the therapeutic gaseous composition which is in excess of 0.3% may sometimes be used, depending upon the condition or disease state to be treated.

In another aspect, the present invention is directed to the use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for emphysema, bronchitis, cystic fibrosis, pneumonia, interstitial lung disease, wound healing, arthritis, Parkinson's disease, and/or Alzheimer's disease.

25

In yet another aspect, the present invention is directed to the use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for localized inflammation of the kidney, spleen, and/or skin.

30

The present invention also provides methods for using carbon monoxide as a biomarker for determining that a patient is suffering from oxidative stress or is at risk for or is suffering from a number of conditions or disease states that are secondary to or result in oxidative stress, for example, asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary

hypertension, secondary pulmonary hypertension, cancers, including lung, larynx and throat cancer, arthritis, wound healing, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and pulmonary vascular thrombotic diseases such as pulmonary embolism, among others. The method comprises detecting carbon

5 monoxide in a patient's breath to determine whether detectable levels of carbon monoxide occur in the breath. If detectable levels of carbon monoxide appear in the patient's breath, the patient may be diagnosed with oxidative stress or for being at risk for oxidative stress. The manifestations of oxidative stress may take the form of one or more of the above-referenced conditions or disease states. Appropriate therapeutic

10 steps or other steps may be taken after such diagnosis to alleviate or treat the condition that is responsible for the oxidative stress in the patient. In one embodiment, the method includes measuring carbon monoxide in breath exhaled by a patient, wherein an amount of carbon monoxide of at least about 1 ppm in the breath is indicative that the patient is at risk for sepsis or septic shock.

15 Another aspect of the present invention relates to the finding that in certain patients, the administration of carbon monoxide in effective amounts to the patient may be used to induce HO-1 enzyme in the patient and prevent or limit oxidative stress in the patient, especially oxidative stress caused by hyperoxia or sepsis. HO-1 enzyme is implicated in maintaining homeostasis in the cells of the patient.

20 Still another aspect of the present invention relates to the use of carbon monoxide to delay the onset of, inhibit or alleviate the effects of oxidative stress which occur in transplant patients, in particular, organ transplant patients, especially, but not exclusively lung transplant patients.

Another aspect of the present invention relates to a method for inhibiting the

25 production of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and MIP-1 β and augmenting the production (expression) of the anti-inflammatory cytokine IL-10 and IL-4 in a patient, the method comprising administering to the patient an effective amount of CO.

Still another aspect of the present invention relates to a method to preserve

30 organs or tissue for transplants comprising adding to media in which the organs or tissue are stored a preservative effective amount or concentration of carbon monoxide. In this aspect of the present invention, the inclusion of carbon monoxide in effective amounts reduces, inhibits or alleviates the formation of reactive oxygen in the stored

organ or tissue, thus extending the period in which organ transplants can be effectively stored without suffering oxidative damage. In one embodiment, the method includes providing a medium comprising carbon monoxide and storing the organ in the medium, wherein the carbon monoxide is present in the
5 medium in an amount sufficient to enhance storage stability of the organ.

Another aspect of the present invention relates to a method to prevent or reduce the likelihood of damage caused by oxidative stress associated with hyperoxia in a patient comprising administering an effective amount of carbon monoxide to a hyperoxic patient.

10 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although suitable methods and materials for the practice or testing of the present invention are described below, other methods and materials similar or equivalent to those described herein, which are well known in the art, can also be used.
15 All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

DETAILED DESCRIPTION

20 The following definitions are used to describe the present invention.

The term "carbon monoxide" (or "CO") as used herein describes molecular carbon monoxide in its gaseous state, compressed into liquid form, or dissolved in aqueous solution. The term "carbon monoxide composition" or "pharmaceutical composition comprising carbon monoxide" is used throughout the specification to
25 describe a gaseous or liquid composition containing carbon monoxide that can be administered to a patient and/or an organ. The skilled practitioner will recognize which form of the pharmaceutical composition, e.g., gaseous, liquid, or both gaseous and liquid forms, is preferred for a given application.

The terms "effective amount" and "effective to treat," as used herein, refer to
30 the administration of carbon monoxide in an amount or concentration and for period of time including acute or chronic administration and periodic or continuous administration that is effective within the context of its administration for causing an

intended effect or physiological outcome. For gases, effective amounts of carbon monoxide generally fall within the range of about 0.0000001% to about 0.3% by weight, e.g., 0.0001% to about 0.25% by weight, preferably at least about 0.001%, e.g., at least about 0.005%, 0.010%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08%, 5 0.10%, 0.15%, 0.20%, 0.22%, or 0.24% by weight carbon monoxide. Preferred ranges include, e.g., 0.001% to about 0.24%, about 0.005% to about 0.22%, about 0.005% to about 0.05%, about 0.010% to about 0.20%, about 0.02% to about 0.15%, about 0.025% to about 0.10%, or about 0.03% to about 0.08%, or about 0.04% to about 0.06%. For liquid solutions of CO, effective amounts generally fall within the range of 10 about 0.0001 to about 0.0044 g CO/100 g liquid, e.g., at least about 0.0001, 0.0002, 0.0004, 0.0006, 0.0008, 0.0010, 0.0013, 0.0014, 0.0015, 0.0016, 0.0018, 0.0020, 0.0021, 0.0022, 0.0024, 0.0026, 0.0028, 0.0030, 0.0032, 0.0035, 0.0037, 0.0040, or 0.0042 g CO/100 g aqueous solution. Preferred ranges include, e.g., about 0.0010 to about 0.0030 g CO/100 g liquid, about 0.0015 to about 0.0026 g CO/100 g liquid, or 15 about 0.0018 to about 0.0024 g CO/100 g liquid. An effective amount of carbon monoxide within the context of reducing the production or effect of inflammatory cytokines can be, for example, an amount sufficient to inhibit production and/or effect of TNF- α , IL-1, IL-6 and MIP-1, among others. Alternatively, it can be an amount sufficient to induce or increase production of anti-inflammatory cytokines such as IL- 20 10, among others. Within the context of transplant patients, an effective amount of carbon monoxide is that amount administered to the transplant patient to reduce the likelihood of rejection through an unfavorable immunological reaction. Within the context of preserving stored organs to be used for transplantation, an effective amount of carbon monoxide can be an amount that is added, e.g., bubbled, into the medium in 25 which the transplant organs are stored in order to enhance preservation of the organ and reduce the likelihood that the organ will be subject to some measure of oxidative damage. A skilled practitioner will appreciate that amounts outside of the ranges described above may be used depending upon the application.

The term "patient" is used throughout the specification to describe an animal, 30 human or non-human, to whom treatment according to the methods of the present invention is provided. Veterinary applications are clearly anticipated by the present invention. The term includes but is not limited to mammals, e.g., humans, other

primates, pigs, rodents such as mice and rats, rabbits, guinea pigs, hamsters, cows, horses, cats, dogs, sheep and goats.

The term "treat(ment)," is used herein to describe delaying the onset of, inhibiting, or alleviating the effects of a condition, e.g., emphysema, bronchitis, arthritis, cystic fibrosis, pneumonia, interstitial lung disease, Parkinson's disease, Alzheimer's disease, or inflammation of the kidneys, spleen, or skin. In the case of wound healing, the term describes the promotion of wound healing, e.g., the promotion of skin wound healing. Individuals considered at risk for developing a condition described herein may benefit particularly from the invention, primarily because prophylactic treatment can begin before there is any evidence of the condition. The skilled practitioner will appreciate that a patient can be determined to be at risk for any of the conditions described herein by any method known in the art, e.g., by a physician's diagnosis.

The term "biomarker" is used to describe carbon monoxide produced in the breath of a patient in minor, detectable amounts, and which provides evidence that the patient is at risk for, is in the early stages of, or is suffering from oxidative stress is at risk for or is suffering from one or more of the conditions or disease states that are secondary to or that may result in oxidative stress. The amount of carbon monoxide in the breath of a patient that may function as a biomarker may be as low as 0.001 ppm, but is generally at least about 0.1 ppm.

Preparation of Gaseous Carbon Monoxide Compositions

A carbon monoxide composition may be a gaseous carbon monoxide composition. Compressed or pressurized gas useful in the methods of the invention can be obtained from any commercial source, and in any type of vessel appropriate for storing compressed gas. For example, compressed or pressurized gases can be obtained from any source that supplies compressed gases, such as oxygen, for medical use. The pressurized gas including carbon monoxide used in the methods of the present invention can be provided such that all gases of the desired final composition (e.g., CO, CO₂, O₂, N₂) are mixed in the same vessel. Optionally, the methods of the present invention can be performed using multiple vessels containing individual gases. For example, a single vessel can be provided that contains carbon monoxide, with or without other gases, the contents of which can be optionally mixed with room air or

with the contents of other vessels, e.g., vessels containing oxygen, nitrogen, carbon dioxide, helium, compressed air, or any other suitable gas or mixtures thereof.

Gaseous compositions administered to a patient according to the present invention typically contain 0% to about 79% by weight nitrogen, about 21% to about 100% by weight oxygen and about 0.0000001% to about 0.3% by weight (corresponding to about 1 ppb or 0.001 ppm to about 3,000 ppm) carbon monoxide. Preferably, the amount of nitrogen in the gaseous composition is about 79% by weight, the amount of oxygen is about 21% by weight, and the amount of carbon monoxide is about 0.0001% to about 0.25% by weight, preferably at least about 0.001%, e.g., at least about 0.005%, 0.010%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08%, 0.10%, 0.15%, 0.20%, 0.22%, or 0.24% by weight of carbon monoxide. Preferred ranges include 0.005% to about 0.24%, about 0.01% to about 0.22%, and about 0.08% to about 0.20%. It is noted that gaseous carbon monoxide compositions having concentrations of carbon monoxide greater than 0.3% (such as 1% or greater) may be used for short periods (e.g., one or a few breaths), depending upon the application.

A gaseous carbon monoxide composition may be used to create an atmosphere that comprises carbon monoxide gas. An atmosphere that includes appropriate levels of carbon monoxide gas can be created, for example, by providing a vessel containing a pressurized gas comprising carbon monoxide gas, and releasing the pressurized gas from the vessel into a chamber or space to form an atmosphere that includes the carbon monoxide gas inside the chamber or space. Alternatively, the gases can be released into an apparatus that culminates in a breathing mask or breathing tube, thereby creating an atmosphere comprising carbon monoxide gas in the breathing mask or breathing tube, ensuring the patient is the only person in the room exposed to significant levels of carbon monoxide.

Carbon monoxide levels in an atmosphere can be measured or monitored using any method known in the art. Such methods include electrochemical detection, gas chromatography, radioisotope counting, infrared absorption, colorimetry, and electrochemical methods based on selective membranes (see, e.g., Sunderman *et al.*, Clin. Chem. 28:2026-2032, 1982; Ingi *et al.*, Neuron 16:835-842, 1996). Sub-parts per million carbon monoxide levels can be detected by, e.g., gas chromatography and radioisotope counting. Further, it is known in the art that carbon monoxide levels in the sub-ppm range can be measured in biological tissue by a midinfrared gas sensor (see,

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e.g., Morimoto *et al.*, Am. J. Physiol. Heart. Circ. Physiol 280:H482-H488, 2001).

Carbon monoxide sensors and gas detection devices are widely available from many commercial sources.

5 Preparation of Liquid Carbon Monoxide Compositions

A carbon monoxide composition may also be a liquid carbon monoxide composition. A liquid can be made into a carbon monoxide composition by any method known in the art for causing gases to become dissolved in liquids. For example, the liquid can be placed in a so-called "CO₂ incubator" and exposed to a
10 continuous flow of carbon monoxide, preferably balanced with carbon dioxide, until a desired concentration of carbon monoxide is reached in the liquid. As another example, carbon monoxide gas can be "bubbled" directly into the liquid until the desired concentration of carbon monoxide in the liquid is reached. The amount of carbon monoxide that can be dissolved in a given aqueous solution increases with
15 decreasing temperature. As still another example, an appropriate liquid may be passed through tubing that allows gas diffusion, where the tubing runs through an atmosphere comprising carbon monoxide (e.g., utilizing a device such as an extracorporeal membrane oxygenator). The carbon monoxide diffuses into the liquid to create a liquid carbon monoxide composition.

20 In one embodiment, the liquid can be any liquid known to those of skill in the art to be suitable administration to a patient (see, for example, Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)). In general, the liquid will be an aqueous solution. Examples of appropriate solutions include Phosphate Buffered Saline (PBS), Celsior™, Perfadex™, Collins solution, citrate solution, and
25 University of Wisconsin (UW) solution (Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)).

Any suitable liquid can be saturated to a set concentration of carbon monoxide via gas diffusers. Alternatively, pre-made solutions that have been quality controlled to contain set levels of carbon monoxide can be used. Accurate control of dose can be
30 achieved via measurements with a gas permeable, liquid impermeable membrane connected to a carbon monoxide analyzer. Solutions can be saturated to desired effective concentrations and maintained at these levels.

Treatment of Patients with Carbon Monoxide Compositions

A patient can be treated with a carbon monoxide composition by any method known in the art of administering gases and/or liquids to patient. Carbon monoxide compositions can be administered to a patient diagnosed with, or determined to be at risk, for example, for emphysema, bronchitis, arthritis, cystic fibrosis, pneumonia, interstitial lung disease, Parkinson's disease, Alzheimer's disease, or inflammation of the kidneys, spleen, or skin; or to promote wound healing, e.g., healing of skin wounds not associated with surgery. The invention contemplates the systemic administration of liquid or gaseous carbon monoxide compositions to patients (e.g., by inhalation and/or ingestion), and the topical administration of the compositions to the patient's lungs (e.g., by inhalation or intratracheal administration), joints (e.g., by infusion or transdermal administration) skin (e.g., by injection or by applying the composition to the surface of the skin), and other organs (e.g., by ingestion, insufflation, and/or introduction into the abdominal cavity).

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Systemic Delivery of Carbon Monoxide

Gaseous carbon monoxide compositions can be delivered systemically to a patient. Gaseous carbon monoxide compositions are typically administered by inhalation through the mouth or nasal passages to the lungs, where the carbon monoxide may exert its effect directly or be readily absorbed into the patient's bloodstream. The concentration of active compound (CO) utilized in the therapeutic gaseous composition will depend on absorption, distribution, inactivation, and excretion (generally, through respiration) rates of the carbon monoxide as well as other factors known to those of skill in the art. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Acute, sub-acute and chronic administration of carbon monoxide are contemplated by the present invention, depending upon, e.g., the severity or persistence of the condition in the patient. Carbon monoxide can be delivered to the patient for a time (including indefinitely) sufficient to treat the condition and exert the intended pharmacological or biological effect.

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The following are examples of some methods and devices that can be utilized to administer gaseous carbon monoxide compositions to patients.

Ventilators

5 Medical grade carbon monoxide (concentrations can vary) can be purchased mixed with air or another oxygen-containing gas in a standard tank of compressed gas (e.g., 21% O₂, 79% N₂). It is non-reactive, and the concentrations that are required for the methods of the present invention are well below the combustible range (10% in air). In a hospital setting, the gas presumably will be delivered to the bedside where it will
10 be mixed with oxygen or house air in a blender to a desired concentration in ppm (parts per million). The patient will inhale the gas mixture through a ventilator, which will be set to a flow rate based on patient comfort and needs. This is determined by pulmonary graphics (i.e., respiratory rate, tidal volumes etc.). Fail-safe mechanism(s) to prevent the patient from unnecessarily receiving greater than desired amounts of carbon
15 monoxide can be designed into the delivery system. The patient's carbon monoxide level can be monitored by studying (1) carboxyhemoglobin (COHb), which can be measured in venous blood, and (2) exhaled carbon monoxide collected from a side port of the ventilator. Carbon monoxide exposure can be adjusted based upon the patient's health status and on the basis of the markers. If necessary, carbon monoxide can be
20 washed out of the patient by switching to 100% O₂ inhalation. Carbon monoxide is not metabolized; thus, whatever is inhaled will ultimately be exhaled except for a very small percentage that is converted to CO₂. Carbon monoxide can also be mixed with any level of O₂ to provide therapeutic delivery of carbon monoxide without consequential hypoxic conditions.

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Face Mask and Tent

A carbon monoxide-containing gas mixture is prepared as above to allow passive inhalation by the patient using a facemask or tent. The concentration inhaled can be changed and can be washed out by simply switching over to 100% O₂.
30 Monitoring of carbon monoxide levels would occur at or near the mask or tent with a fail-safe mechanism that would prevent too high of a concentration of carbon monoxide from being inhaled.

Portable inhaler

Compressed carbon monoxide can be packaged into a portable inhaler device and inhaled in a metered dose, for example, to permit intermittent treatment of a recipient who is not in a hospital setting. Different concentrations of carbon monoxide could be packaged in the containers. The device could be as simple as a small tank (e.g., under 5 kg) of appropriately diluted CO with an on-off valve and a tube from which the patient takes a whiff of CO according to a standard regimen or as needed.

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Intravenous Artificial Lung

An artificial lung (a catheter device for gas exchange in the blood) designed for O₂ delivery and CO₂ removal can be used for carbon monoxide delivery. The catheter, when implanted, resides in one of the large veins and would be able to deliver carbon monoxide at given concentrations either for systemic delivery or at a local site. The delivery can be a local delivery of a high concentration of carbon monoxide for a short period of time at the site of the procedure, e.g., in proximity to the spleen or kidney (this high concentration would rapidly be diluted out in the bloodstream), or a relatively longer exposure to a lower concentration of carbon monoxide (see, e.g., Hattler *et al.*, Artif. Organs 18(11):806-812 (1994); and Golob *et al.*, ASAIO J., 47(5):432-437 (2001)).

Normobaric chamber

In certain instances, it would be desirable to expose the whole patient to carbon monoxide. The patient would be inside an airtight chamber that would be flooded with carbon monoxide (at a level that does not endanger the patient, or at a level that poses an acceptable risk without the risk of bystanders' being exposed. Upon completion of the exposure, the chamber could be flushed with air (e.g., 21% O₂, 79% N₂), and samples could be analyzed by carbon monoxide analyzers to ensure no carbon monoxide remains before allowing the patient to exit the exposure system.

Liquid Compositions

The present invention further contemplates that a liquid composition comprising carbon monoxide can be created for systemic delivery to a patient, e.g., for oral delivery and/or by injection into the body, e.g., intravenously, intra-arterially, intraperitoneally, and/or subcutaneously.

Topical Treatment of Organs with Carbon Monoxide

Alternatively or in addition, carbon monoxide compositions can be applied directly to organs, e.g., the skin, spleen, lung, and/or kidney(s). Gaseous compositions can be applied directly to the interior and/or exterior of the patient's body to treat the patient's organs. A gaseous composition can be directly applied to the internal organs of a patient by any method known in the art for insufflating gases into a patient. For

example, gases, e.g., carbon dioxide, are often insufflated into the abdominal cavity of patients to facilitate examination during laproscopic procedures (see, e.g., Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)). The skilled practitioner will appreciate that similar procedures could be used to administer
5 carbon monoxide compositions directly to an internal organ of a patient. The skin and underlying joints can be treated topically with a gaseous composition by, for example, exposing the affected skin to the gaseous composition in a normobarometric chamber (described above), and/or by blowing the carbon monoxide composition directly onto the skin.

10 Liquid carbon monoxide compositions can also be administered topically to a patient's organs. Liquid forms of the compositions can be administered by any method known in the art for administering liquids to patients. As with gaseous compositions, liquid compositions can be applied directly to the interior and/or exterior of the body to treat a patient's organs. For example, the liquid compositions can be administered
15 orally, e.g., by causing the patient to ingest an encapsulated or unencapsulated dose of the aqueous carbon monoxide composition. As another example, liquids, e.g., saline solutions containing dissolved CO, can be injected into the abdominal cavity of patients during laproscopic procedures. Alternatively or in addition, *in situ* exposures or organs, e.g., kidney(s) and spleen, can be performed by any method known in the art,
20 e.g., by *in situ* flushing of the organ with a liquid carbon monoxide composition (see Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)). The skin can be treated topically with a liquid composition by, for example, injecting the liquid compositions into the skin. As a further example, the skin and underlying joints can be treated topically by applying the liquid composition directly to the surface
25 of the skin, e.g., by pouring or spraying the liquid onto the skin and/or by submerging the skin in the liquid composition.

Disorders and Conditions

Carbon monoxide gas can be used in the preparation of a medicament for use in
30 treating conditions or disease states such as asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary hypertension, secondary pulmonary hypertension, cancers, including lung,

larynx and throat cancer, arthritis, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and pulmonary vascular thrombotic diseases such as pulmonary embolism; and in treating a patient suffering from or at risk for localized inflammation of organs, e.g., the kidney, spleen, and/or skin. The present invention can also be used
5 to aid in wound healing, e.g., skin wound healing. Of particular interest is treatment of wounds not caused by surgery.

The present invention may also be used to delay the onset of, or alleviate the effects of oxidative stress in transplant patients, in particular organ transplant patients, especially lung transplant patients. The carbon monoxide compositions may also be
10 used to treat inflammatory conditions of the lungs or inflammation that occurs secondary to sepsis or rejection in transplant patients. While not being limited by way of theory, low dosage CO is believed to act as an anti-inflammatory agent by inhibiting the production and/or effect of pro-inflammatory cytokines such as TNF- α , IL-1, IL-6, MIP-1, and/or by inducing or promoting the action of anti-inflammatory cytokines IL-4
15 and IL-10.

The term "oxidative stress" is used to describe a condition resulting from the overwhelming production of reactive oxygen which cannot be quenched by endogenous antioxidants. Oxidative stress may result in permanent tissue damage caused by the action of the reactive oxygen species on the tissue. The physiological manifestation of
20 oxidative stress take the form of or occurs during various conditions or disease states that include asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis or septic shock, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary hypertension, secondary pulmonary hypertension, lung cancer and pulmonary vascular thrombotic
25 diseases such as pulmonary embolism or any inflammatory disease of the lungs.

The term "sepsis" is used to describe the presence of various pus-forming and other pathogenic organisms or their toxins (generally, lipopolysaccharides or LPS bacterial cell walls) in the blood tissues. Sepsis will often result in oxidative stress in those tissues exposed to the pathogens or their toxins. Sepsis often manifests itself in
30 the production of pro-inflammatory cytokines such as TNF- α , IL-1, IL-6 and MIP-1, the production of which is reduced or reversed by the administration of effective amounts of carbon monoxide.

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The present invention may be used to treat inflammation. The term "inflammation" is used to describe the fundamental pathological process consisting of a dynamic complex of cytologic and histologic reactions that occur in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical or biologic agent, including the local reactions and resulting morphologic changes, the destruction or removal of the injurious material, and the responses that lead to repair and healing. The term includes various types of inflammation such as acute, allergic, alterative (degenerative), atrophic, catarrhal (most frequently in the respiratory tract), croupous, fibrinopurulent, fibrinous, immune, hyperplastic or proliferative, subacute, serous and serofibrinous. Inflammation localized in the liver, heart, skin (e.g., dermatitis, inflammation due to bacterial, fungal, or viral infections and/or allergic or autoimmune reactions), spleen, brain, kidney (e.g., bacterial pyelonephritis, interstitial nephritis, and/or glomerulonephritis) and pulmonary tract, especially the lungs, and that associated with sepsis or septic shock is favorably treated by the methods according to the present invention.

The term "cancer" is used as a general term to describe any of various types of malignant neoplasms, most of which invade surrounding tissues, may metastasize to several sites and are likely to recur after attempted removal and to cause death of the patient unless adequately treated. Cancers which may be treated using the present compositions and methods include, for example, stomach, colon, rectal, liver, pancreatic, lung, breast, cervix uteri, corpus uteri, ovary, prostate, testis, bladder, renal, brain/CNS, head and neck, throat, Hodgkins disease, non-Hodgkins leukemia, skin melanoma, various sarcomas, small cell lung cancer, choriocarcinoma, mouth/pharynx, oesophagus, larynx, melanoma, kidney and lymphoma, among others.

The present invention can be used to treat conditions or disorders that involve the respiratory system, e.g., emphysema, bronchitis, cystic fibrosis, pneumonia, and interstitial lung disease. The term "emphysema" as used herein refers to a lung disease characterized by an enlargement of lung alveoli. In this condition, alveolar walls are destroyed, causing bronchioles to lose structural support and collapse during exhaling (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 68). The term "bronchitis" refers to a lung disease characterized by inflammation of the tracheobronchial tree. Bronchitis may develop after infections, e.g., viral infections, e.g., the common cold; or bacterial infections, or after exposure to

an irritant (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 69). The term "cystic fibrosis" refers to a genetic disease of the exocrine glands. The disease primarily affects the gastrointestinal tract and respiratory systems, and usually is characterized by chronic obstructive pulmonary disease (COPD) (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 19, Chapter 267). The term "pneumonia" refers to a lung disease affecting the parenchyma, and includes, e.g., bacterial, viral, and aspiration pneumonia (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 73). The terms "interstitial lung disease" or "idiopathic interstitial lung disease" refer to a group of lung diseases of unknown etiology, which produce diffuse pathologic changes usually involving the interalveolar interstitial tissue (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 78).

As used herein, the term "arthritis" refers to a condition characterized by inflammation of the joint and includes, for example, rheumatoid arthritis (RA) (a chronic inflammatory polyarthritis that often leads to destruction of the joints), psoriatic arthritis (inflammatory arthritis associated with psoriasis), ankylosing spondylitis (inflammation of the axial skeleton and large peripheral joints), and ankylosis (immobility or fusion of a joint) (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 5, Chapter 50; Section 5, Chapter 51; Section 5, Chapter 51; and Section 9, Chapter 103, respectively).

The term "Parkinson's disease" refers to "an idiopathic, slowly progressive, degenerative CNS disorder characterized by slow and decreased movement, muscular rigidity, resting tremor, and postural instability." (*The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 14, Chapter 179). The term "Alzheimer's disease" refers to a disease characterized by "a progressive, inexorable loss of cognitive function associated with an excessive number of senile plaques in the cerebral cortex and subcortical gray matter, which also contains b-amyloid and neurofibrillary tangles consisting of tau protein." (*The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 14, Chapter 17).

Low dosage α -tocopherol also be used in the present invention to induce HO-1 enzyme in patients and prevent or limit oxidative stress, especially oxidative stress caused by hyperoxia or hypoxia. Induced HO-1 is implicated in maintaining homeostasis in the cells of the patient.

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Treatment of Organs and Tissues to Enhance Storage Stability.

The present invention also relates to the use of CO as a preservative for storing organs or tissues to be used in transplants. It is an unexpected result that the inclusion of low dosage CO in the medium in which organs to be transplanted are stored will substantially reduce the likelihood of oxidative damage to the organs during storage and substantially enhance the storage time that organs to be transplanted may be safely stored without suffering irreversible oxidative damage. Thus, in one embodiment of the present invention, an effective amount of CO is bubbled into storage medium either before or preferably when an organ is first placed in the media or shortly thereafter. CO may also be used to enhance the storage stability of organs which have been stored for some time in media, in those instances, oxidative damage may have become irreversible, thus limiting the intended effect.

Accordingly, the present invention provides a method for enhancing the storage stability of an organ or tissue. The storage stability is enhanced by exposing the organ or tissue to liquid and/or gaseous carbon monoxide compositions. Exposure of an organ or tissue to gaseous carbon monoxide compositions can be performed in any chamber or area suitable for creating an atmosphere that includes appropriate levels of carbon monoxide gas. Such chambers include, for example, incubators, and chambers built for the purpose of accommodating an organ in a preservation solution. As another example, an appropriate chamber may be a chamber wherein only the gases fed into the chamber are present in the internal atmosphere, such that the concentration of carbon monoxide can be established and maintained at a given concentration and purity, e.g., where the chamber is airtight. For example, a CO₂ incubator may be used to expose an organ to a carbon monoxide composition, wherein carbon monoxide gas is supplied in a continuous flow from a vessel that contains the gas.

With respect to liquid carbon monoxide compositions, the exposure may be performed in any chamber or space having sufficient volume for submerging the organ or tissue, completely or partially, in the carbon monoxide composition. In one embodiment of the present invention, the organ may be exposed to a carbon monoxide composition by placing the organ in any suitable container, and causing the carbon monoxide composition to "wash over" the organ, such that the organ is exposed to a continuous flow of carbon monoxide composition. In another embodiment, the

organ is perfused with a carbon monoxide composition. The term "perfusion" is an art recognized term, and relates to the passage of a liquid, e.g., a carbon monoxide composition, through the blood vessels of an organ or tissue. Methods for perfusing organs *ex vivo* and *in vivo* are well-known in the art. An organ can be perfused with a carbon monoxide composition *ex vivo*, for example, by continuous hypothermic machine perfusion (see *Griffith's Textbook of Surgery*, Morris and Malt, Eds., Oxford University Press (1994)). Optionally, the organ can be perfused with a wash solution, e.g., UW solution without carbon monoxide, prior to perfusion with the carbon monoxide composition to remove the donor's blood from the organ. Such a process could be performed to avoid competition for carbon monoxide by the donor's hemoglobin. As another example, the wash solution can be a carbon monoxide composition. As still another example, an appropriate liquid may be passed through tubing that allows gas diffusion, which runs through an atmosphere comprising carbon monoxide (e.g., through a chamber, such as with extracorporeal membrane oxygenation), to create a liquid carbon monoxide composition, which may then be passed into an organ, e.g., perfused into the organ by connecting the tubing to the organ).

As another example, the organ may be placed, e.g., submerged, in a medium or solution that does not include carbon monoxide, and placed in a chamber such that the medium or solution is made into a carbon monoxide composition via exposure to a carbon monoxide-containing atmosphere as described herein. As still another example, the organ may be submerged in a liquid that does not include carbon monoxide, and carbon monoxide is "bubbled" into the liquid.

The present invention contemplates that any or all of the above methods for exposing an organ to a liquid carbon monoxide composition, e.g., washing, submerging, or perfusing, can be used in a given procedure, e.g., used in a single procedure for enhancing the storage stability of an organ or tissue.

Carbon Monoxide as a Diagnostic Tool

In addition to using CO as a therapeutic agent, the measurement of CO may be a useful diagnostic tool, e.g., a biomarker, to determine whether a patient is in oxidative stress or has a condition or a disease state where CO may be implicated, e.g., sepsis or septic shock. In general, a patient suspected of being in oxidative stress or at risk for

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oxidative stress is monitored to determine whether detectable levels of carbon monoxide may be measured in the exhaled breath of the patient. If detectable levels of carbon monoxide are detected, i.e., an amount of carbon monoxide of at least about 0.01 ppm in the patient's breath, then the attending physician or caregiver may then begin to administer therapeutic doses of carbon monoxide to treat oxidative stress or any one or more of the conditions or disease states which are secondary to or result in oxidative stress.

In one embodiment, a patient will have his or her exhaled breath analyzed for the presence of CO. CO content in a patient's breath is measured by a CO monitor (for example, using a Loprox L42000) which is sensitive to the detection of CO from 0 to about 1000 ppm (with sensitivity as low as 1 ppb). In this method, the subjects exhale slowly from functional FVC into the breath analyzer with a constant flow (5-6 l/m) over a 20-30 second interval. Two successful recordings are made and mean values will be used for calculations. Ambient CO levels are recorded before each breath in order to provide control or background values. While any elevation in CO levels from background numbers may implicate an actual or incipient state of oxidative stress, an amount of CO of at least about 1 ppm provides a clear indication that the patient is in or is about to suffer oxidative stress.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. Use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for at least one disorder selected from the group consisting of: emphysema, bronchitis, cystic fibrosis, pneumonia, interstitial lung disease, wound healing, arthritis, Parkinson's disease, and Alzheimer's disease; or to promote wound healing in the patient.
2. The method of claim 1, wherein the disorder is emphysema.
3. The method of claim 1, wherein the disorder is bronchitis.
4. The method of claim 1, wherein the disorder is cystic fibrosis.
5. The method of claim 1, wherein the disorder is pneumonia.
6. The method of claim 1, wherein the disorder is interstitial lung disease.
7. The method of claim 1, wherein the disorder is arthritis.
8. The method of claim 1, wherein the disorder is Parkinson's disease.
9. The method of claim 1, wherein the disorder is Alzheimer's disease.
10. The method of claim 1, wherein the carbon monoxide gas is used in the preparation of a medicament for use in promoting wound healing in the patient.
11. Use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for localized inflammation of at least one organ selected from the group consisting of: kidney, spleen, and skin.
12. The method of claim 11, wherein the organ is a kidney.

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13. The method of claim 11, wherein the organ is a spleen.

14. The method of claim 11, wherein the organ is skin.

5 15. A method of enhancing the storage stability of an organ in a medium,
comprising:
 providing a medium comprising carbon monoxide; and
 storing the organ in the medium, wherein the carbon monoxide is
present in the medium in an amount sufficient to enhance storage stability of the organ.

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16. A method of determining whether a patient is at risk for sepsis or septic
shock, comprising:

 measuring carbon monoxide in breath exhaled by a patient, wherein an amount
of carbon monoxide of at least about 1 ppm in the breath is indicative that the patient is
15 at risk for sepsis or septic shock.

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AMENDED CLAIMS

**[Received by the International Bureau on 02 September 2003 (02.09.2003);
original claims 1-14 and 16 unchanged, original claim 15 cancelled
(1 page)]**

13. The method of claim 11, wherein the organ is a spleen.

14. The method of claim 11, wherein the organ is skin.

15. A method of determining whether a patient is at risk for sepsis or septic shock, comprising:

measuring carbon monoxide in breath exhaled by a patient, wherein an amount of carbon monoxide of at least about 1 ppm in the breath is indicative that the patient is at risk for sepsis or septic shock.

Título: MONOXIDO DE CARBONO COMO UN BIOMARCADOR Y AGENTE TERAPÉUTICO

Resumen: La presente invención describe el uso de monóxido de carbono (CO) como un biomarcador y agente terapéutico de enfermedades del corazón, pulmón, hígado, bazo, cerebro, piel y riñón y otras condiciones y estados de enfermedad que incluyen, por ejemplo, asma, enfisema, bronquitis, síndrome de dificultad respiratoria del adulto, sepsis, fibrosis quística, neumonía, enfermedades pulmonares intersticiales, enfermedades pulmonares idiopáticas, otras enfermedades pulmonares que incluyen hipertensión pulmonar primaria, hipertensión pulmonar secundaria, cánceres, incluyendo cáncer de pulmón, de laringe y de garganta, artritis, cicatrización de heridas, la enfermedad de Parkinson, la enfermedad de Alzheimer, la enfermedad vascular periférica y enfermedades pulmonares relacionadas con trombosis vasculares tales como la embolia pulmonar. El CO puede ser utilizado para proporcionar alivio anti-inflamatorio en pacientes que sufren de estrés oxidativo y otras condiciones incluyendo especialmente sepsis y shock séptico. Adicionalmente, el monóxido de carbono puede ser usado como biomarcador o agente terapéutico para la reducción de la dificultad respiratoria en pacientes con trasplantes de pulmón y para reducir o inhibir el estrés oxidativo e inflamación en pacientes con trasplantes.

MONOXIDO DE CARBONO COMO UN BIOMARCADOR Y AGENTE TERAPÉUTICO

CAMPO TECNICO

La presente invención describe el uso de monóxido de carbono (CO) como un biomarcador y agente terapéutico de enfermedades del corazón, pulmón, hígado, bazo, cerebro, piel y riñón y otras condiciones y estados de enfermedad que incluyen, por ejemplo, asma, enfisema, bronquitis, síndrome de dificultad respiratoria del adulto, sepsis, fibrosis quística, neumonía, enfermedades pulmonares intersticiales, enfermedades pulmonares idiopáticas, otras enfermedades pulmonares que incluyen hipertensión pulmonar primaria, hipertensión pulmonar secundaria, cánceres, incluyendo cáncer de pulmón, de laringe y de garganta, artritis, cicatrización de heridas, la enfermedad de Parkinson, la enfermedad de Alzheimer, la enfermedad vascular periférica y enfermedades pulmonares relacionadas con trombosis vasculares tales como la embolia pulmonar. El CO puede ser utilizado para proporcionar alivio anti-inflamatorio en pacientes que sufren de estrés oxidativo y otras condiciones incluyendo especialmente sepsis y shock séptico. Adicionalmente, el monóxido de carbono puede ser usado como biomarcador o agente terapéutico para la reducción de la dificultad respiratoria en pacientes con trasplantes de pulmón y para reducir o inhibir el estrés oxidativo e inflamación en pacientes con trasplantes.

ANTECEDENTES

La Hemo oxigenasa (HO) cataliza el primer paso, y limitante de la velocidad, en la degradación del hemo para producir cantidades equimoleculares de biliverdina IXa, monóxido de carbono (CO) y hierro (Choi et al., *Am. J. Respir. Cell Mol. Biol.* 15: 9-19; y Maines, *Annu. Rev. Pharmacol. Toxicol.* 37: 517-554). Existen tres isoformas de HO; HO-1 es altamente inducible mientras que HO-2 y HO-3 son expresadas constitutivamente (Choi et al., *supra*, Maines, *supra* y McCoubrey et al., *E. J. Bioch.* 247: 725-732). Aunque el hemo es el principal sustrato de la HO-1, una variedad de agentes no-hemo que incluyen metales pesado, citoquinas, hormonas, endotoxinas y choques de calor son también fuertes introductores de la expresión de HO-1 (Choi et al., *supra*, Maines, *supra* y Tenhunen et al., *J. Lab. Clin. Med.* 75: 410-421). Esta diversidad de introductores de HO-1 ha proporcionado un sustento adicional para la hipótesis de que la HO-1, además de su rol en la degradación del hemo, puede jugar también un papel vital en mantener la homeostasis celular. Adicionalmente, la HO-1 es altamente inducida por una variedad de agentes que causan estrés oxidativo incluyendo peróxido de hidrógeno, reductores de glutatona, radiación UV, endotoxina e hiperoxia (Choi, et al. *supra*, Maines, *supra* y Keyse, et al. *Proc. Natl. Acad. Sci. USA.* 86: 99-103). Una interpretación de este hallazgo es que la HO-1 puede servir como una molécula biológica clave en la adaptación y/o defensa contra el estrés oxidativo (Choi, et al., *supra*, Lee, et al., *Proc Natl Acad Sci USA* 93: 10393-10398; Otterbein et al., *Am. J. J. Respir. Cell Mol. Biol.* 13: 595-601; Poss et al., *Proc. Natl. Acad. Sci. USA.* 94: 10925-10930; Vile, et al., *Proc. Natl. Acad. Sci.* 91: 2607-2610; Abraham et al., *Proc. Natl. Acad. Sci. USA.* 92: 6798-6802; y Vile y Tyrrell, *J. Biol. Chem.* 268: 14678-14681. Nuestro laboratorio y otros han demostrado que la inducción de la HO-1 endógena

proporciona protección tanto *in vivo* como *in vitro* contra el estrés oxidativo asociado con hiperoxia y lesiones de tejido inducidas por lipopolisacáridos (Lee et al., supra, Otterbein, et al., supra y Taylor et al., *Am. J. Physiol.* 18: L582- L591). Nosotros también hemos demostrado que la administración de HO-1 exógena vía transferencia de genes puede proporcionar protección contra las lesiones tisulares producidas por la oxidación y producir tolerancia al estrés hiperóxico (Otterbein, et al., *Am. J. Resp. Crit. Care Med.* 157: A565 (Resumen)).

El monóxido de carbono (CO) es una molécula gaseosa con toxicidad conocida y letal para organismos vivos (Haldane, *Biochem. J.* 21: 1068-1075; y Chance, et al., 1970, *Ann. NY Acad Sci.* 174: 193-204). Sin embargo, en contra del conocido paradigma de la toxicidad del CO, se ha generado en los últimos años un renovado interés en el comportamiento del CO como una molécula reguladora en procesos celulares y biológicos según lo indican varias observaciones determinantes. Las células de los mamíferos tiene la capacidad de generar CO endógeno principalmente mediante la catálisis del hemo por parte de la enzima hemo oxigenasa (HO) (Choi et al., supra y Maines, supra). La totalidad de la producción del CO es generada principalmente vía la degradación del hemo mediante HO (Marilena, *Biochem. Mol. Med.* 61: 136-142 y Verma, et al., 1993, *Science* 259: 381-384). Por otra parte, CO, al igual que la molécula gaseosa de óxido nítrico, juega un papel importante interviniendo en la transmisión neuronal (Verma et al., supra y Xhuo, et al., *Science* 260: 1946-1950) y en la regulación del tono vasomotor (Morita, y Kourembanas, 1995, *J. Clin. Invest.* 96: 2676-2682.; Morita et al., 1995 *Proc. Natl. Acad. Sci. USA* 92:-1479 ; y Goda et al., 1998, *J. Clin. Inv.*

101: 604-12). Otras publicaciones relacionadas con la actividad biológica del CO incluyen Pinsky et al., Patente E.U. No. 6,316, 403; Sato et al., *J. Immunol.* 166: 4185-4194 (2001); Fujita et al., *Nat. Med.* 7 (5): 598-604 (2001); Nachar et al., *High Altitude Medicine & Biology* 2: 377-385 (2001); Vassalli et al., *Crit. Care. Med.* 29: 359-366 (2001); Otterbein et al., *Am. J. Physiol. Lung Cell Mol. Physiol.* 276: L688-L694 (1999); Cardell et al., *Brit. J. Pharmacol.* 124: 1065-1068 (1998); Otterbein et al., *Nat. Med.* 6 (4): 422-428 (2000); y Otterbein et al., *Am. J. Physiol Lung Cell Mol. Physiol*, 279: L1029-L1037 (2000).

El shock séptico y síndrome de sepsis, son causados por la estimulación excesiva de células inmunes, particularmente monocitos y macrófagos, continúan siendo una de las principales causas de muerte en pacientes hospitalizados. Parillo, et al., *Ann. Intern. Med.* 113:991-992 (1992). Las alteraciones patofisiológicas observadas durante la sepsis frecuentemente no son debidas al organismo infeccioso por si mismo, si no mas bien a la producción incontrolada de citoquinas y quemoquinas pro-inflamatorias incluyendo TNF- α , IL-1 y MIP-1 que conllevan al reclutamiento leucocitario, a la fuga capilar y que en última instancia participar en el proceso de muerte por sepsis. Beutler, et al., 232, 977-980 (1986); Netea, et al., *Immunology* 94, 340-344 (1998); y Wolpe, et al., *J. Exp. Med.* 167, 570-581 (1988). El lipopolisacárido (LPS), un componente de la pared celular de las bacterias gram negativas, es la principal causa de sepsis, y cuando es administrado a macrófagos o ratones, estos reproducen la misma respuesta inflamatoria. Tras la administración del LPS, se presenta un incremento rápido pero transitorio en estos intermediarios pro-inflamatorios

los cuales son posteriormente modulados hacia abajo por una batería de citoquinas anti-inflamatorias que incluyen interleuquina-10(IL-10) e interleuquina-4 (IL-4), las cuales inhiben la síntesis de citoquinas y quemoquinas pro-inflamatorias. *J. Exp. Med.* 177,1205-1208 (1993). El LPS inicialmente se unen a las CD14 y a los receptores tipo peaje (RTP) 2 (o 4) en la superficie de la célula, (Yang, et al., *Nature*. 395: 284- 288 (1998) y Chow, et al., *J. Biol. Chem.* 274: 10689-10692 (1999)) y ha sido demostrado que entonces van a activar las quinasas, proteínas activadas por mitógenos (PAM), mecanismo que incluye quinasas (MAP) p38, p42/p44 ERK y JNK. Liu et al., *J. Immunol.* 153, 2642-2652 (1994); Hambleton et al., *Proc. Natl. Acad. Sci. USA.* 93, 2274-2778 (1996); Han et al., *J. Biol. Chem.* 268, 25009-25014 (1993); Han et al., *Science* 265, 808- 811 (1994); Sanghera et al. *J. Immunol.* 156, 4457-4465 (1996), y Raingeaud et al., *J. Biol. Chem.* 270, 7420-7426 (1995). La relación entre la activación de estas moléculas transmisoras, la posterior expresión de la citoquina y su función fisiológica constituyen una línea activa de investigación.

El gobierno de Estados Unidos ha proporcionado fondos para investigaciones las cuales han permitido la presente invención bajo una o más transferencias del Instituto Nacional de Salud de los Estados Unidos (NIH por sus siglas en ingles) con números HL60234, AI42365 y HL55330. En consecuencia, el gobierno conserva ciertos derechos en esta invención.

RESUMEN

La presente invención describe formulaciones farmacéuticas novedosas para ser administradas a pacientes que sufren los efectos del estrés oxidativo, las formulaciones contienen concentraciones efectivas de monóxido de carbono en una mezcla gaseosa que consta de oxígeno y opcionalmente, de nitrógeno gaseoso (lo mismo que de algunos componentes gaseosos opcionales en una menor proporción). Un aspecto adicional de la presente invención está enfocado a un método para retardar el inicio de, inhibir o aliviar los efectos del estrés oxidativo, el método consiste en administrar un gas terapéutico que contiene monóxido de carbono en una cantidad y por un periodo de tiempo efectivo para retardar el inicio de, inhibir o aliviar los efectos del estrés oxidativo en el paciente. Ha sido descubierto de forma inesperada que la administración de un gas terapéutico que contiene bajas concentraciones (por ejemplo, concentraciones en el intervalo comprendido entre 1 ppb (parte por billón) y 3,000 ppm (preferiblemente por encima de 0.1 ppm dentro de este rango) del gas, preferiblemente el intervalo entre 1 ppm y 2,800 ppm, con una mayor preferencia por el intervalo entre 25 ppm y 750 ppm, aún una mayor preferencia por el intervalo entre 50 ppm y 500 ppm, e.g., alrededor de 250 ppm) de monóxido de carbono es un método extremadamente efectivo para retardar el inicio de, inhibir o reversar los efectos del estrés oxidativo en el paciente. Este es un resultado inesperado. Se resalta en este punto que dentro del método de la presente invención, una cantidad de monóxido de carbono que exceda el 0.3% en una formulación terapéutica puede ser usada algunas veces, dependiendo de la condición o el estado de la enfermedad a ser tratado.

En otro aspecto, la presente invención está enfocada al uso de monóxido de carbono gaseoso en la preparación de un medicamento para ser usado en el tratamiento de pacientes que padecen de o están en riesgo de padecer enfisema, bronquitis, fibrosis quística, neumonía, enfermedad pulmonar intersticial, cicatrización de heridas, artritis, la enfermedad de Parkinson o la enfermedad de Alzheimer.

Aún en otro aspecto, la presente invención está enfocada al uso de monóxido de carbono gaseoso en la preparación de un medicamento para ser usado en el tratamiento de pacientes que padecen de o están en riesgo de padecer inflamación localizada del riñón, del bazo y/o de la piel.

La presente invención también proporciona métodos para el uso de monóxido de carbono como un biomarcador para determinar si un paciente padece de estrés oxidativo o está en riesgo de padecerlo o está presentando un número de condiciones o estados de enfermedad que son subsiguientes a o conllevan al estrés oxidativo, por ejemplo, asma, enfisema, bronquitis, síndrome de dificultad respiratoria del adulto, sepsis, fibrosis quística, neumonía, enfermedades pulmonares intersticiales, enfermedades pulmonares idiopáticas, otras enfermedades pulmonares que incluyen hipertensión pulmonar primaria, hipertensión pulmonar secundaria, cánceres, incluyendo cáncer de pulmón, de laringe y de garganta, artritis, cicatrización de heridas, la enfermedad de Parkinson, la enfermedad de Alzheimer, la enfermedad vascular periférica y enfermedades pulmonares relacionadas con trombosis vasculares tales

como la embolia pulmonar, entre otras. El método consiste en la detección de monóxido de carbono en la respiración para determinar en que casos se presentan niveles detectables de monóxido de carbono en la respiración. Si se presenta niveles detectables de monóxido de carbono en la respiración del paciente, es posible diagnosticar estrés oxidativo en el paciente o que este está en riesgo de presentar estrés oxidativo. Las manifestaciones de estrés oxidativo pueden tomar la forma de una o mas de las condiciones o estados de enfermedad anteriormente mencionadas. Después de este diagnóstico las medidas terapéuticas apropiadas u otras medidas pueden ser adoptadas para aliviar o tratar la condición que es responsable del estrés oxidativo en el paciente. En un modo de ejecución, el método incluye la medición del monóxido de carbono en la respiración exhalada por un paciente, en donde una cantidad de monóxido de carbono de por lo menos 1 ppm en la respiración es un indicio de que el paciente está en riesgo de presentar sepsis o shock séptico.

Otro aspecto de la presente invención describe el descubrimiento de que en ciertos pacientes, la administración de monóxido de carbono al paciente en cantidades efectivas puede ser usado para inducir la enzima HO-1 en el paciente y prevenir o limitar el estrés oxidativo en el paciente, especialmente el estrés oxidativo causado por hiperoxia o sepsis. La enzima HO-1 está involucrada en el mantenimiento de la homeostasis en las células de de el paciente.

Incluso otro aspecto de la presente invención describe el uso de monóxido de carbono para retardar el inicio de, inhibir o aliviar los efectos del estrés oxidativo los cuales ocurren en pacientes con transplantes, en particular, en pacientes con transplantes de órganos, especialmente, pero no exclusivamente en pacientes con transplantes de pulmón.

Otro aspecto de la presente invención describe un método para inhibir la producción de citoquinas pro-inflamatorias tales como $\text{TNF-}\alpha$, $\text{IL-1}\beta$, IL-6 y $\text{MIP-1}\beta$ y aumentar la producción (expresión) de las citoquinas anti-inflamatorias IL-10 e IL-4 en un paciente, el método consiste en administrar al paciente una cantidad efectiva de CO .

Incluso otro aspecto de la presente invención describe un método para preservar órganos o tejidos para transplantes que consiste en adicionar al medio en el cual los órganos o tejidos están almacenados una cantidad o concentración efectiva de monóxido de carbono capaz de preservar. En este aspecto de la presente invención, la inclusión de monóxido de carbono en cantidades efectivas reduce, inhibe o alivia la formación de oxígeno reactivo e el órgano o tejido almacenado, prolongando de esta forma el periodo en el cual los transplantes de órgano pueden ser efectivamente almacenados sin sufrir daño oxidativo. En un modo de ejecución, el método incluye la provisión de un medio que contiene monóxido de carbono y el almacenamiento del órgano en el medio, en donde el monóxido de carbono está presente en el medio en una cantidad suficiente para ampliar la estabilidad de almacenamiento del órgano.

Otro aspecto de la presente invención describe un método para prevenir o reducir la probabilidad de daño causado por estrés oxidativo asociado con hiperoxia en pacientes que consiste en administrar una cantidad efectiva de monóxido de carbono al paciente hiperóxico.

A menos que sea definido de otra manera, todos los términos técnicos y científicos usados en este documento tienen el mismo significado de acuerdo con lo entendido comúnmente por alguien con el conocimiento habitual en el arte al cual pertenece la invención. A pesar de que los métodos y materiales adecuados para la práctica o ensayo de la presente invención son descritos en este documento, otros métodos y materiales similares o equivalentes a los descritos aquí, los cuales son bien conocidos en el estado del arte, también pueden ser usados. Todas las publicaciones, aplicaciones de patentes, patentes y otras referencias mencionadas dentro de este documento son incorporadas por la referencia en su totalidad. En caso de conflicto, la presente descripción, incluyendo definiciones, será usada como control. Adicionalmente, los materiales, los métodos y los ejemplos son solamente ilustrativos y no pretenden ser limitantes.

DESCRIPCIÓN DETALLADA

Las siguientes definiciones son usadas para describir la presente invención.

El término "monóxido de carbono" (o "CO") según lo utilizado aquí describe monóxido de carbono molecular en su estado gaseoso, comprimido a forma líquida o disuelto en soluciones acuosas. El término "formulación de monóxido de carbono" o "formulación farmacéutica que contiene monóxido de carbono" es usado a través de la descripción para describir una formulación gaseosa o líquida que contiene monóxido de carbono que puede ser administrado a un paciente y/o a un órgano. Un médico con la experiencia adecuada reconocerá cual de las formas de la formulación farmacéutica, e.g., gaseosa, líquida o ambas formas gaseosa y líquida, es adecuada para una aplicación dada.

Los términos "cantidad efectiva" o "efectivas para tratamiento", de acuerdo con lo usado aquí, se refiere a la administración de monóxido de carbono en una cantidad o concentración y por un periodo de tiempo que incluye la administración aguda o crónica y la administración periódica o continua que es efectiva dentro del contexto de su administración para causar un efecto previsto o un resultado fisiológico. Para gases las cantidades efectivas de monóxido de carbono generalmente se encuentran dentro del intervalo comprendido entre 0.0000001% y alrededor del 0.3% en peso, e.g., entre 0.0001% y alrededor del 0.25% en peso, preferiblemente por lo menos alrededor del 0.001%, e.g., por lo menos alrededor del 0.005%, 0.010%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08%, 0.10%, 0.15%, 0.20%, 0.22%, o 0.24% de monóxido de carbono en peso. Los intervalos preferidos incluyen, e.g., entre 0.001% y alrededor del 0.24%, de alrededor del 0.005% a alrededor del 0.22%, de alrededor del 0.005% a alrededor del 0.05%, de alrededor del 0.010% a alrededor del 0.20%, de alrededor del

0.02% a alrededor del 0.15%, de alrededor del 0.025% a alrededor del 0.10%, o de alrededor del 0.03% a alrededor del 0.08%, o de alrededor del 0.04% a alrededor del 0.06%. Para las soluciones líquidas del CO, las cantidades efectivas se encuentran generalmente dentro del intervalo comprendido entre aproximadamente 0.0001 a alrededor de 0.0044 g CO/100 g de líquido, e.g., por lo menos alrededor de 0.0001, 0.0002, 0.0004, 0.0006, 0.0008, 0.0010, 0.0013, 0.0014, 0.0015, 0.0016, 0.0018, 0.0020, 0.0021, 0.0022, 0.0024, 0.0026, 0.0028, 0.0030, 0.0032, 0.0035, 0.0037, 0.0040 o 0.0042 g CO/100 g de solución acuosa. Los intervalos preferidos incluyen, e.g., de alrededor de 0.0010 a alrededor de 0.0030 g CO/100 g de líquido, de alrededor de 0.0015 a alrededor de 0.0026 g CO/100 g de líquido o de alrededor de 0.0018 a alrededor de 0.0024 g CO/100 g de líquido. Una cantidad efectiva de monóxido de carbono dentro del contexto de reducir la producción o efecto inflamatorio de las citoquinas puede ser, por ejemplo, una cantidad suficiente para inhibir la producción y/o efecto de la TNF- α , IL-1 y MIP-1, entre otras. Alternativamente, esta puede ser una cantidad suficiente inducir o aumentar la producción de citoquinas anti-inflamatorias tales como IL-10, entre otros. Dentro del contexto de los pacientes con trasplantes, una cantidad efectiva de monóxido de carbono es aquella cantidad administrada al paciente con transplante para reducir la probabilidad de rechazo mediante una reacción inmunológica desfavorable. Dentro del contexto de preservación de órganos almacenados para ser usados en trasplantes, una cantidad de efectiva de monóxido de carbono puede ser una cantidad que es adicionada, por ejemplo, burbujeada, dentro del medio en el cual los órganos a ser transplantados son almacenados teniendo como objetivo aumentar la preservación del órgano y reducir la probabilidad de que el órgano esté sujeto en alguna medida a daño oxidativo. Un médico con la experiencia

adecuada decidirá si cantidades por fuera de los intervalos anteriormente descritos pueden ser usadas dependiendo de la aplicación.

El término paciente es usado a través de la descripción para describir un animal, humano o no-humano, al quien es suministrado el tratamiento de acuerdo con los métodos de la presente invención. Aplicaciones veterinarias son anticipadas claramente para la presente invención. El término incluye pero no está limitado a mamíferos, por ejemplo, seres humanos, otros primates, cerdos, roedores tales como ratones y ratas, conejos, conejillos de indias, hámsteres, vacas, caballos, gatos, perros, ovejas y cabras.

El término "tratar(o tratamiento)", es usado aquí para describir la posposición del inicio de, la inhibición o el alivio de los efectos de una condición, por ejemplo, enfisema, bronquitis, artritis, fibrosis quística, neumonía, enfermedad pulmonar intersticial, la enfermedad de Parkinson, la enfermedad de Alzheimer o una inflamación de los riñones, el bazo, o la piel. En el caso de cicatrización de heridas, el término describe la promoción de la cicatrización de las heridas, e.g., la promoción de la cicatrización de las heridas en la piel. Los individuos considerados en el riesgo de desarrollar alguna de las condiciones descritas aquí pueden verse particularmente beneficiados por esta invención, principalmente porque los tratamientos profilácticos pueden ser iniciados antes de que haya cualquier evidencia de la condición. El médico con la experiencia adecuada determinará si un paciente puede estar en riesgo de presentar cualquiera de

las condiciones aquí descritas mediante cualquiera de los métodos conocidos en el estado del arte, por ejemplo, mediante un diagnóstico médico.

El término "biomarcador" es usado para describir monóxido de carbono producido en la respiración del paciente en una proporción menor, dentro del intervalo de cantidades detectables, y la cual proporciona evidencia de que el paciente está en riesgo de presentar, esta en las instancias iniciales de, o está padeciendo de estrés oxidativo está en riesgo de presentar o está padeciendo de una o mas de las condiciones o estados de enfermedad que son subsiguientes a o conllevan al estrés oxidativo. La cantidad de monóxido de carbono en la respiración de un paciente que puede funcionar como un biomarcador puede llegar a niveles tan bajos como 0.001 ppm, pero es generalmente de por lo menos 0.1 ppm.

Preparación de Formulaciones Gaseosas de Monóxido de Carbono

Una formulación de monóxido de carbono puede ser una formulación gaseosa de monóxido de carbono. El gas comprimido o presurizado útil en los métodos de la invención puede ser obtenido de cualquier fuente comercial, y en cualquier tipo de recipiente apropiado para almacenar gas comprimido. Por ejemplo, gases comprimidos o presurizados pueden ser obtenidos de cualquier fuente que suministre gases comprimidos, tales como oxígeno, para uso médico. El gas presurizado que contiene

monóxido de carbono usado en los métodos de la presente invención puede ser suministrado de forma tal que todos los gases de la formulación final deseada (e.g., CO, CO₂, O₂, N₂) están mezclados en el mismo recipiente. Opcionalmente, los métodos de la presente invención pueden ser llevadas a cabo usando múltiples recipientes que contienen los gases por separado. Por ejemplo, puede ser suministrado un único recipiente que contenga monóxido de carbono, junto con o sin otros gases, el contenido del cual puede ser opcionalmente mezclado con aire del lugar o con el contenido de otros recipientes, por ejemplo, recipientes que contengan oxígeno, nitrógeno, dióxido de carbono, helio, aire comprimido o cualquier otro gas adecuado o mezcla de estos.

Las formulaciones gaseosas administradas a un paciente de acuerdo con la presente invención contienen típicamente de 0% a alrededor del 79% en peso de nitrógeno, de alrededor del 21% a alrededor del 100% en peso de oxígeno y de alrededor del 0.0000001% a alrededor del 0.3% en peso (que corresponde de alrededor de 1 ppb o 0.001 ppm a alrededor de 3,000 ppm) de monóxido de carbono.

Preferiblemente la cantidad de nitrógeno en la formulación gaseosa está alrededor del 79% en peso, la cantidad de oxígeno está alrededor del 21% en peso y la cantidad de monóxido de carbono está entre alrededor del 0001% y alrededor de 0.25% en peso, preferiblemente por lo menos alrededor del 0.001%, e.g., por lo menos alrededor de 0.005%, 0.010%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08%, 0.10%, 0.15%, 0.20%, 0.22% o 0.24% en peso de monóxido de carbono. Los intervalos

preferidos incluyen de 0.005% a alrededor del 0.24%, de alrededor del 0.01% a alrededor del 0.22% y de alrededor del 0.08% a alrededor del 0.20%. Se resalta en este punto que formulaciones gaseosas de monóxido de carbono que tienen concentraciones de monóxido de carbono por encima del 0.3% (tales como el 1% o superiores) pueden ser usadas por periodos cortos (e.g., unas pocas aspiraciones), dependiendo de la aplicación.

Una formulación gaseosa de monóxido de carbono puede ser usada para crear una atmósfera que conste de monóxido de carbono gaseoso. Una atmósfera que incluye niveles apropiados de monóxido de carbono gaseoso puede ser creada, por ejemplo, mediante la provisión de un recipiente que contenga un gas presurizado que conste de monóxido de carbono gaseoso y la liberación del gas presurizado desde el recipiente en un compartimiento o un espacio para formar una atmósfera que contenga el monóxido de carbono gaseoso en el interior del compartimiento o del espacio. Alternativamente, los gases se pueden ser liberados en un aparato que culmine en una máscara de respiración o un tubo respirador, de tal modo que se cree una atmósfera que contenga el gas del monóxido de carbono en la máscara de respiración o el tubo respirador, asegurando que el paciente es la única persona en la habitación expuesto a niveles significativos de monóxido de carbono.

Los niveles de monóxido de carbono en una atmósfera pueden ser medidos o monitoreados usando cualquier método conocido en el estado del arte. Tales métodos

incluyen detección electroquímica, cromatografía de gases, conteo de radioisótopos, absorción infrarroja, colorimetría y métodos electroquímicos basados en membranas selectivas (véase, e.g., Sunderman et al., *Clin. Chem.* 28: 2026-2032, 1982 ; Ingi et al., *Neuron* 16: 835-842, 1996). Niveles de monóxido de carbono por debajo de las partes por millón pueden ser detectados mediante, por ejemplo, cromatografía de gases y conteo de radioisótopos. Adicionalmente, es conocido en el estado del arte que los niveles de monóxido de carbono en el intervalo por debajo de las ppm puede ser medidos en tejidos biológicos mediante un sensor de gas en el infrarrojo medio (véase, e.g., Morimoto et al., *Am. J. Physiol. Heart. Circ. Physiol* 280: H482-H488, 2001). Sensores de monóxido de carbono y dispositivos para la detección de gases están ampliamente disponibles en muchas fuentes comerciales.

Preparación de Formulaciones Líquidas de monóxido de carbono

Una formulación de monóxido de carbono también puede ser una formulación líquida de monóxido de carbono. Una formulación líquida que contenga monóxido de carbono puede ser elaborada por cualquiera de los métodos conocido en el estado del arte para hacer que un gas se disuelva en un líquido. Por ejemplo, el líquido puede ser colocado en un dispositivo conocido como "incubador de CO₂" y expuesto a un flujo continuo de monóxido de carbono, preferiblemente equilibrado con dióxido de carbono, hasta que una concentración deseada de monóxido de carbono es alcanzada en el líquido. Como otro ejemplo, el monóxido de carbono gaseoso puede ser "burbujeado"

directamente dentro del líquido hasta que una concentración deseada de monóxido de carbono es alcanzada en el líquido. La cantidad de monóxido de carbono que puede ser disuelta en una solución acuosa dada se incrementa con la disminución de la temperatura. Aún como otro ejemplo, un líquido apropiado puede ser pasado a través de una tubería que permite la difusión del gas, en donde la tubería pasa a través de una atmósfera que contiene monóxido de carbono (e.g., utilizando un dispositivo tal como un oxigenador de membrana extracorporal). El monóxido de carbono se difunde dentro del líquido para crear una formulación líquida de monóxido de carbono.

En un modo de ejecución, el líquido puede ser cualquiera conocido por una persona con experiencia en el estado del arte que sea adecuado par la administración al paciente (véase, por ejemplo, *Oxford Textbook of Surgery*, Morris y Malt, Eds., Oxford University Press (1994)) En general, el líquido será una solución acuosa. Ejemplos de soluciones apropiadas incluyen soluciones salinas amortiguadoras de fosfato (PBS por sus siglas en ingles), Celsior™, Perfadex™, la solución de Collins, solución de citrato y la solución de la Universidad de Wisconsin (UW) (*Oxford Textbook of Surgery*, Morris y Malt, Eds., Oxford University Press (1994))

Cualquier líquido adecuado puede ser saturado hasta una concentración determinada de monóxido de carbono mediante difusores de gas. Alternativamente, soluciones pre-elaboradas que hayan sido sujetas a control de calidad para asegurar que contengan niveles determinados de monóxido de carbono pueden ser usadas. El

control exacto de las dosis puede ser alcanzado mediante mediciones con membranas permeables a gas e impermeables a líquidos conectadas a un analizador de monóxido de carbono. Las soluciones pueden ser saturadas hasta concentraciones efectivas deseadas y mantenidas en estos niveles.

Tratamiento de Pacientes con Formulaciones de Monóxido De Carbono

Un paciente puede ser tratado con una formulación de monóxido de carbono por cualquier método conocido en el estado del arte de administrar gases y/o líquidos al paciente. Las formulaciones de monóxido de carbono se pueden administrar a un paciente diagnosticado con, o que se ha determinado está en riesgo de, por ejemplo, presentar enfisema, bronquitis, artritis, fibrosis quística, neumonía, enfermedad pulmonar intersticial, la enfermedad de Parkinson o la enfermedad de Alzheimer o una inflamación de los riñones, el bazo, o la piel, o promover la cicatrización de heridas, e.g., cicatrización de herida en la piel que no están asociadas con cirugías. La invención contempla la administración sistémica de formulaciones líquidas o gaseosas de monóxido de carbono a pacientes (e.g. por inhalación o ingestión), y la administración tópica de las formulaciones a los pulmones (e.g. por inhalación o administración intratraqueal), las articulaciones (e.g., por la infusión o la administración transdérmica), la piel (e. g., por la inyección o aplicación de la formulación a la superficie de la piel) y a otros órganos (e. g., por ingestión, insuflación, y/o la introducción en la cavidad abdominal) del paciente.

Administración Sistémica de Monóxido de Carbono

Las formulaciones gaseosas de monóxido de carbono pueden ser administradas sistemáticamente al paciente. Las formulaciones gaseosas de monóxido de carbono son administradas normalmente mediante la inhalación a través de la boca o fosas nasales a los pulmones, en donde el monóxido de carbono puede ejercer su efecto directamente o ser fácilmente absorbido en la corriente sanguínea del paciente. La concentración del compuesto activo (CO) utilizada en la formulación terapéutica gaseosa dependerá de la absorción, distribución, desactivación y de la velocidad de excreción (generalmente, a través de la respiración) del monóxido de carbono así como otros factores conocidos por personas con experiencia en el estado del arte. Debe ser entendido que para cualquier sujeto en particular, tratamientos de dosis específicas deben ser ajustados dentro de un cierto plazo de acuerdo con las necesidades individuales y al juicio profesional de la persona que administra o supervisa la administración de las formulaciones, y que los intervalos de las concentraciones definidos aquí son solamente ejemplares y con ellas no se pretende limitar el alcance o la práctica de la formulación reivindicada. La administración aguda, sub-aguda y crónica de monóxido de carbono están contempladas por la presente invención, dependiendo de, e.g., la severidad o persistencia de las condiciones en el paciente. El monóxido de carbono puede ser administrado por un periodo de tiempo suficiente (incluyendo indefinidamente) para tratar la condición y ejercer el efecto farmacológico o biológico pretendido.

Los siguientes son algunos ejemplos de algunos métodos y dispositivos que pueden ser utilizados para administrar la formulación gaseosa a los pacientes.

Respiradores

Monóxido de carbono de grado médico (las concentraciones pueden variar) puede ser comprado mezclado con aire u otro gas que contenga oxígeno en un cilindro estándar de gas comprimido (e.g., 21% de O₂, 79% de N₂). Esta mezcla es no-reactiva, y las concentraciones que son requeridas para los métodos de la presente invención se ubican en un intervalo bastante inferior al combustible (10% en aire). En el montaje de un hospital, el gas será llevado probablemente a la cabecera en donde será mezclado con oxígeno o con aire del ambiente en un mezclador hasta obtener la concentración deseada en ppm (partes por millón). El paciente inhalara la mezcla de gas a través de un respirador, el cual será ajustado para permitir un flujo con base en la comodidad y necesidades del paciente. Este será determinado por los parámetros pulmonares (por ejemplo, frecuencia respiratoria, volumen corriente etc.). Mecanismo(s) a prueba de fallas para prevenir al paciente de recibir una cantidad innecesariamente mayor que las cantidades deseadas de monóxido de carbono pueden ser diseñados dentro del sistema de administración. El nivel de monóxido de carbono del paciente puede ser monitoreado mediante el estudio de (1) carboxihemoglobina (COHb), la cual puede ser medida en sangre venosa, y (2) el monóxido de carbono exhalado recolectado de un puerto lateral del respirador. El grado de exposición al monóxido de carbono puede ser ajustado con base en el status de salud del paciente y sobre la base de los marcadores. Si es necesario, el monóxido de carbono puede ser eliminado del paciente cambiando a

una inhalación de 100% de oxígeno. El monóxido de carbono no es metabolizado, de esta forma lo que es inhalado será en última instancia exhalado con excepción de un porcentaje muy pequeño que es convertido a CO_2 . El monóxido de carbono también puede ser mezclado con cualquier nivel de O_2 para proporcionar una administración terapéutica de monóxido de carbono sin generar condiciones de hiperoxia.

Mascarilla y Cámara

Una mezcla de gases que contiene monóxido de carbono es preparada como se indicó anteriormente para permitir la inhalación pasiva por parte del paciente usando una mascarilla o una cámara. La concentración inhalada puede ser cambiada y puede ser eliminada simplemente cambiando hasta obtener el 100% de O_2 . El control de los niveles de monóxido de carbono podría llevarse a cabo dentro de o cerca de la mascarilla o la cámara con un mecanismo a prueba de fallas que evitaría la inhalación de concentraciones muy altas de monóxido de carbono.

Inhalador Portátil

Monóxido de carbono comprimido puede ser almacenado en un dispositivo inhalador portátil e inhalado en una dosis regulada, por ejemplo, para permitir el

tratamiento intermitente desde un recipiente que no esta en el montaje de un hospital. Diferentes concentraciones de monóxido de carbono podrían ser almacenadas en los contenedores. El dispositivo podría ser tan simple como un pequeño tanque (e.g. inferior a 5 Kg) de CO diluido apropiadamente con una válvula tipo abre-cierra y un tubo desde el cual el paciente aspira CO de acuerdo a un régimen estándar o según lo necesitado.

Pulmones Artificiales Intravenosos

Un pulmón artificial (un catéter para el intercambio de gas en la sangre) diseñado para llevar oxígeno y retirar CO₂ puede ser usado para la administración de monóxido de carbono. El catéter, cuando es implantado, se ubica en una de las venas principales y podría transportar monóxido de carbono de una concentración dada para una administración sistémica o en un punto local. El suministro de puede ser de tipo local con una concentración elevada de monóxido de carbono por un periodo de tiempo en el sitio del procedimiento, e.g., en las proximidades del bazo o el riñón (esta concentración elevada podría ser diluida rápidamente en la corriente sanguínea), o por un periodo de exposición relativamente mas largo con una concentración de monóxido de carbono mas baja (véase, e.g., Hattler et al., *Artif. Organs* 18 (11): 806-812 (1994); y Golob et al., *ASAIO J.*, 47 (5): 432-437 (2001)).

Cámaras Normobáricas

En ciertas instancias, puede ser deseable exponer al paciente en su totalidad al monóxido de carbono. El paciente estaría dentro de una cámara hermética que sería inundada con monóxido de carbono (en un nivel que no ponga en peligro la vida del paciente, o en un nivel que posea un riesgo aceptable sin el riesgo de que las personas presente sean expuestas. Una vez completada la exposición la cámara puede ser evacuada con aire (e.g. 21% O₂, 79% N₂), y unas muestras podrían ser analizadas por un analizador de monóxido de carbono para asegurar que no queda monóxido de carbono antes de permitir que el paciente sea expuesto al sistema.

Formulaciones Líquidas

La presente invención adicionalmente contempla que una formulación líquida que contenga monóxido de carbono pueda ser creada para la administración sistémica al paciente, e.g., para administración oral y/o por inyección en el cuerpo, e.g., vía intravenosa, intra-arterial, intraperitoneal y/o subcutánea.

Tratamientos Tópicos de Órganos con Monóxido de Carbono

Alternativamente o adicionalmente, formulaciones de monóxido de carbono pueden ser aplicadas, e.g., a la piel, al bazo, al pulmón y/o al (los) riñón (es). Formulaciones gaseosas pueden ser aplicadas directamente al interior y/o al exterior del cuerpo del paciente para tratar los órganos del paciente. Una formulación gaseosa puede ser aplicada directamente a los órganos internos del paciente por cualquiera de los métodos conocidos en el estado del arte para insuflar gases en el paciente, por ejemplo, gases e.g. dióxido de carbono, son frecuentemente insuflados dentro de la cavidad abdominal de pacientes para facilitar el examen durante procedimientos laparoscópicos (véase, e.g., *Oxford Textbook of Surgery*, Morris y Malt, Eds., Oxford University Press (1994)). El médico con la experiencia adecuada decidirá que procedimientos similares podrían ser usados para administrar formulaciones de monóxido de carbono a un órgano interno del paciente. La piel y las articulaciones subyacentes pueden ser tratadas tópicamente con una formulación gaseosa mediante, por ejemplo, la exposición de la piel afectada a la formulación gaseosa en una cámara normbarométrica (anteriormente descrita), y/o soplando la formulación de monóxido de carbono directamente sobre la piel.

Formulaciones líquidas de monóxido de carbono pueden también ser administradas tópicamente al órgano del paciente. Formas líquidas de las formulaciones pueden ser administradas por cualquiera de los métodos conocidos en

estado del arte para la administración de líquidos a pacientes. Como con las formulaciones gaseosas, las formulaciones líquidas pueden ser aplicadas directamente al interior y/o al exterior del cuerpo del paciente para tratar los órganos del paciente. Por ejemplo una formulación líquida puede ser administrada oralmente, e.g. haciendo que el paciente ingiera una dosis encapsulada o no encapsulada de la formulación acuosa de monóxido de carbono. Como otro ejemplo, líquidos, e.g. soluciones salinas que contengan CO disuelto pueden ser inyectadas dentro de la cavidad abdominal de los pacientes durante procedimientos laparoscópicos. Alternativamente o adicionalmente, exposiciones *in situ* de órganos, e.g., riñón (es) y bazo, pueden ser llevada a cabo por cualquier método conocido en el estado del arte, e.g., mediante el enjuague *in situ* del órgano con una formulación líquida de monóxido de carbono (véase, *Oxford Textbook of Surgery*, Morris y Malt, Eds., Oxford University Press (1994)). La piel puede ser tratada tópicamente con una composición líquida mediante, por ejemplo, inyección de las composiciones líquidas en la piel. Como un ejemplo adicional, la piel y las articulaciones subyacentes pueden ser tratadas tópicamente mediante la aplicación de las formulaciones líquidas directamente a la superficie de la piel, e.g. vertiendo o esparciendo el líquido sobre la piel y/o sumergiendo la piel en la formulación líquida.

Trastornos y Condiciones

El monóxido de carbono gaseoso puede ser usado en la preparación de un medicamento para ser usado en el tratamiento de condiciones o estados de enfermedad tales como asma, enfisema, bronquitis, síndrome de dificultad respiratoria del adulto, sepsis, fibrosis quística, neumonía, enfermedades pulmonares intersticiales, enfermedades pulmonares idiopáticas, otras enfermedades pulmonares que incluyen hipertensión pulmonar primaria, hipertensión pulmonar secundaria, cánceres, incluyendo cáncer de pulmón, de laringe y de garganta, artritis, la enfermedad de Parkinson, la enfermedad de Alzheimer, la enfermedad vascular periférica y enfermedades pulmonares relacionadas con trombosis vasculares tales como la embolia pulmonar; y el tratamiento de pacientes que padecen de o están en riesgo de padecer inflamación localizada de órganos, e.g. el riñón, el bazo y/o de la piel. La presente invención también puede ser usada para ayudar en la cicatrización de heridas e.g. cicatrización de heridas en la piel. Recibe un interés particular el tratamiento de heridas que no son causadas por cirugías.

La presente invención también puede ser usada para retardar el inicio de, o aliviar los efectos del estrés oxidativo en pacientes con transplantes, en particular, en pacientes con transplantes de órganos, especialmente, pero no exclusivamente en pacientes con transplantes de pulmón. Las formulaciones de monóxido de carbono pueden también ser usadas para el tratamiento de condiciones inflamatorias de los

pulmones o inflamaciones que ocurren como consecuencia de la sepsis o rechazo en pacientes con transplantes. Mientras que no sea limitada por la teoría, se cree que dosis bajas de CO actúan como un agente anti-inflamatorio al inhibir la producción y/o efecto de las citoquinas pro-inflamatorias tales como TNF- α , IL-1, IL-6 y MIP-1 y al aumentar la producción de las citoquinas anti-inflamatorias IL-4 e IL-10.

El término "estrés oxidativo" es usado para describir una condición que resulta de la producción abrumadora de oxígeno reactivo la cual no puede ser detenida por antioxidantes endógenos. El estrés oxidativo puede dar lugar a un daño permanente en el tejido causado por la acción de especies de oxígeno reactivo sobre el tejido. Las manifestaciones fisiológicas del estrés oxidativo toman la forma de u ocurren durante varias condiciones de estados de enfermedad que incluyen asma, enfisema, bronquitis, síndrome de dificultad respiratoria del adulto, sepsis o shock séptico, fibrosis quística, neumonía, enfermedades pulmonares intersticiales, enfermedades pulmonares idiopáticas, otras enfermedades pulmonares que incluyen hipertensión pulmonar primaria, hipertensión pulmonar secundaria, cáncer de pulmón, y enfermedades pulmonares relacionadas con trombosis vasculares tales como la embolia pulmonar o cualquier enfermedad inflamatoria de los pulmones.

EL término "sepsis" es usado para describir la presencia de varios formadores de pus y otros organismos patogénicos o sus toxinas (generalmente, lipopolisacáridos o LPS, componentes de las paredes de las células bacterianas) en los tejidos

sanguíneos. La sepsis dará lugar a menudo al estrés oxidativo en aquellos tejidos expuestos a los patógenos o a sus toxinas. La sepsis se manifiesta a menudo como la producción de citoquinas pro-inflamatorias tales como $\text{TNF-}\alpha$, IL-1, IL-6 y MIP-1, cuya producción es reducida o reversada por la administración de cantidades efectivas de monóxido de carbono.

La presente invención puede ser usada para tratar la inflamación. El término "inflamación" es usado para describir el proceso fundamental patológico que consta de una compleja dinámica de reacciones citológicas e histológicas que ocurren en los vasos sanguíneos afectados y los tejidos adyacentes en respuesta a una lesión o un estímulo anormal causado por un agente físico, químico o biológico, que incluyen las reacciones locales y los cambios morfológicos resultantes, la destrucción o remoción de los materiales perjudiciales y las respuestas que conducen a la reparación y cicatrización. El término incluye varios tipos de inflamación tales como aguda, alérgica, alteradora (degenerativa), atróficas, catarral (la mayoría de las veces en el tracto respiratorio), crupal, fibrinopurulenta, fibrinosa, inmune, hiperplástica o proliferativa, subagudas, serosa y serofibrinosa. La inflamación localizada en el hígado, el corazón, la piel (e. g., dermatitis, inflamación causada por infecciones bacterianas, fúngicas o virales y/o a reacciones alérgicas o autoinmunes), el bazo, el cerebro, el riñón (e. g., pielonefritis bacterial, nefritis intersticial, y/o glomerulonefritis) y el tracto pulmonar, especialmente los pulmones, y que están asociadas con sepsis o shock séptico es tratada de forma favorable por los métodos de acuerdo con la presente invención.

El término "cáncer" es usado como un término general para describir cualquiera de varios tipos neoplasmas malignos, la mayoría de los cuales invaden los tejidos circundantes, pueden hacer metástasis a varios sitios y son probablemente replicados después intentar removerlos y causan la muerte del paciente a menos que sean tratados adecuadamente. Los cánceres que pueden ser tratados usando las formulaciones y métodos de la presente invención incluyen, por ejemplo, estomacal, de colón, rectal, de hígado, pancreático, de pulmón, de seno, de cuello uterino, de cuerpo uterino, de ovario, de próstata, de testículos, de vejiga, renal, cerebral/SNC, de cabeza y de cuello, de garganta, enfermedad de Hodgkins, leucemia de tipo no-Hodgkins, melanoma de la piel, varios sarcomas, cáncer de pulmón de células pequeñas, coriocarcinoma, de boca/faringe, de esófago, de laringe, melanoma, de riñón y linfoma, entre otros.

La presente invención puede ser usada para tratar condiciones o trastornos que involucran el sistema respiratorio, e.g., enfisema, bronquitis, fibrosis quística, neumonía y enfermedades pulmonares intersticiales. El término "enfisema" según lo usado aquí se refiere a una enfermedad pulmonar caracterizada por un alargamiento del alveolo pulmonar. En esta condición, las paredes alveolares son destruidas, causando la pérdida de la estructura de soporte de los bronquiolos y el colapso durante la exhalación (véase, e.g. *The Merck Manual of Diagnosis and Therapy*, 17th Edición, Sección 6, Capítulo 68). El término "bronquitis" se refiere a una enfermedad pulmonar caracterizada por la inflamación del árbol traqueobronquial. La bronquitis puede ser desarrollada después de infecciones, e.g., infecciones virales, e.g. el resfriado común; o

infecciones bacterianas, o tras la exposición a un irritante (véase, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edición, Sección6, Capítulo 69). El término "fibrosis quística" se refiere a una enfermedad genética de las glándulas exocrinas. La enfermedad afecta principalmente el tracto gastrointestinal y el sistema respiratorio, y es usualmente caracterizado como una enfermedad pulmonar obstructiva crónica (EPOC) (véase, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edición, Sección19, Capítulo 267). El término "neumonía" se refiere a una enfermedad pulmonar que afecta el parénquima y que incluye, e.g., neumonía bacteriana, viral y por aspiración (véase, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edición, Sección6, Capítulo 73). El término "enfermedad pulmonar intersticial" o "enfermedad pulmonar idiopática" se refiere al grupo de enfermedades pulmonares de etiología desconocida, que producen cambios patológicos difusos que generalmente involucran el tejido intersticial interalveolar (véase, e.g., *Merck Manual of Diagnosis and Therapy*, 17th Edición, Sección6, Capítulo 78).

Como se usa aquí el término "artritis" se refiere a una condición caracterizada por la inflamación de las articulaciones, e incluye, por ejemplo, artritis reumatoide (AR) (una poliartritis inflamatoria crónica que conduce a menudo a la destrucción de las articulaciones), artritis soriática (artritis inflamatoria asociada a soriasis), espondilitis anquilosante (inflamación del esqueleto axial y de las articulaciones periféricas grandes), y la anquilosis (inmovilidad o fusión de la articulación) (véase, e. g., *The Merck Manual of Diagnosis and Therapy*, 17th Edición, Sección5, Capítulo 50; Sección5, Capítulo 51 ; Sección5, Capítulo 51; y Sección9, Capítulo 108, respectivamente).

El término "enfermedad de Parkinson" se refiere a "una enfermedad idiopática, lentamente progresiva, degenerativa del SNC caracterizada por un movimiento lento y disminuido, rigidez muscular, temblor de reposo e inestabilidad de la postura". (*The Merck Manual of Diagnosis and Therapy*, 17ª Edición, Sección 14, Capítulo 179). El término "enfermedad de Alzheimer" se refiere a una enfermedad caracterizada por "una pérdida progresiva, inexorable de las funciones cognitivas asociadas con un número excesivo de placas seniles en la corteza cerebral y materia gris subcortical la cual también contiene las b-amiloides y la maraña neurofibrilar que están formadas por proteínas tau," (*The Merck Manual of Diagnosis and Therapy*, 17th Edición, Sección 14, Capítulo 171).

Dosis bajas de CO pueden también ser usadas en la presente invención para inducir la enzima HO-1 en pacientes y prevenir o limitar el estrés oxidativo, especialmente estrés oxidativo causado por hiperoxia o sepsis. La HO-1 inducida está involucrada en el mantenimiento de la homeóstasis en las células del paciente.

Tratamiento de Órganos y Tejidos para Ampliar la Estabilidad de Almacenamiento

La presente invención también describe el uso de CO como un preservativo para el almacenamiento de órganos o tejidos que serán usados en transplantes. Es un resultado inesperado que la adición de dosis bajas de CO en el medio en el cual los

órganos a ser transplantados están almacenados reducirá sustancialmente la probabilidad de daño oxidativo de los órganos durante el almacenamiento y ampliará sustancialmente el tiempo de almacenamiento para el cual los órganos a ser transplantados pueden ser almacenados en forma segura sin sufrir daño oxidativo irreversible. De esta forma, en un modo de ejecución de la presente invención una cantidad efectiva de CO es burbujeado dentro del medio de almacenamiento antes o preferiblemente cuando un órgano es colocado por primera vez en un medio o justo después de esto. El CO puede ser usado para ampliar la estabilidad de almacenamiento de los órganos que han sido almacenados por algún tiempo en otro medio, pero en estos casos, el daño oxidativo pudo haber sido irreversible y de esta forma queda limitado el efecto pretendido.

De acuerdo con la presente invención se proporciona un método para ampliar la estabilidad de almacenamiento de un órgano o tejido. La estabilidad de almacenamiento es ampliada mediante la exposición del órgano o tejido a formulaciones líquidas o gaseosas de monóxido de carbono. La exposición de un órgano o tejido a formulaciones gaseosas de monóxido de carbono puede ser llevada a cabo en cualquier cámara o espacio adecuado para la creación de una atmósfera que contiene niveles apropiados de monóxido de carbono gaseoso. Tales cámaras incluyen por ejemplo, incubadores, y cámaras construidas para el propósito de acomodar un órgano en una solución preservadora. Como otro ejemplo una cámara apropiada puede ser una cámara donde solamente los gases alimentados dentro de la cámara están presentes en la atmósfera interna, de tal forma que la concentración de monóxido

de carbono pueda ser establecida y mantenida a una concentración y pureza dada, e.g., en donde la cámara es hermética. Por ejemplo, un incubador de CO₂ puede ser usado para exponer un órgano a una formulación de monóxido de carbono, donde el monóxido de carbono gaseoso es alimentado con un flujo continuo desde un recipiente que contiene el gas.

Con relación a las formulaciones líquidas de monóxido de carbono, la exposición puede ser llevada a cabo en cualquier cámara o espacio que tenga el volumen suficiente para sumergir el órgano o el tejido, completamente o parcialmente, en una formulación de monóxido de carbono. En un modo de ejecución de la presente invención, el órgano puede ser expuesto a la formulación de monóxido de carbono mediante la ubicación del órgano en cualquier contenedor adecuado y haciendo que la composición de monóxido de carbono "bañe los" órganos, de manera que el órgano esté expuesto a un flujo continuo de la formulación de monóxido de carbono. En otro modo de ejecución el órgano es perfundido con una formulación de monóxido de carbono. El término perfusión es un término reconocido en el estado del arte, y describe el paso de un líquido, e.g., una formulación de monóxido de carbono, que pasa través de los vasos sanguíneos de un órgano o tejido. Métodos para la perfusión de órganos *ex vivo* e *in situ* son bien conocidos en el estado del arte. Un órgano puede ser perfundido con una formulación de monóxido de carbono *ex vivo*, por ejemplo, mediante una máquina de perfusión hipotérmica continua (véase, *Oxford Textbook of Surgery*, Morris y Malt, Eds., Oxford University Press (1994)). Opcionalmente, el órgano puede ser perfundido con una solución de lavado, e.g., solución UW sin monóxido de carbono,

antes de la perfusión con la formulación del monóxido de carbono para remover del órgano la sangre del donante. Un proceso como este podría ser realizado para evitar la competencia por parte de la hemoglobina del donante por el monóxido de carbono. Como otra opción, la solución de lavado puede ser una formulación de monóxido de carbono. Incluso como otro ejemplo, un líquido apropiado puede ser pasado a través de una tubería que permite que el gas se difunda, la cual pasa a través de una atmósfera que contiene monóxido de carbono. (e.g., a través de una cámara, tal que cuente con un membrana de oxigenación extracorporeal), para crear una formulación líquida de monóxido de carbono, la cual puede ser entonces transferida dentro del un órgano (e.g., perfundida dentro del órgano mediante la conexión del tubería al órgano).

Como otro ejemplo, el órgano puede ser ubicado, e.g., sumergido, en un medio o solución que no contenga monóxido de carbono, y colocado en una cámara tal que el medio o la solución pueda ser transformado en una formulación de monóxido de carbono mediante la exposición a una atmósfera que contenga monóxido de carbono como las que se han descrito aquí. Incluso como otro ejemplo, el órgano puede ser sumergido en un líquido que no contenga monóxido de carbono, y el monóxido de carbono puede ser burbujeado dentro del líquido.

La presente invención contempla que cualquiera de o todos los métodos anteriormente descritos para la exposición de un órgano a una formulación líquida de monóxido de carbono, e.g., lavando, sumergiendo o perfundiendo, puede ser usado en

un procediendo dado, e.g., usado en un procedimiento único para ampliar la estabilidad de almacenamiento de un órgano o tejido.

Monóxido de Carbono como una Herramienta de Diagnóstico

Adicional al uso de CO como un agente terapéutico, la medición de CO puede ser una herramienta de diagnóstico útil, e.g., como un biomarcador, para determinar si un paciente presenta estrés oxidativo o tiene una condición o estado de enfermedad en donde el CO puede estar involucrado, e.g., sepsis o shock séptico. En general, es monitoreada la respiración exhalada por un paciente del cual se sospecha presenta estrés oxidativo o está en riesgo de presentar estrés oxidativo para determinar si pueden ser medidos niveles de monóxido de carbono. Si son observados niveles detectables de monóxido de carbono (i.e., una cantidad de monóxido de carbono de por lo menos alrededor de 0.01 ppm en la respiración del paciente), entonces el médico que atiende o la persona encargada del cuidado puede entonces comenzar a administrar dosis terapéuticas de monóxido de carbono para tratar el estrés oxidativo o alguna o algunas de las condiciones de los estados de enfermedad, las cuales son subsecuentes a o resultado del estrés oxidativo.

En un modo de ejecución, la respiración exhalada por un paciente será analizada para determinar la presencia de CO. El contenido de CO en la respiración de un

paciente es medido mediante un analizador de CO (por ejemplo, usando un Logan LR2000) el cual es sensible para la detección de CO entre 0 y alrededor de 1000 ppm (con una sensibilidad tan baja como 1 ppb). En este método los sujetos exhalan lentamente desde CVF funcional hacia adentro del analizador de respiración con un flujo constante (5-6 l/min) durante un intervalo de 20-30 segundos. Se llevan a cabo dos medidas exitosas y el promedio de estos valores será usado para todos los cálculos. Los niveles de CO ambientales son medidos antes de cada respiración con el objetivo de proporcionar los valores de control y de fondo. Mientras que cualquier elevación en los niveles de CO a partir de los valores de fondo puede implicar un estado actual o incipiente de estrés oxidativo, una cantidad de por lo menos alrededor de 1 ppm proporciona un indicio claro de que el paciente padece o esta próximo a padecer estrés oxidativo.

Un número de modos de ejecución de la invención ha sido descrito. No obstante, se entenderá que pueden ser realizadas varias modificaciones sin apartarse del espíritu y alcance de la invención. Por consiguiente, otros modos de ejecución están contenidos dentro del alcance y las siguientes reivindicaciones.

SE REIVINDICA:

1. El uso de monóxido de carbono gaseoso en la preparación de un medicamento para ser usado en el tratamiento de pacientes que padecen o están en riesgo de padecer al menos un trastorno seleccionado del grupo conformado por: enfisema, bronquitis, fibrosis quística, neumonía, enfermedad pulmonar intersticial, cicatrización de heridas, artritis, la enfermedad de Parkinson y la enfermedad de Alzheimer; o promover la cicatrización de heridas en el paciente.

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2. El método de la reivindicación 1, en donde el trastorno es enfisema.

3. El método de la reivindicación 1, en donde el trastorno es bronquitis.

4. El método de la reivindicación 1, en donde el trastorno es fibrosis quística.

5. El método de la reivindicación 1, en donde el trastorno es neumonía.

6. El método de la reivindicación 1, en donde el trastorno es una enfermedad pulmonar intersticial.

7. El método de la reivindicación 1, en donde el trastorno es artritis.
8. El método de la reivindicación 1, en donde el trastorno es la enfermedad de Parkinson.
9. El método de la reivindicación 1, en donde el trastorno es la enfermedad de Alzheimer.
10. El método de la reivindicación 1, en donde el monóxido de carbono gaseoso es usado en la preparación de un medicamento para el uso en la promoción de cicatrización de heridas en el paciente. 350
11. El uso de monóxido de carbono gaseoso en la preparación de un medicamento para el uso en el tratamiento de un paciente que padece o esta en riesgo de padecer inflamación localizada en por lo menos uno de los órganos seleccionado del grupo conformado por: riñón, bazo y piel. 350
12. El método de la reivindicación 11, en donde el órgano es un riñón.

Revisando folio 79

13. El método de la reivindicación 11, en donde el órgano es el bazo.

14. El método de la reivindicación 11, en donde el órgano es la piel.

15. Un método para ampliar la estabilidad de almacenamiento de un órgano en un medio, que consiste en:

Proporcionar un medio que contenga monóxido de carbono; y almacenar el órgano en el medio, en donde el monóxido de carbono está presente en el medio en una cantidad suficiente para ampliar la estabilidad de almacenamiento del órgano.

16. Un método para determinar si un paciente está en el riesgo de presentar sepsis o el shock séptico, que consiste en:

Medir el monóxido de carbono en la respiración exhalada por un paciente, en donde una cantidad de por lo menos aproximadamente 1 ppm de monóxido de carbono en la respiración está indicando que el paciente está en riesgo de presentar sepsis o shock séptico.

PATENT COOPERATION TREATY

JKE
TEB
MLB

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
IANIS K. FRASER
FISH & RICHARDSON P.C.
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BOSTON, MA 02110

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BOSTON OFFICE

PCT

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

04 OCT 2004

Applicant's or agent's file reference

13681-003WO1

IMPORTANT NOTIFICATION

International application No.

PCT/US02/14836

International filing date (day/month/year)

09 May 2002 (09.05.2002)

Priority date (day/month/year)

Applicant

YALE UNIVERSITY

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Docketed By Practice Systems
Foreign App. 113-01
(003001)
Initials: JKE

Docketed By Billing Secretary
Due Date:
Deadline: 11/2/04
Initials: NAB

Name and mailing address of the IPEA/US
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Commissioner for Patents
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Alexandria, Virginia 22313-1430
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Form PCT/IPEA/416 (July 1992)

Authorized officer
John Pak
Telephone No. (571) 272-1600

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 13681-003WO1	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IEPA/416)	
International application No. PCT/US02/14836	International filing date (day/month/year) 09 May 2002 (09.05.2002)	Priority date (day/month/year)
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 33/00 and US Cl.: 424/613,699,718		
Applicant YALE UNIVERSITY		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2.	This REPORT consists of a total of <u>7</u> sheets, including this cover sheet. ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of <u>1</u> sheets.
3.	This report contains indications relating to the following items: - I ☒ Basis of the report - II ☐ Priority - III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability - IV ☐ Lack of unity of invention - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement - VI ☐ Certain documents cited - VII ☐ Certain defects in the international application - VIII ☒ Certain observations on the international application

Date of submission of the demand 09 December 2003 (09.12.2003)	Date of completion of this report 18 August 2004 (18.08.2004)
Name and mailing address of the IEPA/US Mail Stop PCT, Attn: IEPA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer John Pak Telephone No. (571) 272-1600

Form PCT/IEPA/409 (cover sheet)(July 1998)

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US02/14836

I. Basis of the report

1. With regard to the elements of the international application:*

- ☐ the international application as originally filed.
- ☒ the description:
pages 1-21 _____ as originally filed
pages NONE _____, filed with the demand
pages NONE _____, filed with the letter of _____.
- ☒ the claims:
pages 22 _____, as originally filed
pages NONE _____, as amended (together with any statement) under Article 19
pages NONE _____, filed with the demand
pages 23-25 _____, filed with the letter of 18 August 2004 _____.
- ☐ the drawings:
pages NONE _____, as originally filed
pages NONE _____, filed with the demand
pages NONE _____, filed with the letter of _____.
- ☐ the sequence listing part of the description:
pages NONE _____, as originally filed
pages NONE _____, filed with the demand
pages NONE _____, filed with the letter of _____.

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☐ the description, pages NONE
- ☒ the claims, Nos. 16
- ☐ the drawings, sheets/fig NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT		International application No. PCT/US02/14836
V. Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement		
1. STATEMENT		
Novelty (N)	Claims <u>15</u>	YES
	Claims <u>1-14</u>	NO
Inventive Step (IS)	Claims <u>NONE</u>	YES
	Claims <u>1-15</u>	NO
Industrial Applicability (IA)	Claims <u>1-15</u>	YES
	Claims <u>NONE</u>	NO
2. CITATIONS AND EXPLANATIONS Please See <u>Continuation Sheet</u>		

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT	International application No. PCT/US02/14836
VIII. Certain observations on the international application	
The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made: Claims 1-14 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims 1-14 are indefinite for the following reason(s): The claims are directed to use claims and the language does not clearly indicate whether a method of use or a method of preparation was intended.	

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US02/14836

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Claims 1-15 meet the criteria set out in PCT Article 33(4), and thus meet industrial applicability because the subject matter claimed can be made or used in industry.

Claims 1-14 lack novelty under PCT Article 33(2) as being anticipated by Abidin et al. Abidin et al. explicitly disclose the use of carbon monoxide gas as a ~~medicament~~ (Abstract). Claims 1-14 are drafted as use claims, as such, it is uncertain whether the claims are intended to be a method of use or method of making. Therefore, the claims are read to be method of making claims, in which case, the intended use does not appear to patentably distinguish the claims.

Claims 1-14 lack novelty under PCT Article 33(2) as being anticipated by Otterbein et al. Otterbein et al. explicitly disclose the use of carbon monoxide gas as a ~~medicament~~ (Abstract). Claims 1-14 are drafted as use claims, as such, it is uncertain whether the claims are intended to be a method of use or method of making. Therefore, the claims are read to be method of making claims, in which case, the intended use does not appear to patentably distinguish the claims.

Examiner has duly considered Applicant's arguments but deems them ~~unpersuasive~~. Applicant's claims are directed to use of carbon monoxide gas in the preparation of a ~~medicament~~ for the use in a patient. All claims limitations have been considered but based on the interpretation of the claims as a method of preparation claims the intended use of the ~~medicament~~ has been determined not to distinguish the claims from the prior art. The burden is on Applicant to show that the ~~medicament~~ prepared would not be capable of being used in a patient for treatment of the various conditions/disease.

Claim 15 lacks inventive step under PCT Article 33(3) as being obvious over Yaum et al. in view of Stone (US Pat. 5,293,875). Yaum et al. discloses a method for determining whether one is at risk of sepsis or septic shock by measuring for elevated levels of endogenous carbon monoxide (CO) in the lungs (Abstract).

Stone discloses an apparatus for measuring CO in gas from the lungs by measuring end-tidal breath where the apparatus is capable of measuring CO in the range of 0 to 500 ppm (See entire reference, especially, Column 2, lines 15-28)

The difference between the prior art and the claimed invention is that the prior art does not expressly disclose measuring exhaled CO or a CO level of at least about 1 ppm. However, the prior art amply suggests the same as it is taught to measure the level of endogenous carbon monoxide in the lungs, that increased endogenous is indicative of sepsis and septic shock and a method and apparatus for measuring CO in the lungs by measuring end-tidal breath is taught. As such, it would have been well within the skill of and one of ordinary skill in the art would have been motivated to measure endogenous carbon monoxide in the breath as it would be expected that carbon monoxide in the lungs could be measured by measuring the end-tidal breath of the patient. Further, the prior art discloses that increased carbon monoxide levels is indicative of sepsis or septic shock, as such, it would have been well within the skill of one of ordinary skill in the art to arrive at the optimum level of carbon monoxide. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 105 USPQ 233, 235 (CCPA 1955) (Claimed process which was performed at a temperature between 40°C and 80°C and an acid

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US02/14836

Supplementary Box

(To be used when the space in any of the preceding boxes is not sufficient)

concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); In re Peterson, 65 USPQ2d 1379, 1382-83 (Fed. Cir. 2003). ("The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages.")

Examiner has duly considered Applicant's arguments but deems them unpersuasive for the reasons above and the further reasons below. Applicant cites to Yuan at column 2, lines 7, 9, however, the Yuan et al. reference cited herein is only an abstract. Applicant does not appear to provide a copy of the Yuan reference on which Applicant's assertions are based. In any case, according to Applicant's Specification, the term "patient" includes rodents such as mice and rats and there is nothing in the claims which exclude sacrificing rats (Specification, Pg. 7, lines 29-32, Pg. 8, lines 1,2).

Claims 1-14 lack an inventive step under PCT Article 33(3) as being obvious over WO 98/08523 in view of Choi et al. (1996), Lefter et al. (1993) and Vassalli et al. (1998).

WO 98/08523 teaches methods of treating inflammatory processes in humans and animals with mixtures of gases, including carbon monoxide, nitric oxide and nitrous oxide, which is effective in treating cancer, pneumonia, asthma, acute respiratory distress syndrome, pulmonary hypertension, bronchitis and sepsis (See entire document).

Choi et al. (1996) teaches that oxidative stress plays a central role in the pathogenesis of many pulmonary diseases including adult respiratory distress syndrome, emphysema, asthma, bronchopulmonary dysplasia and interstitial pulmonary fibrosis (Abstract).

Lefter et al. (1993) teach that carbon monoxide has cytoprotective effect in low concentrations in inflammatory states (Abstract).

Vassalli et al. (1998) teaches that carbon monoxide inhibits acute pulmonary vasoconstriction and would be effective in the treatment of acute pulmonary hypertension (Abstract).

The difference between the prior art and the claimed invention is that the prior art does not expressly disclose a method of treating the claimed disorders which are secondary or resulting from oxidative stress, a method of treating asthma, a method of treating cancer, a method of treating inflammation in at least one organ, a method of treating inflammation secondary to sepsis or sepsis or a method of promoting wound healing, with carbon monoxide. However, the prior art amply suggests the same as it is known that oxidative stress plays a central role in pulmonary disorders and that carbon dioxide is effective in treating various conditions, including inflammation, hypertension and vasoconstriction and sepsis. As such, it would have been well within the skill of and one of ordinary skill in the art would have expected that administration of carbon dioxide would be effective in treating the above conditions.

Examiner has duly considered Applicant's arguments but deems them unpersuasive. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See In re Keller, 208 USPQ 871 (CCPA 1981); In re Merck & Co., 231 USPQ 375 (Fed. Cir. 1986). Further, the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See In re Keller, 208 USPQ 871 (CCPA 1981).

Lefter et al. discloses that CO has anti-inflammatory activity, as such, one of ordinary skill in the art would be motivated to use CO in the process disclosed by WO 98/08523. WO 98/08523, Lefter et al. and Vassalli et al. all disclose or suggest the therapeutic use of exogenous CO, as such, one of ordinary skill in the art would expect from the teaching of Choi et al. that administration of exogenous CO would be effective in treating the pulmonary diseases above. Lefter is cited for the teaching that carbon monoxide has cytoprotective effects in inflammatory states. The fact that the study in Lefter et al. is in vitro, that Lefter does not teach or suggest using CO to treat localized inflammation of the kidney, spleen or skin, or that CO could be administered to a patient without toxic effects does not make the claims non-obvious as Lefter is not cited for the same but for the reasons above. Further, the teachings of Lefter et al. must be viewed in light of the teachings of WO '523 and Vassalli et al. which do disclose administration of CO to a patient. Contrary to Applicant's arguments Vassalli et al. does have a bearing the obviousness of Applicant's claims in that Vassalli discloses the therapeutic administration of CO as a medicament. Examiner notes that Applicant claims are directed to the preparation of a medicament for treatment of a patient. With respect to Applicant's arguments that one or more of the prior art does not disclose the treatment of a specific condition/disease, all claim limitations have been considered based on the interpretation of the claims as a method of preparation claims. As such, the intended use of the medicament has been determined not to distinguish the claims from the prior art. The burden is on Applicant to show that the medicament prepared would not be capable of being used in a patient for treatment of the various conditions/disease.

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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PCT/US02/14836

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

NEW CITATIONS
US 5,293,875 A (STONE) 15 March 1994 (15.03.1994), see entire references, especially column 2, lines 15-28.

PCT/US 02/14836

IPERUS 08 AUG 2004

13. The method of claim 11, wherein the organ is a spleen.

14. The method of claim 11, wherein the organ is skin.

5 15. A method of determining whether a patient is at risk for sepsis or septic shock, comprising:

measuring carbon monoxide in breath exhaled by a patient, wherein an amount of carbon monoxide of at least about .1 ppm in the breath is indicative that the patient is at risk for sepsis or septic shock.

AMENDED SHEET

REIVINDICACIONES ADICIONALES

[Recibidas por el International Bureau el 02 de septiembre de 2003 (02.09.2003); las reivindicaciones originales 1-14 y 16 no son modificadas, la reivindicación 15 original es cancelada (1 página)]

13. El método de la reivindicación 11, en donde el órgano es el bazo. =

14. El método de la reivindicación 11, en donde el órgano es la piel. =

15 cancelado

15. Un método para determinar si un paciente está en el riesgo de presentar sepsis o el shock séptico, que consiste en: = 16

Medir el monóxido de carbono en la respiración exhalada por un paciente, en donde una cantidad de monóxido de carbono de por lo menos alrededor de 1 ppm en la respiración está indicando que el paciente está en riesgo de presentar sepsis o shock séptico.

J.K.F.
TELT

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITYTo:
JANIS K. FRASER
FISH & RICHARDSON P.C.
225 FRANKLIN STREET
BOSTON, MA 02110

RECEIVED

MAR 17 2004

FISH & RICHARDSON, P.C.
BOSTON OFFICE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing
(day/month/year)

15 MAR 2004

Applicant's or agent's file reference

13681-003WO1

REPLY DUE

within 1 months/days from
the above date of mailing

International application No.

PCT/US02/14836

International filing date (day/month/year)

09 May 2002 (09.05.2002)

Priority date (day/month/year)

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

IPC(7): A61K 33/00 and US Cl.: 424/613,699,718

Applicant

YALE UNIVERSITY

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2 (a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension. See rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 09 September 2004 (09.09.2004).

Name and mailing address of the IPEA/US

Mail Stop PCT, Attn: IPEA/US

Commissioner for Patents

P.O. Box 1430

Alexandria, Virginia 22313-1430

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Form PCT/IPEA/408 (cover sheet)(July 1998)

MAR 18 2004

Docketed By Practice Systems

Action Code: Wnt. DP

Base Date: 3.15.04

Due Date: 4.15.04

Docketed By Billing Secretary

Due Date:

Deadline: 4/15/04

WRITTEN OPINION

International application No.

PCT/US02/14836

Basis of the opinion

With regard to the elements of the international application:*

- ☒ the international application as originally filed
- ☒ the description:
 pages 1-21, as originally filed
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____
- ☒ the claims:
 pages 22 and 23, as originally filed
 pages NONE, as amended (together with any statement) under Article 19
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____
- ☐ the drawings:
 pages NONE, as originally filed
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____
- ☐ the sequence listing part of the description:
 pages NONE, as originally filed
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
 These elements were available or furnished to this Authority in the following language _____ which is:
- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:
- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages NONE
- ☐ the claims, Nos. NONE
- ☐ the drawings, sheets/fig NONE

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed."

WRITTEN OPINION

International application No.
PCT/US02/14836

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT			
Novelty (N)	Claims	15	YES
	Claims	1-14 and 16	NO
Inventive Step (IS)	Claims	15	YES
	Claims	1-14 and 16	NO
Industrial Applicability (IA)	Claims	1-16	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

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WRITTEN OPINION

International application No.

PCT/US02/14836

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Claims 1-14 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims 1-14 are indefinite for the following reason(s): The claims are directed to use claims and the language does not clearly indicate whether a method of use or a method of preparation was intended.

~~Supplemental Box~~

(To be used when the space in any of the preceding boxes is not sufficient)

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

V. 2. Citations and Explanations:

Claim 15 meets the criteria set out in PCT Article 33(2), because no single prior art reference explicitly discloses a method of enhancing the storage stability of an organ in a medium comprising providing a medium comprising carbon monoxide and storing the organ in the medium where the carbon monoxide is present in an amount sufficient to enhance storage stability of the organ.

Claim 15 meets the criteria set out in PCT Article 33(3), because the prior art does not teach or fairly suggest a method of enhancing the storage stability of an organ in a medium comprising providing a medium comprising carbon monoxide and storing the organ in the medium where the carbon monoxide is present in an amount sufficient to enhance storage stability of the organ.

Claims 1-16 meet the criteria set out in PCT Article 33(4), and thus meet industrial applicability because the subject matter claimed can be made or used in industry.

Claims 1-14 lack novelty under PCT Article 33(2) as being anticipated by Abidin et al. Abidin et al. explicitly disclose the use of carbon monoxide gas as a medicament (Abstract). Claims 1-14 are drafted as use claims, as such, it is uncertain whether the claims are intended to be a method of use or method of making. Therefore, the claims are read to be method of making claims, in which case, the intended use does not appear to patentably distinguish the claims.

Claims 1-14 lack novelty under PCT Article 33(2) as being anticipated by Omerbein et al. Omerbein et al. explicitly disclose the use of carbon monoxide gas as a medicament (Abstract). Claims 1-14 are drafted as use claims, as such, it is uncertain whether the claims are intended to be a method of use or method of making. Therefore, the claims are read to be method of making claims, in which case, the intended use does not appear to patentably distinguish the claims.

Claim 16 lacks novelty under PCT Article 33(2) as being anticipated by Yaun et al. Yaun et al. explicitly disclose a method for determining whether one is at risk of sepsis or septic shock by measuring for elevated levels of endogenous carbon monoxide.

Claims 1-14 lack an inventive step under PCT Article 33(3) as being obvious over WO 98/08523 in view of Choi et al. (1996), Lefer et al. (1993) and Vaasalli et al. (1998).

WO 98/08523 teaches methods of treating inflammatory processes in humans and animals with mixtures of gases, including carbon monoxide, nitric oxide and nitrous oxide, which is effective in treating cancer, pneumonia, asthma, acute respiratory distress syndrome, pulmonary hypertension, bronchitis and sepsis (See entire document).

Choi et al. (1996) teaches that oxidative stress plays a central role in the pathogenesis of many pulmonary diseases including

WRITTEN OPINION

International application No.
PCT/USD2/14836

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

adult respiratory distress syndrome, emphysema, asthma, bronchopulmonary dysplasia and interstitial pulmonary fibrosis (Abstract).
Lefer et al. (1993) teach that carbon monoxide has cytoprotective effective in low concentrations in inflammatory states (Abstract).

Vassalli et al. (1998) teaches that carbon monoxide inhibits acute pulmonary vasoconstriction and would be effective in the treatment of acute pulmonary hypertension (Abstract).

The difference between the prior art and the claimed invention is that the prior art does not expressly disclose a method of treating the claimed disorders which are secondary or resulting from oxidative stress, a method of treating asthma, a method of treating cancer, a method of treating inflammation in at least one organ, a method of treating inflammation secondary to sepsis or sepsis or a method of promoting wound healing, with carbon monoxide. However, the prior art amply suggests the same as it is known that oxidative stress plays a central role in pulmonary disorders and that carbon dioxide is effective in treating various conditions, including inflammation, hypertension and vasoconstriction and sepsis. As such, it would have been well within the skill of and one of ordinary skill in the art would have expected that administration of carbon dioxide would be effective in treating the above conditions.

Full

IN THE UNITED STATES RECEIVING OFFICE

Applicant : Yale University et al.
Serial No. : US02/14836
Filed : May 9, 2002
Title : CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT

MAIL STOP PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO WRITTEN OPINION

In response to the Written Opinion dated March 15, 2004, applicants submit the remarks below.

CERTIFICATE OF MAILING BY EXPRESS MAIL

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April 15, 2004
Date of Deposit

Applicant : Yale University et al.
Serial No. : US02/14836
Filed : May 9, 2002
Page : 2 of 5

Attorney's Docket No.: 13681-003WO1

REMARKS

As an initial matter, applicants wish to point out that original claims 1 to 14 and 16 (now renumbered as claim 15) are pending in this application. Applicants canceled original claim 15 (which was directed to a method of enhancing the storage stability of an organ in a medium) by way of an Amendment Under Article 19, which was filed on September 2, 2003.

The Written Opinion states that claims 1 to 14 lack novelty under PCT Article 33(2) because they are allegedly anticipated by both Abidin et al. (Kosmicheskaya Biologiya i Aviakosmicheskaya Meditsina (1978) 6:63-67) and Otterbein et al. (Am. J. Physiol. 276 (Lung Cell Mol. Physiol. 20): L688-L694 (1999)). In support these conclusions, the Written Opinion states (at page 5, section V):

Claims 1-14 are drafted as use claims, as such, it is uncertain whether the claims are intended to be a method of use or method of making. Therefore, the claims are read to be method of making claims, in which case, the intended use does not appear to patentably distinguish the claims.

Applicants respectfully disagree and point out that claims 1 to 14 are written in standard "second medical use" format. Applicants submit that every limitation recited in these claims should be considered during examination. Neither Abidin nor Otterbein describes using carbon monoxide (CO) gas to prepare a medicament for treating emphysema, bronchitis, cystic fibrosis, pneumonia, interstitial lung disease, wound healing, arthritis, Parkinson's disease, or Alzheimer's disease, or to promote wound healing, in a patient. Further, neither of these references describes using CO gas to prepare a medicament for treating localized inflammation of a patient's kidney, spleen, or skin. As such, applicants submit that these references do not anticipate claims 1 to 14.

The Written Opinion also states that claim 16 (which has been renumbered as claim 15) lacks novelty under PCT Article 33(2) as allegedly anticipated by Yuan et al. (Chinese Medical Sciences Journal, Vol. 12(4):212-215 (1997)). In support of this conclusion, the Written Opinion states (at page 5, section V):

Yuan et al. explicitly disclose a method for determining whether one is at risk of sepsis or septic shock by measuring for elevated levels of endogenous carbon monoxide.

Applicants respectfully disagree with both the above-quoted interpretation of Yuan et al. and the Written Opinion's conclusion for the reasons below.

Yuan describes a study carried out to determine whether there is evidence of abnormal endogenous CO production in newborn rats during endotoxemia (see Yuan at column 2, lines 7 to 9). To that end, Yuan simply injected rats with endotoxin and, at various time points following such injection (e.g., 1, 2, 3, and 4 hours after injection), sacrificed the rats and measured endogenous CO levels in the rats' plasma, livers, kidneys, and lungs. Yuan concluded that endogenous CO levels increased in the plasma and vital organs of those animals. Nowhere does Yuan teach that his protocols could be used to determine whether a patient is at risk of sepsis or septic shock. Indeed, it is absurd to propose that Yuan considered his method, which involved killing the experimental subjects, was a clinically useful technique.

Note also that claim 16 requires measuring CO in breath exhaled by the patient. Nowhere does Yuan teach that a patient's risk for sepsis or septic shock can be determined by measuring exhaled CO. Nor is there any suggestion that a CO level of at least about 1 ppm might be significant. Since Yuan fails to teach any (much less all) of the limitations recited in claim 16, Yuan does not anticipate this claim.

The Written Opinion further states that claims 1 to 14 lack an inventive step under PCT Article 33(3) as being allegedly obvious over WO 98/08523 (hereinafter "Eschwey") in view of Choi et al. (Am. J. Respir. Cell. Mol. Biol. Jul;15(1):9-19 (1996)), Lefer et al. (Methods and Findings in Experimental and Clinical Pharmacology, 15(9):617-622 (1993)), and Vassalli et al. (Eur. Resp. J. 12, Suppl. 28, 273a (1998)). Applicants respectfully disagree with this conclusion for the reasons discussed below.

Eschwey describes the use of "hydrogenous medications," i.e., gaseous mixtures containing hydrogen (e.g., protium, deuterium, or tritium), for the treatment of inflammatory processes and cancer in animals. Eschwey states that hydrogen-containing mixtures have anti-tumor and anti-inflammatory activities and are therefore useful for treating cancer and various inflammatory conditions. Eschwey includes a list of other compounds that can optionally be

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included in the hydrogenous medications, such as "NO (nitric oxide), N₂O (nitrous oxide), acetylene (ethine, C₂H₂), ethylene (ethene, C₂H₄) or carbon monoxide (CO)" (Eschwey translation (attached hereto as Exhibit A) at page 7, lines 16 to 18, see also page 14, lines 20 to 24). There is no indication in Eschwey that CO has anti-inflammatory activity, or that it would be useful in and of itself to treat the diseases and conditions recited in claims 1 to 14. In fact, Eschwey does not describe specific activities of, or uses for, any of the listed optional compounds except nitric and nitrous oxide. Eschwey's discussion is directed exclusively to the uses of medications containing hydrogen or hydrogen plus nitric oxide to treat inflammatory diseases (see, e.g., page 8, line 3, to page 9, line 16, and in particular page 8, line 15 to page 9, line 5). Eschwey merely refers to the optional compounds as "pharmacologically active" gases, without elaboration. Accordingly, a skilled practitioner would not have been motivated by Eschwey to administer CO to treat the diseases and conditions recited in applicants' claims, because he or she would have had no reason to expect such a treatment to be successful.

The Written Opinion also cites Choi, Lefer, and Vassalli. However, none of these additional references provides the information missing in the primary reference. Choi is a review article that describes the physiological significance of heme oxygenase-1 in lung injury caused by oxidative stress. Choi also describes the role of endogenously-produced CO (i.e., CO produced in the body via heme oxygenase-1 degradation of heme). Nowhere does Choi teach, or even suggest, that exogenously-delivered CO gas could be used as a therapeutic agent. Thus, Choi has no bearing upon the patentability of the pending claims.

Lefer discloses *in vitro* experiments investigating the effects of nitric oxide- and CO-saturated aqueous solutions on isolated blood vessels and leukocytes. Specifically, Lefer discloses that nitric oxide- and CO-saturated Krebs-Henseleit solutions relax isolated rat superior mesenteric arteries (SMA) rings *in vitro*, and that these solutions attenuate adherence of cat polymorphonuclear leukocytes to cat SMA endothelium *in vitro*. Lefer does not teach, or even suggest, using CO to treat the various diseases recited in claim 1 or to promote wound healing (also recited in claim 1). Further, Lefer does not teach, or even suggest, using CO to treat localized inflammation of the kidney, spleen or skin. Lefer does not even suggest that CO could be administered to a patient without toxic effects. Contacting pieces of blood vessels with an

aqueous solution is conceptually far removed from administration of CO to a patient. Thus, Lefer provides no motivation whatsoever to use CO as recited in claims 1 to 14.

Vassalli is a publication cited in the Written Opinion because, according to the Written Opinion (at page 6, section V): "Vassalli et al. (1998) teaches that carbon monoxide inhibits acute pulmonary vasoconstriction and would be effective in the treatment of acute pulmonary hypertension." Applicants respectfully submit that not one of claims 1 to 14 recites using CO to treat acute pulmonary vasoconstriction or acute pulmonary hypertension. Thus, Vassalli has no bearing on the patentability of the pending claims.

Applicants submit that none of the publications cited in the Written Opinion, singly or in combination, teaches or suggests administering CO as a therapeutic agent to treat the diseases and conditions recited in the pending claims. Thus, a skilled practitioner would find no motivation, nor a reasonable expectation of success, for using CO as described in the present application in these or any other publications. For all of the reasons discussed above, applicants submit that claims 1 to 14 do not lack inventive step in view of these references.

No fees are believed to be due at this time. If any are due, please apply them to Deposit Account No. 06-1050.

Respectfully submitted,

Date: April 15, 2004

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PCT

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changes having been made

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[Code Key: The countries listed in the document, page 2, are already recognizable from the German text, except perhaps for the following: - Translator]

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YU	Yugoslavia

Hydrogen gas normally contains molecular hydrogen. Hydrogen gas consists for example of P₂, PD or D₂ or mixtures such as P₂/PD, PD/D₂, P₂/D₂, P₂/PD/D₂, where the standard hydrogen gas (H₂) contains more than 99% by vol. of P₂. Hydrogen gas may also contain atomic hydrogen (hydrogen in the process of development). Molecular hydrogen exists in two forms: ortho-hydrogen (with parallel-oriented nuclear spin) and para-hydrogen (with antiparallel-oriented nuclear spin). Both forms coexist in thermal equilibrium. At temperatures above 200K a mixture of constant composition with 75% by vol. ortho-hydrogen and 25% by vol. para-hydrogen. appears. Hydrogen gas with this composition is called n hydrogen.

The content data (e.g. vol. %), volume data and pressure data are referenced to normal conditions (20°C, 1.013 bar). The unit ppm is defined as one part by volume per 10⁶ parts by volume. The term "hydrogenous gas-containing mixture" also includes pure hydrogen gas.

Hydrogen gas is available on the market in steel tanks at a pressure below 200 bar, for example, or is stored in liquid form (cryogen) at -253°C in ultra-insulated containers.

The medication according to the invention can be used in a variety of forms. The medication may be a hydrogenous gas-containing mixture, or a liquid preparation (e.g. hydrogenous gas-containing mixture or pure hydrogen gas dispersed in a fat emulsion), or a solid preparation (e.g., a gas stored in a clathrate).

Liquid medications are preferably administered either intravenously for systemic use or topically. Solid medications or medications containing solids are preferably applied topically (on the body surface). The gas mixture may consist, for example, of one or more inert gases and hydrogen, or of one or more inert gases, oxygen and hydrogen. Inert gases are nitrogen and the noble

Parent gases, hydrogenous gas mixtures or gaseous medications are usually compressed and stored in compressed gas containers. Storage of a hydrogenous gas mixture in a compressed gas container is advantageous to the extent that dosage is thereby enhanced. The hydrogen for a hydrogenous gas mixture may also be produced or prepared through electrolysis.

The action of hydrogen gas is not limited to the gas phase. In accordance with Henry's law, a certain portion of hydrogen gas is physically dissolved in liquids. As a result, hydrogen gas is active also in aqueous solutions (e.g. bodily fluids).

In addition, hydrogen gas can be conditionally active in lipophilic media because of its solubility. Dissolved hydrogen gas demonstrates its medicinal effect, for example, in and on the membranes of biological systems, including both cytoplasmic membranes and those of the endoplasmic reticulum, Golgi complex, lysosomes, nuclear membrane and the mitochondria. This is all the more important in that decisive biological reactions occur in such membrane systems, which are distinguished, *inter alia*, by their structured character.

Besides the basic possibility that the medication according to the invention already contains hydrogen gas in gaseous form, the medication may also contain a source for hydrogen gas. Hydrogen exists in chemically or physically bound form in hydrogen gas sources, from which hydrogen gas is released at the selected point in time (e.g. hydrogen-cleaving compounds, metal hydride, clathrates, microparticles).

Hydrogenous gas-containing medications contribute to the treatment of inflammatory processes in the body, for example organ inflammation (e.g. lungs) or inflammation of the skin or mucosa. Hydrogenous gas-containing medications are especially appropriate for the treatment of inflammatory processes in the lungs, which usually involves all

For example, an inhalant gas with 10 – 1000 ppm, especially 10 – 100 ppm, D₂, PD or their mixture, can be used in the prevention of lung cancer. A portable device with deuterium-containing gas for mixing with inhalant gas can be used, for example, with heavy smokers. Inhalant gases are usually humidified prior to inspiration.

The appropriate inflammation and explosion limits must be observed in setting the dosage of hydrogen gas in suitable gas mixtures. These limits vary in relation to the degree of oxygen content and the gas humidity, as experts in the field are aware of.

The beneficial pharmacological action of hydrogenous gas mixtures can also be exploited in working with cell, tissue and organ cultures. For example, cell cultures with hydrogenous gas mixtures can be gassed. However, liquid, gel or solid hydrogenous gas-containing preparations or sources for hydrogen gas can be used as components of or additions to the nutrient medium or nutrient solutions for cultures from cells, microorganisms, tissues or organs.

Oxygen uptake in the lungs can be enhanced through treatment with a hydrogenous gas mixture as a medication. Because of the small molecular size of hydrogen and the beneficial transport features associated with this, expressed in lower viscosity and elevated creep and diffusion potential, respiration performance and its concomitant metabolism can be improved, especially in the case of airway obstruction (presence of atelectasis). Without being restricted to this observation, the improved metabolism appears therefore to be related to the fact that oxygen molecules are carried along in the wake of hydrogen molecules.

preferably from 1 ppm to 50% by vol., and most preferably from 1 ppm to 2.5% by vol. in medications destined for inhalation.

It has been serendipitously found that gas mixtures of hydrogen gas and NO are not only advantageously enhanced in their action, but that the action of NO is also increased in the inhalation therapy of lung diseases. NO is therefore particularly preferred as a pharmacologically active gas for addition to a hydrogenous gas mixture. The pharmacological action of NO, which is also found endogenously, is described in "Nüssler A.K.: PZ, No. 2, Vol. 141, Published 1-11-1996, pp. 11-20," to which reference is made. The NO used originated generally from a gas mixture as a source of NO, which is available on the market in compressed gas tanks, for example as a mixture of 900 ppm NO in nitrogen (for medical use).

Substances that give off NO (NO donors) are also suitable as NO sources, e.g., S-nitroso-N-acetyl-penicillamine, S-nitroso-cysteine, sodium prusside, nitroguanidine, glycerol trinitrate, isoamyl nitrate, inorganic nitrite, azide and hydroxylamine.

Hydrogenous gas mixtures with NO or a NO source contribute to the production of a medication for the treatment of reversible and irreversible pulmonary vasoconstriction (pulmonary vessel spasm), bronchoconstriction (spasm-like narrowing of the bronchi) and inflammatory processes in the lungs, in particular for the treatment or prevention of the diseases pneumonia (lung inflammation), pulmonary trauma, bronchial asthma, ARDS (Acute Respiratory Distress Syndrome), PPHN (Persistent Pulmonary Hypertension of the Newborn), COPD (Chronic Obstructive Pulmonary Disease), bronchitis (bronchial inflammation), hypoxia-mediated vasoconstriction (vascular spasm secondary to oxygen deficiency), fat embolus in the lung (obstruction of pulmonary arteries), acute pulmonary edema (acute accumulation of fluid in the lung), acute

in mixture), hydrogen gas and NO, or hydrogen gas and a pharmaceutically active gas.

In the production of liquid pharmaceutical compounds (medications), the hydrogenous gas mixture can be dissolved or dispersed in an aqueous phase under atmospheric pressure or applied pressure. The aqueous phase can contain sugar, sugar alcohol, dextrose, dextrane, polysaccharides, (e.g., starch, cellulose, amylose, pectin, agar), derivative polysaccharides (e.g., methylcellulose), proteins (e.g. albumin), surfactants and/or salts. Aqueous gas-containing compounds are described in EP 0122624-A1 and EP0123235-A1, to which reference is made. The viscosity of the aqueous phase can be set through additives (e.g., polyethylene glycol, methylcellulose, etc.). The aqueous phase is preferably suited to infusion and meets physiological conditions (isotonic solutions). Aqueous compounds with carbohydrates (e.g., sugar, polysaccharides) and/or protein or protein mixtures (e.g. albumin) can also be used in the production of hydrogenous gas-containing microbubbles. The production of hydrogenous gas-containing microbubbles is described in WO 96/38181-A1, to which reference is made. Aqueous compounds with hydrogenous gas-containing microbubbles may be obtained, for example, from ultrasound treatment of a solution with 1 part of aqueous solution with 5% by weight human serum albumin and 3 parts of 5% by weight dextrose in an hydrogenous gas-containing environment. The gas-containing microbubbles usually have a diameter of 1 – 10 microns.

The production of gas-containing microdroplets is described in US 4622219, to which reference is made.

Liquid or gel pharmaceutical compounds may also be produced by utilizing the fact that the hydrogenous gas mixture is dissolved or dispersed in a lipophilic phase under atmospheric pressure, or preferably under applied pressure.

Mixtures from lipophilic and hydrophilic phases (e.g. fatty emulsion) are fed gas by conducting or bubbling the gas, for example, through the mixture under atmospheric pressure or applied pressure (e.g., 1 – 300 bar) at a temperature of, for example, 0 – 50°C. Preferably, the hydrogenous gas-containing mixture is produced in high-pressure autoclaves by commingling the mixture and gas at a pressure of 100 – 300 bar. But it is also possible to load the lipophilic phase with a hydrogenous gas-containing mixture first, followed by the mixture from the hydrophilic phase.

Liquid or gel pharmaceutical compounds can be hydrogenous gas-containing liposomes or hydrogenous gas-containing, liposome-containing liquids. Hydrogenous gas-containing liposomes can be produced by conventional methods. The production of gas-containing liposomes is described in US 5334381, to which reference is made. The pharmaceutical compounds with hydrogenous gas-containing liposomes are generally used as injectable medications. Ointments with hydrogenous gas-containing liposomes can also be produced. The ointments are applied externally, for example.

Conventional methods such as ultrasound treatment or mechanical homogenization can be used in the production of hydrogenous gas-containing solutions or emulsions, in particular liposomes. For example, the Ultra-Turrax® from the Jahnke & Kunkel company can be used as a homogenization device. Liquid hydrogenous gas-containing compounds can also be produced by bubbling the liquid (e.g., through fine particle sinters in a gas-washing bottle). Loading with hydrogenous gas-containing gas is preferably performed under pressure.

Liquid or gel pharmaceutical compounds can contain hydrogenous gas-containing microparticles. The microparticles generally have a particle diameter in the range of 0.1 – 40 microns. The microparticles are constructed, for example, of polymers and contain the gas in a polymer capsule.

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The high-pressure autoclave is usually then maintained at a set temperature for some time (e.g., 80°C for 2 hours). Next, the high-pressure autoclave is gradually cooled (e.g. over 5 days). The precipitated crystals are then separated and usually washed in the solvent. The nuclear size of the crystal can vary, depending on production conditions or the mechanical processes of particle comminution. The crystalline clathrates may be coated with hydrophilic, lipophilic or amphiphilic adjuvants. For application, the clathrates are placed (e.g. suspended) in a sterile aqueous system with additives to set the viscosity, surface tension, pH and osmotic pressure. In the case of hydroquinone as a host substance, an aqueous system with the following compound is preferably suitable: 1% gelatin solution, 1% albumin solution, 10% glycerol solution, 15% propylene glycol solution, mixtures of sodium cholate and phosphatidyl choline in water, 0.01-1% phosphatidyl choline dispersion (aqueous), 1% methylcellulose, 1-2% dextrane solution, 1% agar solution, 2% Tween solution (Tween 80) and 1% gum arabic.

Dissolution of the clathrate releases its gas content. Among other factors, the rapidity of release of the gas is dependent on the host substance, nuclear size and the aqueous system employed; it can be set within a wide range of limits. Thus with the help of a clathrate, injectable hydrogenous pharmaceutical preparations can be obtained in a simple manner.

For example, 10 mg of the hydroquinone/H₂ complex (3:1 complex) releases about 0.03 mg H₂.

Hydrogenous gas medications (for example, with or without NO, CO, N₂O, acetylene or ethylene) are used, *inter alia*, in the form of suppositories, ointments, solutions, dispersions, emulsions, microdroplets, microbubbles, liposomes, microparticles, aerosols, foams, particulate agents, pills, pastilles, capsules, microcapsules, types of chewing gum and in carriers or as part of a plaster.

During the experiment the test animals were first anesthetized with a barbiturate (thiopental) and then an narcotic inhalant (isoflurane). The animals were put on a pressure-controlled ventilator to maintain breathing (Servo SV 900, Siemens, Germany).

The gas mixture of 3.5% by vol. H_2 and the remainder nitrogen (pressure gas tank from Messer Griesheim) was introduced into the respiratory system of the test animals via a so-called disk-type gas mixer, which is normally used for anesthesia with N_2O . It was possible to control for a precise H_2 concentration by using a density conversion factor, as well as externally via the disk-type gas mixer. The H_2 concentration of the inspiratory gas (gas breathed in) and the expiratory gas (gas breathed out) was monitored. Electronic sensor measuring devices (Compur) and heat conduction-based measuring devices (Hydros, Rosemount) were used to measure H_2 . Once the fixed value determination was made, the respiration parameters were not adjusted again during the procedure.

The procedure with hydrogenous inhalant gas lasted 60 minutes. Afterwards, ARDS was induced with oleic acid (see: Schuster, Daniel P.: Clinical Lessons from the Oleic Acid Model of Acute Lung Injury, Am. J. Respir. Crit. Care Med., Vol. 149, 1994, pp. 245-260).

The results are summarized in the Table below.

This damage to the cell barrier precipitates an increased accumulation of fluid in the interstitial and alveolar tissue of the lung, which leads to severe gas metabolism disturbance. The test results indicate that hydrogenous gas therapy produces a reduction in gas metabolism disturbance, and an increase in lymph flow, as well as a decrease in lung fluid, which benefits the cell barrier.

8. Use of the medications according to Claims 1 through 7, characterized in that the medication is administered locally, intracutaneously or transcutaneously for therapy; intravenously for systemic use; intrarterially orally or rectally through partial exchange of bodily fluid or lymph components; intrapleurally, intrathecally, intraventricularly, intraperitoneally, intracavitarily in an OR environment for use in cavities.
9. Use of a hydrogenous gas-containing compound or gas mixture for the production of a medication for the treatment or prevention of ARDS (Adult Respiratory Distress Syndrome), bronchial asthma, COPD (Chronic Obstructive Pulmonary Disease), bronchitis, pneumonia, pulmonary trauma, hypoxia-mediated vasoconstriction and for the treatment of airway obstruction (atelectasis) or inflammatory processes.
10. Use of a hydrogenous gas-containing and a nitric oxide-containing compound or gas mixture for the production of a medication for the treatment or prevention of pulmonary vasoconstriction, bronchoconstriction or airway obstruction.
11. Use of a hydrogenous gas-containing and a nitric oxide-containing compound for the production of a medication for the treatment of reversible and irreversible pulmonary vasoconstriction (pulmonary vessel spasm), bronchoconstriction (spasm-like narrowing of the bronchi) and inflammatory processes in the lungs.
12. Use of a hydrogenous gas-containing and a nitric oxide-containing compound or gas mixture for the production of a medication for the treatment or prevention of the diseases pneumonia (lung inflammation), pulmonary trauma, bronchial asthma, ARDS (Acute Respiratory Distress Syndrome), PPHN (Persistent Pulmonary

17. Use of a hydrogenous gas mixture or a hydrogenous gas compound for the production of a medication to reduce the concentration of peroxonitrites or hydroxyl radicals in the bodies of mammals or humans.
18. Use of a gas mixture composed of hydrogen gas, helium and oxygen gas for the production of an inhalable medication in the treatment of gas metabolism disturbance in the lungs and the treatment of airway obstruction.
19. Use of a liquid containing carbohydrates, lipids, peptides or proteins for the production of a hydrogenous gas medication.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (if desired) (12 characters maximum) 13681-003WO1

Box No. I TITLE OF INVENTION
CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT

Box No. II APPLICANT ☐ This person is also inventor

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)

Beth Israel Deaconess Medical Center
One Deaconess Road
Boston, Massachusetts 02115
United States of America

Telephone No.

Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State (that is, country) of nationality:
US

State (that is, country) of residence:
US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)

Yale University
433 Temple Street
New Haven, Connecticut 06520-8336
United States of America

This person is:

☒ applicant only

☐ applicant and inventor

☐ inventor only (If this check-box is marked, do not fill in below.)

Applicant's registration No. with the Office

State (that is, country) of nationality:
US

State (that is, country) of residence:
US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent

☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

FRASER, Janis K.; BRENNAN, Jack
Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110

Telephone No.
617-542-5070

Facsimile No.
617-542-8906

Teleprinter No.

Agent's registration No. with the Office
34,819

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) GOSE, Jeann ✓		This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)
		Applicant's registration No. with the Office

State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	

Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) CHOI, Augustine M.K. a 2 Pole Bridge Lane Gulford, Connecticut 06437 United States of America		This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)
		Applicant's registration No. with the Office

State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	

Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) BACH, Fritz H.		This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)
		Applicant's registration No. with the Office

State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	

Name and address: (Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) OTTERBEIN, Leo E. 93 Mather Street Hamden, Connecticut 06517 United States of America		This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)
		Applicant's registration No. with the Office

State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

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Box No.V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

- ☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP European Patent:** AT Austria, BE Belgium, CH & LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | | |
|---|--|--|
| ☒ AE United Arab Emirates | ☒ GH Ghana | ☒ MX Mexico |
| ☒ AG Antigua and Barbuda | ☒ GM Gambia | ☒ MZ Mozambique |
| ☒ AL Albania | ☒ HR Croatia | ☒ NO Norway |
| ☒ AM Armenia | ☒ HU Hungary | ☒ NZ New Zealand |
| ☒ AT Austria | ☒ ID Indonesia | ☒ PL Poland |
| ☒ AU Australia | ☒ IL Israel | ☒ PT Portugal |
| ☒ AZ Azerbaijan | ☒ IN India | ☒ RO Romania |
| ☒ BA Bosnia and Herzegovina | ☒ IS Iceland | ☒ RU Russian Federation |
| ☒ BB Barbados | ☒ JP Japan | |
| ☒ BG Bulgaria | ☒ KE Kenya | ☒ SD Sudan |
| ☒ BR Brazil | ☒ KG Kyrgyzstan | ☒ SE Sweden |
| ☒ BY Belarus | ☒ KP Democratic People's Republic of Korea | ☒ SG Singapore |
| ☒ BZ Belize | ☒ KR Republic of Korea | ☒ SI Slovenia |
| ☒ CA Canada | ☒ KZ Kazakhstan | ☒ SK Slovakia |
| ☒ CH & LI Switzerland and Liechtenstein | ☒ LC Saint Lucia | ☒ SL Sierra Leone |
| ☒ CN China | ☒ LK Sri Lanka | ☒ TJ Tajikistan |
| ☒ CO Colombia | ☒ LR Liberia | ☒ TM Turkmenistan |
| ☒ CR Costa Rica | ☒ LS Lesotho | ☒ TR Turkey |
| ☒ CU Cuba | ☒ LT Lithuania | ☒ TT Trinidad and Tobago |
| ☒ CZ Czech Republic | ☒ LU Luxembourg | ☒ TZ United Republic of Tanzania |
| ☒ DE Germany | ☒ LV Latvia | ☒ UA Ukraine |
| ☒ DK Denmark | ☒ MA Morocco | ☒ UG Uganda |
| ☒ DM Dominica | ☒ MD Republic of Moldova | ☒ US United States of America |
| ☒ DZ Algeria | | |
| ☒ EC Ecuador | ☒ MG Madagascar | ☒ UZ Uzbekistan |
| ☒ EE Estonia | ☒ MK The former Yugoslav Republic of Macedonia | ☒ VN Viet Nam |
| ☒ ES Spain | ☒ MN Mongolia | ☒ YU Yugoslavia |
| ☒ FI Finland | ☒ MW Malawi | ☒ ZA South Africa |
| ☒ GB United Kingdom | | ☒ ZW Zimbabwe |
| ☒ GD Grenada | | |
| ☒ GE Georgia | | |

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☒ All eligible states ☐ ☐
- ☐ ☐ ☐

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

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Sheet No. 4...

Box No. VI PRIORITY CLAIM				
The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: regional Office	international application: receiving Office
item (1)				
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office) identified above as:

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

* Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):

Box No. VII INTERNATIONAL SEARCHING AUTHORITY	
Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):	
ISA/ US.....	
Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):	
Date (day/month/year)	Number Country (or regional Office)

Box No. VIII DECLARATIONS	
The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):	
☐ Box No. VIII (i)	Declaration as to the identity of the inventor : Number of declarations
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent : Number of declarations
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application : Number of declarations
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America) : Number of declarations
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty : Number of declarations

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Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains:		This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item):		Number of items
(a) the following number of sheets in paper form:		1. ☒ fee calculation sheet		:
request (including declaration sheets)	: 5	2. ☐ original separate power of attorney		:
description (excluding sequence listing part)	: 21	3. ☐ original general power of attorney		:
claims	: 2	4. ☐ copy of general power of attorney; reference number, if any:		:
abstract	: 1	5. ☐ statement explaining lack of signature		:
drawings	: 0	6. ☐ priority document(s) identified in Box No. VI as item(s):		:
Sub-total number of sheets	: 29	7. ☐ translation of international application into (language):		:
sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (b) below)	:	8. ☐ separate indications concerning deposited microorganism or other biological material		:
Total number of sheets	: 29	9. ☐ sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other))		:
(b) sequence listing part of description filed in computer readable form		(i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)		:
(i) ☐ only (under Section 801 (a)(i))		(ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter		:
(ii) ☐ in addition to being filed in paper form (under Section 801 (a)(ii))		(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column		:
Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained (additional copies to be indicated under item 9(ii), in right column):		10. ☒ other (specify): check; postcard		:
Figure of the drawings which should accompany the abstract:		Language of filing of the international application: English		

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).


Jack Brennan, Reg. No. 47,443

For receiving Office use only		2. Drawings: ☐ received: ☐ not received:
1. Date of actual receipt of the purported international application:	6. ☐ Transmittal of search copy delayed until search fee is paid	
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA/		

For International Bureau use only
Date of receipt of the record copy by the International Bureau:

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This sheet is not part of and does not count as a sheet of the international application.

PCT
FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's
file reference

13681-003WO1

Applicant

Beth Israel Deaconess Medical Center

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE 240.00 **T**

2. SEARCH FEE 700.00 **S**

International search to be carried out by

US

(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

Where item (b) of Box No. IX applies, enter Sub-total number of sheets } 29
Where item (b) of Box No. IX does not apply, enter Total number of sheets }

b1 first 30 sheets 407.00 **b1**

b2 0 x 9.00 = 0.00 **b2**
number of sheets fee per sheet

b3 additional component (only if sequence listing part of description
is filed in computer readable form under Section 801(a)(i), or both
in that form and on paper, under Section 801(a)(ii)):

400 x = **b3**
fee per sheet

Add amounts entered at b1, b2 and b3 and enter total at B ... 407.00 **B**

Designation Fees

The international application contains all designations.

5 x 88.00 = 440.00 **D**

number of designation fees amount of designation fee
payable (maximum 6)

Add amounts entered at B and D and enter total at I ... 847.00 **I**

(Applicants from certain States are entitled to a reduction of 75% of the
international fee. Where the applicant is (or all applicants are) so entitled, the

4. FEE FOR PRIORITY DOCUMENT (if applicable) **P**

5. TOTAL FEES PAYABLE 1,787.00

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

TOTAL

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☒ authorization to charge ☐ postal money order ☐ cash ☐ coupons

☒ cheque ☐ bank draft ☐ revenue stamps ☐ other (specify):

AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

(This mode of payment may not be available at all receiving Offices)

☐ Authorization to charge the total fees indicated above.

☒ (This check-box may be marked only if the conditions for deposit accounts of
the receiving Office so permit) Authorization to charge any deficiency or
credit any overpayment in the total fees indicated above.

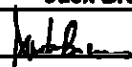
☐ Authorization to charge the fee for priority document.

Receiving Office: RO/ US

Deposit Account No.: 08-1050

Date: May 9, 2002

Name: Jack Brennan

Signature: 

From the INTERNATIONAL BUREAU

PCT**NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES**

(PCT Rule 47.1(c), first sentence)

To:

RECEIVEDFRASER, Janis, K.
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
ETATS-UNIS D'AMERIQUE

DEC 02 2003

**FISH & RICHARDSON, P.C.
BOSTON OFFICE**

Date of mailing (day/month/year) 11 November 2003 (11.11.03)		
Applicant's or agent's file reference 13681-003WO1		
IMPORTANT NOTICE		
International application No. PCT/US02/14836	International filing date (day/month/year) 09 May 2002 (09.05.02)	Priority date (day/month/year)
Applicant YALE UNIVERSITY et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

AU, AZ, BY, CH, CN, CO, DE, DZ, GH, HU, JP, KG, KP, KR, MD, MK, MZ, RU, TM, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BZ, CA, CR, CU, CZ, DK, DM, EA, EC, EE, EP, ES, FI, GB, GD, GE, GM, HR, ID, IL, IN, IS, KE, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MG, MN, MW, MX, NO, NZ, OA, PL, PT, RO, SD, SE, SG, SI, SK, SL, TJ, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 20 November 2003 (20.11.03) under No. 03/094932

4. **TIME LIMITS** for filing a demand for International preliminary examination and for entry into the national phase

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be 30 MONTHS from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 19 months from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/index.html>.

For filing a demand for international preliminary examination, see the *PCT Applicant's Guide*, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

It is the applicant's sole responsibility to monitor all these time limits.

* No Docketing Required *	
Reviewed by Practice Systems	
Initials: <u> JML </u>	
Reviewed by Billing Secretary	
Initials: <u> </u>	

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Judith Zahra

Facsimile No. (41-22) 740.14.35

Telephone No. (41-22) 338.91.11

Attorney's Docket No. 13681-003WO1	Express Mail Label No. EL965903432US	Mailing Date April 15, 2004	For PTO Use Only <i>Do Not Mark in This Area</i>
Application No. US02/14836	Filing Date May 9, 2002	Attorney/Secretary Init TEG/kzh	
Title of the Invention Carbon monoxide as a biomarker and therapeutic agent			
Applicant Yale University et al.			
Enclosures Response to Written Opinion			

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Mailing Label
Label 1-9 June 2008

ORIGIN (POSTAL USE ONLY)			DELIVERY (POSTAL USE ONLY)		
PO ZIP Code			Post Office To Address		
Date in	Day of Delivery	Flat Rate Envelope	No. of Delivery Attempts	Time	Employee Signature
Mo. Day Year	☐ Next ☐ Second ☐ Third	Insured	Mo. Day	☐ AM ☐ PM	
Weight	1st Day 2nd Day 3rd Day	Return Receipt For	Mo. Day	☐ AM ☐ PM	Employee Signature
☐ AM ☐ PM	1st Day 2nd Day 3rd Day	COD Fee Insurance Fee	Mo. Day	☐ AM ☐ PM	Employee Signature
No Delivery	Acceptance Clerk Initials				
FROM: (PLEASE PRINT)			TO: (PLEASE PRINT)		
FISH, RICHARDSON PC 225 FRANKLIN ST. FL 32 BOSTON MA 02110-2809			MAIL STOP COMMISSIONER OF PATENTS PO BOX 1430 ALEXANDRIA VA 22313-1450		
13681-003601 TE6/KSL					

FOR PICKUP OR TRACKING CALL 1-800-232-1811 www.usps.com

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The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only		
Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 13681-003WO1
International application No. PCT/US02/14836	International filing date (day/month/year) 9 May 2002 (09/05/02)	(Earliest) Priority date (day/month/year) ()
Title of invention CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) YALE UNIVERSITY Two Whitney Avenue New Haven, Connecticut 06511 United States of America		Telephone No.
		Facsimile No.
		Teleprinter No.
		Applicant's registration No. with the Office
State (that is, country) of nationality: US		State (that is, country) of residence: US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) JOHNS HOPKINS UNIVERSITY 111 Marketplace Suite 906 Baltimore, Maryland 21202 United States of America		
State (that is, country) of nationality: US		State (that is, country) of residence: US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) CHOI, Augustine M.K. 43 Long Meadow Drive Pittsburgh, Pennsylvania 15238 United States of America		
State (that is, country) of nationality: US		State (that is, country) of residence: US
☒ Further applicants are indicated on a continuation sheet.		

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Sheet No. 2.		International application No. PCT/US02/14836
Continuation of Box No. II APPLICANT(S) <i>If none of the following sub-boxes is used, this sheet should not be included in the demand.</i>		
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)</i> OTTERBEIN, Leo E. 910 Normandy Drive New Kensington, Pennsylvania 15068 United States of America		
State (that is, country) of nationality: US	State (that is, country) of residence: US	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)</i>		
State (that is, country) of nationality:	State (that is, country) of residence:	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)</i>		
State (that is, country) of nationality:	State (that is, country) of residence:	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)</i>		
State (that is, country) of nationality:	State (that is, country) of residence:	
☐ Further applicants are indicated on another continuation sheet.		

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative
 and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.
☐ is hereby appointed and any earlier appointment of (an) agent(s) /common representative is hereby revoked.
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: (Family name followed by given name; for a legal entity, full official
 The address must include postal code and name of country.)

FRASER, Janis K., Ph.D., J.D.
 Fish & Richardson P.C.
 225 Franklin Street
 Boston, Massachusetts 02110
 United States of America

Telephone No.

617-542-5070

Facsimile No.

617-542-8908

Teleprinter No.

Agent's registration No. with the Office
34,819

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION**Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed.

the description ☐ as originally filed
☐ as amended under Article 34

the claims ☐ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☐ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). (This check-box may be marked only where the time limit under Article 19 has not yet expired.)

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

- ☒ which is the language in which the international application was filed.
☐ which is the language of a translation furnished for the purposes of international search.
☐ which is the language of publication of the international application.
☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States (that is, all States which have been designated and which are bound by Chapter II of the PCT)

excluding the following States which the applicant wishes not to elect:

47
146

Sheet No. 4.

International application No.
PCT/US02/14838

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | |
|--|---|--------|
| 1. translation of international application | : | sheets |
| 2. amendments under Article 34 | : | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets |
| 5. letter | : | sheets |
| 6. other (specify) | : | sheets |

For International Preliminary Examining Authority use only

received not received

- | | |
|--------------------------|--------------------------|
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |

The demand is also accompanied by the item(s) marked below:

- | | |
|--|--|
| 1. ☒ fee calculation sheet | 5. ☐ statement explaining lack of signature |
| 2. ☐ original separate power of attorney | 6. ☐ sequence listing in computer readable form |
| 3. ☐ original general power of attorney | 7. ☒ other (specify): check; postcard; copy of 92bis |
| 4. ☐ copy of general power of attorney; reference number, if any: | |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).


Janis K. Fraser, Ph.D., J.D., Reg. No. 34,819

For International Preliminary Examining Authority use only

- | | |
|--|---|
| 1. Date of actual receipt of DEMAND: | |
| 2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b): | |
| 3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. | ☐ The applicant has been informed accordingly. |
| 4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5. | |
| 5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82. | |

For International Bureau use only

Demand received from IPEA on:

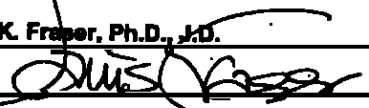
118
117

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand

International application No. PCT/US02/14838		For International Preliminary Examining Authority use only	
Applicant's or agent's file reference 13681-003WO1		Date stamp of the IPEA	
Applicant Yale University and Johns Hopkins University			
CALCULATION OF PRESCRIBED FEES			
1. Preliminary examination fee		490.00	P
2. Handling fee <i>(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)</i>		172.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box		662.00	
		TOTAL	
MODE OF PAYMENT			
☒ authorization to charge deposit account with the IPEA (see below)	☐ cash		
☒ cheque	☐ revenue stamps		
☐ postal money order	☐ coupons		
☐ bank draft	☐ other (specify):		
AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT <i>(This mode of payment may not be available at all IPEAs)</i>			
☐ Authorization to charge the total fees indicated above.	IPEA/ US	Deposit Account No.: 06-1050	
☒ <i>(This check-box may be marked only if the conditions for deposit accounts of the IPEA so permit.)</i> Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.	Date: December 8, 2003	Name: Janis K. Fraser, Ph.D., J.D.	
	Signature: 		

FISH & RICHARDSON P.C.

118
225 Franklin Street
Boston, Massachusetts
02110-2804

Telephone
617 542-5070

Facsimile
617 542-8906

Web Site
www.fr.com

Date December 9, 2003

To International Bureau
World Intellectual Property Organization
International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
Switzerland
Telephone: 011 41 22 338 9111

Facsimile number 13681-00391631 / 011 41 22 733 54 28

From Nicole L. Babine
Foreign Patent Paralegal

Re CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT
Applicant: Yale University et al.
Application No.: US02/14836
Filing Date: May 9, 2002
Country: WIPO
Our Ref.: 13681-003WO1

Number of pages
including this page 7

Message Please see attached 92 bis.

NOTE: This facsimile is intended for the addressee only and may contain privileged or confidential information. If you have received this facsimile in error, please immediately call us collect at 617 542-5070 to arrange for its return. Thank you.

IN THE INTERNATIONAL BUREAU

Applicant : Yale University et al.
Serial No. : US02/14836
Filed : May 9, 2002
Title : CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT

International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
SWITZERLAND

CHANGE OF APPLICANT INFORMATION UNDER 92 bis

Effective immediately, please amend the information in the above noted matter of the
Inventor/Applicant **CHOI, Augustine M.K.** to:

43 Long Meadow Drive
Pittsburgh, Pennsylvania 15238
United States of America,

Inventor/Applicant **OTTERBEIN, Leo E.** to:

910 Normandy Drive
New Kensington, Pennsylvania 15068
United States of America,

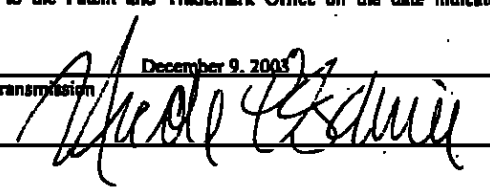
Attached hereto are the relevant power of attorney documents.

Please do not hesitate to contact us if further documentation is needed.

CERTIFICATE OF TRANSMISSION BY FACSIMILE

I hereby certify that this correspondence is being transmitted by
facsimile to the Patent and Trademark Office on the date indicated
below.

Date of Transmission December 9, 2003

Signature 

Nicole L. Babine
Typed or Printed Name of Person Signing Certificate

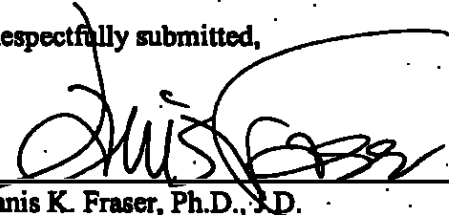
Applicant : Yale University et al.
Serial No. : US02/14836
Filed : May 9, 2002
Page : 2 of 2

Attorney's Docket No.: 13681-003WO1

The telephone and facsimile numbers are telephone: (617) 542-5070 and facsimile:
(617) 542-8906, respectively.

Respectfully submitted,

Date: 9 December 2003


Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

20768868.doc

**GENERAL POWER OF ATTORNEY
(CONCERNING SEVERAL INTERNATIONAL APPLICATIONS)**

**PATENT COOPERATION TREATY
APPOINTMENT OF AGENT OR COMMON REPRESENTATIVE**

The undersigned applicant:

YALE UNIVERSITY

hereby appoints

Janis K. Fraser, Ph.D., J.D., Reg. No. 34,819
Harold H. Fox, Reg. No. 41,498
John W. Freeman, Reg. No. 29,066
J. Peter Fasse, Reg. No. 32,983
Timothy A. French, Reg. No. 30,175

of

FISH & RICHARDSON P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804

☒ as agent(s)

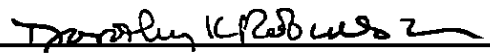
☐ as common representative(s)

to act on applicant's behalf before the competent International Authorities in connection with any and all international applications filed by the applicant with the United States Patent and Trademark Office as receiving Office and to make and receive payments on applicant's behalf.

The undersigned, whose title appears below, is empowered to sign the General Power of Attorney on behalf of the applicant.

Place: New Haven, CT

Date: January 23, 2003

Signature: 
YALE UNIVERSITY

Printed Name: Dorothy E. Robinson

Title: Vice President and General Counsel

**GENERAL POWER OF ATTORNEY
(CONCERNING SEVERAL INTERNATIONAL APPLICATIONS)**

**PATENT COOPERATION TREATY
APPOINTMENT OF AGENT OR COMMON REPRESENTATIVE**

The undersigned applicant:

CHOI, Augustine M.K. e

hereby appoints

Janis K. Fraser, Ph.D., J.D., Reg. No. 34,819
Harold H. Fox, Reg. No. 41,498
John W. Freeman, Reg. No. 29,066
J. Peter Fasse, Reg. No. 32,983
Timothy A. French, Reg. No. 30,175

of

**FISH & RICHARDSON P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804**

☒ as agent(s)

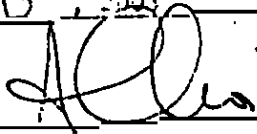
☐ as common representative(s)

to act on applicant's behalf before the competent International Authorities in connection with any and all international applications filed by the applicant with the United States Patent and Trademark Office as receiving Office and to make and receive payments on applicant's behalf.

The undersigned, whose title appears below, is empowered to sign the General Power of Attorney on behalf of the applicant.

Place: University of Pittsburgh

Date: January 13, 2003

Signature: 

Printed Name: Augustine M.K. Choi

**GENERAL POWER OF ATTORNEY
(CONCERNING SEVERAL INTERNATIONAL APPLICATIONS)**

**PATENT COOPERATION TREATY
APPOINTMENT OF AGENT OR COMMON REPRESENTATIVE**

The undersigned applicant:

OTTERBURN, Leo E.

hereby appoints

Janis K. Fraser, Ph.D., J.D., Reg. No. 34,819
Harold H. Fox, Reg. No. 41,498
John W. Freeman, Reg. No. 29,066
J. Peter Fosse, Reg. No. 32,983
Timothy A. French, Reg. No. 30,175

of

FISH & RICHARDSON P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804

☒ as agent(s)

☐ as common representative(s)

to act on applicant's behalf before the competent International Authorities in connection with any and all international applications filed by the applicant with the United States Patent and Trademark Office as receiving office and to make and receive payments on applicant's behalf.

The undersigned, whose title appears below, is empowered to sign the General Power of Attorney on behalf of the applicant.

Place: UNIVERSITY OF ILLINOIS

Date: January 13, 2003

Signature: *Leo E. Otterburn*

Printed Name: Leo E. C.

**GENERAL POWER OF ATTORNEY
(CONCERNING SEVERAL INTERNATIONAL APPLICATIONS)**

**PATENT COOPERATION TREATY
APPOINTMENT OF AGENT OR COMMON REPRESENTATIVE**

The undersigned applicant:

JOHNS HOPKINS UNIVERSITY

hereby appoints:

Janis K. Fraser, Ph.D., J.D., Reg. No. 34,819
Harold H. Fox, Reg. No. 41,498
John W. Freeman, Reg. No. 29,066
J. Peter Pavesi, Reg. No. 32,983
Timothy A. ... Reg. No. 30,175

of

FISH & RICHARDSON P.C.
225 Franklin Street
Boston, MA 02110-2804

☒ as agent(s)

☐ as common representative(s)

to act on applicant's behalf before the competent International Authorities in connection with any and all international applications filed by the applicant with the United States Patent and Trademark Office and to make and receive payments on applicant's behalf. For ... /14136 filed May 09, 2002 entitled Carbon ... Marker and Therapeutic Agent.

The undersigned, whose title appears below, is empowered to sign the General Power of Attorney on behalf of the applicant.

Place: Baltimore, MD, U.S.A.

Date: 8/15/02

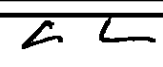
Signature: [Signature]
Johns Hopkins University

Printed Name: _____

Title: President

726
125

PATENT COOPERATION TREATY

PCT		From the INTERNATIONAL BUREAU	
NOTIFICATION OF THE RECORDING OF A CHANGE		To:	
(PCT Rule 92bis.1 and Administrative Instructions, Section 422)		FRASER, Janis, K. Fish & Richardson P.C. 225 Franklin Street Boston, MA 02110 United States of America	
Date of mailing (day/month/year) 09 September 2003 (09.09.03)			
Applicant's or agent's file reference 13681-003WO1	IMPORTANT NOTIFICATION		
International application No. PCT/US02/14836	International filing date (day/month/year) 09 May 2002 (09.05.02)		
1. The following indications appeared on record concerning:			
☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative			
Name and Address BETH ISRAEL DEACONESS MEDICAL CENTER		State of Nationality	State of Residence
		Telephone No.	
		Facsimile No.	
		Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:			
☒ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence			
Name and Address JOHNS HOPKINS UNIVERSITY 111 Marketplace, Suite 906 Baltimore, MD 21202 United States of America		State of Nationality US	State of Residence US
		Telephone No.	
		Facsimile No.	
		Teleprinter No.	
3. Further observations, if necessary: The person in Box 1 has been deleted as an applicant. The person in Box 2 has been added as an applicant.			
4. A copy of this notification has been sent to:			
☒ the receiving Office ☐ the designated Offices concerned			
☐ the International Searching Authority ☐ the elected Offices concerned			
☐ the International Preliminary Examining Authority ☐ other:			
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland		Authorized officer  Anman QIU	
Facsimile No. (41-22) 338.89.65		Telephone No. (41-22) 338 8088	

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

FRASER, Janis, K.
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
United States of America

Date of mailing (day/month/year)

10 September 2003 (10.09.03)

Applicant's or agent's file reference

13681-003WO1

International application No.

PCT/US02/14836

IMPORTANT NOTIFICATION

International filing date (day/month/year)

09 May 2002 (09.05.02)

1. The following indications appeared on record concerning:



the applicant



the inventor



the agent



the common representative

Name and Address

GOSE, Jeann

BACH, Fritz, H.

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:



the person



the name



the address



the nationality



the residence

Name and Address

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

The persons in Box 1 have been deleted as inventors and applicants for US only.

4. A copy of this notification has been sent to:



the receiving Office



the International Searching Authority



the International Preliminary Examining Authority



the designated Offices concerned



the elected Offices concerned



other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.89.85

Authorized officer

Anman QIU

Telephone No. (41-22) 338 9088

127 128

PCT/US02/14836

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

FRASER, Janis, K.
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
United States of America

Date of mailing (day/month/year) 11 September 2003 (11.09.03)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 13681-003WO1	
International application No. PCT/US02/14836	International filing date (day/month/year) 09 May 2002 (09.05.02)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address CHOI, Augustine, M., K., e 2 Pole Bridge Lane Guilford, CT 06437 United States of America	State of Nationality US	State of Residence US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☒ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address CHOI, Augustine, M., K. 2 Pole Bridge Lane Guilford, CT 06437 United States of America	State of Nationality US	State of Residence US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
Correction of indication of name.

4. A copy of this notification has been sent to:

☒ the receiving Office	☐ the designated Offices concerned
☐ the International Searching Authority	☐ the elected Offices concerned
☐ the International Preliminary Examining Authority	☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338.89.85	Authorized officer Anman QIU Telephone No. (41-22) 338 9088
--	---

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

FRASER, Janis, K.
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
United States of America

Date of mailing (day/month/year)

12 September 2003 (12.09.03)

Applicant's or agent's file reference

13681-003WO1

International application No.

PCT/US02/14836

International filing date (day/month/year)

09 May 2002 (09.05.02)

IMPORTANT NOTIFICATION

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address

YALE UNIVERSITY
433 Temple Street
New Haven, CT 06520-8336
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

YALE UNIVERSITY
Two Whitney Avenue
New Haven, CT 06511
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☐ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.89.85

Authorized officer

Anman QIU

Téléphone No. (41-22) 338 9088

PATENT COOPERATION TREATY

PCT/US02/14836

NLB

JLF
TEG

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PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

RECEIVED

FRASER, Janis, K.
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
United States of America

JAN 05 2004

**FISH & RICHARDSON, P.C.
BOSTON OFFICE**

Date of mailing (day/month/year) 19 December 2003 (19.12.03)	
Applicant's or agent's file reference 13681-003WO1	IMPORTANT NOTIFICATION
International application No. PCT/US02/14836	International filing date (day/month/year) 09 May 2002 (09.05.02)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address CHOI, Augustine, M., K. 2 Pole Bridge Lane Guilford, CT 06437 United States of America	State of Nationality US	State of Residence US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address CHOI, Augustine, M., K. 43 Long Meadow Drive Pittsburgh, PA 15238 United States of America	State of Nationality US	State of Residence US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☒ the designated Offices concerned

☐ the International Searching Authority ☐ the elected Offices concerned

☐ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Frederick SAMUELS
Facsimile No. (41-22) 338.89.65	Telephone No. (41-22) 338 8471

130

PATENT COOPERATION TREATY

PCT NOTIFICATION OF THE RECORDING OF A CHANGE (PCT Rule 92bis.1 and Administrative Instructions, Section 422)		From the INTERNATIONAL BUREAU To: FRASER, Janis, K. Fish & Richardson P.C. 225 Franklin Street Boston, MA 02110 United States of America	
Date of mailing (day/month/year) 19 December 2003 (19.12.03)			
Applicant's or agent's file reference 13681-003WO1		IMPORTANT NOTIFICATION	
International application No. PCT/US02/14836		International filing date (day/month/year) 09 May 2002 (09.05.02)	
1. The following indications appeared on record concerning: ☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative			
Name and Address OTTERBEIN, Leo, E. 93 Mather Street Hamden, CT 06517 United States of America		State of Nationality US	State of Residence US
		Telephone No.	
		Facsimile No.	
		Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: ☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence			
Name and Address OTTERBEIN, Leo, E. 910 Normandy Drive New Kensington, PA 15068 United States of America		State of Nationality US	State of Residence US
		Telephone No.	
		Facsimile No.	
		Teleprinter No.	
3. Further observations, if necessary:			
4. A copy of this notification has been sent to: ☒ the receiving Office ☒ the designated Offices concerned ☐ the International Searching Authority ☐ the elected Offices concerned ☐ the International Preliminary Examining Authority ☐ other:			
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338.89.85		Authorized officer Frederick SAMUELS Telephone No. (41-22) 338 9471	

PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING
THE FILING OF AMENDMENTS OF THE CLAIMS
(PCT Administrative Instructions, Section 417)

From the INTERNATIONAL BUREAU

To:

FRASER, Janis, K.
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110
United States of AmericaDate of mailing
(day/month/year)

10 September 2003 (10.09.03)

Applicant's or agent's file reference

13681-003WO1

IMPORTANT NOTIFICATION

International application No.

PCT/US02/14836

International filing date
(day/month/year)

09 May 2002 (09.05.02)

Applicant

YALE UNIVERSITY et al

1. The applicant is hereby notified that amendments to the claims under Article 19 were received by the International Bureau on:

02 September 2003 (02.09.03)

2. This date is within the time limit under Rule 48.1.

Consequently, the International publication of the international application will contain the amended claims according to Rule 48.2(f), (h) and (i).

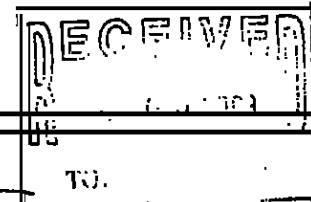
3. The applicant is reminded that the international application (description, claims and drawings) may be amended during the international preliminary examination under Chapter II, according to Article 34, and in any case, before each of the designated Offices, according to Article 28 and Rule 52, or before each of the elected Offices, according to Article 41 and Rule 78.

RECEIVED

SEP 24 2003

FISH & RICHARDSON, PC
BOSTON OFFICE

Amu

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.89.85

Authorised officer

Anman QIU

Telephone No. (41-22) 338 8088

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/14836

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A61K 33/00 US CL : 424/613,699,718 According to International Patent Classification (IPC) up to both national classification and IPC			
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/613,699,718 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) Please See Continuation Sheet			
C. DOCUMENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
X	Database NIOSHTIC on STN/CAS, Acc. No. 1997:109846, Doc. No. NIOSH-00153659, ABIDIN et al, The combined effect of carbon monoxide and normobaric hyperoxia on animals. Koericheskaya Biologiya i Aviakosmicheskaya Meditsina, 1978, No. 6, pp. 63-67, abstract.	1-14	
X	OTTERBEIN et al, Carbon monoxide provides protection against hyperoxic lung injury. Am. J. Physiol., 1999, 276, L688-L694, see entire document.	1-14	
X	Database BIOSIS on STN/CAS, Acc. No. 1998:178713, Doc. No. PREV199800178713, YUAN et al., Evidence of increased endogenous carbon monoxide production in newborn rat endotoxemia. Chinese Medical Sciences Journal, 1997, Vol. 12, No. 4, pp. 212-215, abstract.	16	
Y	WO 98/08523 A1 (MESSER GRIESHEIM GMBH) 05 March 1998 (05.03.98), See entire document, especially the abstract, claims 4, 5, 7-12, see entire document.	1-14	
Y	Database BIOSIS on STN/CAS, Acc. No. 1996:420959, Doc. No. PREV199699143315, CHOI et al, Heme oxygenase 1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. American Journal of Respiratory Cell and Molecular Biology, 1996, Vol. 15, No. 1, pp. 9-19, abstract.	1-14	
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.			
* Special categories of cited documents:			
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent published on or after the international filing date	"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
"L"	document which may throw doubts on priority of the application (as specified)	"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
"O"	document referring to an oral disclosure, use, or other means	"A"	document member of the same patent family
"P"	document published prior to the international filing date but after the priority date claimed		
Date of the actual completion of the international search 08 August 2002 (08.08.2002)		Date of mailing of the international search report 30 SEP 2002	
Name and mailing address of the ISA/US Commissioner of Patents and Trademark Box PCT Washington, D.C. 20531 Facsimile No. (703)305-3230		Authorized officer Valerie Bell-Harris for Frank Choi Telephone No. (703) 308-1235	

INTERNATIONAL SEARCH REPORT

PCT/US02/14836

Continuation of B. FIELDS SEARCHED Item 3:
WEST,STN/CAS
Search terms: carbon monoxide, disease, wound, emphysema, bronchitis, cystic fibrosis, pneumonia, interstitial lung disease, arthritis, Parkinson's, Alzheimer's, inflammation, organ, storage, sepsis, septic

INTERNATIONAL SEARCH REPORT

PCT/US02/14836

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database SCISEARCH on STN/CAS, Acc. No. 94:51937, LEFER et al, A comparison of vascular biological actions of carbon-monoxide and nitric-oxide. Methods and Findings in Experimental and Clinical Pharmacology, 1993, Vol. 15, No. 9, pp. 617-622), abstract.	1-14
Y	Database DRUGU on STN/CAS, Doc. No. 1999-19188, VASSALLI et al, Inhibition of hypoxic pulmonary vasoconstriction by carbon monoxide in dogs. Eur. Resp. J., 1998, 12, Suppl. 28, 237a), abstract.	1-14

136
135

SUPERINDUSTRIA Y COMERCIO
Radicacion : 84123586 88888888 Folios: 135
Fecha (AMD): 2884-12-89 16:14:01
Tramite : 811 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2828 DIVISION DE NUEVAS CREACIONES

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION ()	()	()
MODELO DE UTILIDAD ()	()	()
- Indicación de que se solicita la concesión de una patente.	()	()
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	()	()
- Descripción de la invención.	()	()
- Dibujos, de ser estos pertinentes.	()	()
- Comprobante de Pago de las tasas establecidas.	()	()
COMPLETA ()	INCOMPLETA ()	()
DISEÑO INDUSTRIAL ()		
- Indicación de que se solicita el registro de Diseño Industrial.	()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.	()	()
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	()	()
- Comprobante de pago de las tasas establecidas.	()	()
COMPLETA ()	INCOMPLETA ()	()
ESQUEMA DE TRAZADO ()		
- Indicación de que solicita el registro de un Esquema de trazado.	()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.	()	()
- Representación gráfica del esquema de trazado.	()	()
- Comprobante de pago de las tasas establecidas.	()	()
COMPLETA ()	INCOMPLETA ()	()

Firma Responsable 

Fecha:

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Bogotá D C
Doctor(a)
DILIA MARIA RODRIGUEZ D'ALEMAN
Apoderado(a)
YALE UNIVERSITY Y JOHNS HOPKINS UNIVERSITY

11873

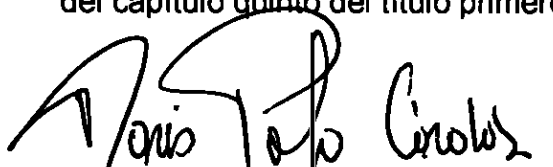
REFERENCIA:	Radicación N°	04-123506
	Solicitud internacional	PCT/US02/14836
	Fecha límite inicio fase nacional	2004-12-09
	Trámite	011
	Evento	379
	Actuación	430
	Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

- La solicitud no contiene el poder debidamente otorgado, con el lleno de requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando éste fuere una persona jurídica
- La solicitud no contiene copia del documento en que consta la cesión del derecho de patente del inventor al solicitante o a su causante (Art. 26-k Decisión 486).

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


DORIS ELENA POLO CORDOBA
Jefe de la División de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista de fecha,
14 ENE. 2005

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