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(54) Title: NITRIC OXIDE INDUCING AGENTS

(57) Abstract: The present invention provides a method to promote the production of nitric oxide in cells found in the epidermal layer. The method is effective to prevent and treat a wide range of cancers. The method is also effective for promoting pain relief and controlling diabetes in patients.



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NITRIC OXIDE INDUCING AGENTS

Background of the Invention

Nitric oxide is a chemical that has been implicated in many processes in the body, including regulation of blood pressure, defense against infection, function of the platelets and transmission of some types of nerve impulses. Nitric oxide has been implicated in neurotoxicity associated with stroke and neurodegenerative diseases, neuronal regulation of smooth muscle including peristalsis, and penile erections. Nitric oxide has been proposed to be a messenger molecule for its diversified effects in various physiologic and pathologic events Ignarro, 1990 Ann. Rev. Pharmacol Toxicol 30:535-560. Unlike typical neurotransmitters, nitric oxide is not stored in synaptic vesicle and does not act on membrane receptors.

Incubation of various tissues including heart, liver, kidney, muscle and intestine from mice and erythrocytes or their membrane fractions from humans with physiologic concentrations of insulin has resulted in activation of a membrane-bound nitric oxide synthase (NOS). Activation of NOS and synthesis of nitric oxide were stimulated by the binding of insulin to specific receptors on the cell surface. Kahn et al. Life 49:441-450, 2000. It was further demonstrated that a membrane bound form of nitric oxide synthase of human erythrocytes could be activated by insulin. Bhattacharya et al., Archives of Physiology and Biochemistry, 2001 1009:(5)441-449. Insulin has been established to have an essential role in carbohydrate metabolism. Currently, insulin treatment is used for blood sugar related conditions.

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The present invention provides a method to promote the production of nitric oxide in cells found in the epidermal layer. The method is effective to prevent and treat a wide range of cancers. The method is also effective
5 to promote pain relief in patients, and to control blood sugar in Type I and Type II diabetics.

Summary of the Invention

The present invention provides a method for preventing and treating cancer without causing significant
10 side effects to a human patient in need thereof, comprising topically administering an agent which modulates the production of nitric oxide by cells found in the epidermal tissue layer of a patient.

The present invention further provides a method for
15 relieving pain in a patient without causing significant side effects comprising topically administering an agent which modulates the production of nitric oxide by red blood cells.

The present invention further provides a method for
20 decreasing the systemic side effects of cancer treatment in a patient comprising topically administering an agent which modulates the production of nitric oxide by cells found in the epidermal tissue layer of a patient.

The present invention further provides a method for
25 controlling or preventing diabetes in a patient comprising topically administering an agent which modulates the production of nitric oxide by cells found in the epidermal tissue layer of a patient.

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Detailed Description of the Invention

Nitric Oxide (NO) has been reported to possess a wide range of antineoplastic properties. However, until the present invention, the identity of the physiologic
5 stimulator of NO synthesis remained obscure. The present invention demonstrates the existence of an insulin activated nitric oxide synthase (IANOS) in various cell membranes. In cancerous tumors an antibody is produced that blocks insulin activated nitric oxide synthase
10 (IANOS). The enzymatic activity of the erythrocyte membranes from patients with different types of neoplastic conditions is markedly inhibited due to the presence of an antibody which results in the diminished synthesis of NO in the patient's system. The present invention identifies
15 agents which are able to neutralize the antibody *in vitro* from both biologic sources and from non-biologic sources. These antineoplastic agents act through *in situ* generation of nitric oxide which results in not only amplification of the enzymatic activity but also the neutralization of the
20 antibody *in vivo*.

It is believed that neoplastic cells elicit the aid of an antibody in the system capable of blocking the production of nitric oxide through the activation of IANOS by insulin. Unlike normal cells, cancer cells do not
25 produce nitric oxide when treated with insulin. Further, cancer cells do not need insulin for the stimulation of carbohydrate metabolism. Nitric oxide only stimulates carbohydrate metabolism in normal cells. However, nitric oxide acts as a potent tumoricide in cancerous cells. The
30 antibody against IANOS plays a crucial role in the pathophysiology of cancer through the blocking of IANOS. The antibody against IANOS is the light chain part of IgG. This antibody occurs in both humans and animals with neoplastic diseases and cancers.

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The present invention provides a method for treating cancer without causing significant side effects to a human patient in need thereof, comprising providing an agent which modulates the production of nitric oxide by cells found in the epidermal layer in a patient. The agent is topically applied to the patient. In a preferred embodiment the agent which modulates production of nitric oxide is applied to the skin of the patient via a dermal patch. The dermal patch may comprise an adhesive backing for attachment to the skin of the patient. The dermal patch may further comprise a backing material which renders the patch impermeable to oils and water. The patch should be applied near the tumor site in the patient. It is further preferred that the agent which modulates the production of nitric oxide synthase by the red blood cells of a patient comprises insulin or $\text{Na}_2\text{Fe}[(\text{CN})_5\text{NO}]$ in water.

Importantly, the neutralization of IANOS antibody is completed only by dermal application of antineoplastic agents which modulate the production of nitric oxide by the red blood cells of a patient. The agents themselves do not need to enter into circulation in the patient. Dermal application allows the antineoplastic agents to penetrate the skin of the patient and activate NO synthesis in red blood cells. In cancer patients, application of the agents in manners other than topical or transdermal application have not been found to be effective.

The IANOS which is present in various cell membranes is actually an insulin receptor. In cancer cells, IANOS is blocked by an antibody (light chain of IgG). However, in the skin cell membranes the antibody does not block IANOS because the antibody is not present in the skin surface. Like insulin, nitric oxide activates IANOS, but unlike insulin, the activation of IANOS by nitric oxide is initiated even in the presence of the antibody. Hence,

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nitric oxide can subsequently diffuse into the circulation without the modulating agent entering into the circulation. However, the diffusion of nitric oxide into the circulation in turn activates erythrocyte membrane IANOS. This
5 amplification of IANOS activity of the erythrocyte membrane further results in increased plasma nitric oxide levels in the patient.

When a dermal patch comprising the modulating agent is applied to the patient's skin, synthesis of nitric oxide
10 in cells in the epidermal layer is induced by the agent. The patch is not intended to accomplish transdermal delivery of the agent into the circulation of the patient. Rather, nitric oxide production is achieved. Increased nitric oxide activates enzymes which block the anti-IANOS
15 antibody. Once the anti-IANOS antibody is blocked, the nitric oxide begins to act as a tumoricide in cancer cells.

An agent which modulates the production of nitric oxide by red blood cells, is any agent which induces systemic production of nitric oxide, and which counteracts
20 the antibody to IANOS. The induction of nitric oxide reactivates antibody inhibited IANOS in cancer patients. Preferred agents which modulate the production of nitric oxide by cells found in the epidermal layer are insulin and $\text{Na}_2\text{Fe}[(\text{CN})_5\text{NO}]$ in water. In a further preferred embodiment,
25 the insulin is of bovine pancreatic origin.

The duration of treatment varies with each patient. However, a typical range of topical application is between about 30 and 45 days. After 30 days of treatment via a dermal patch, some patients continued to neutralize the
30 anti-IANOS antibody despite a discontinuation of patch application. This continued neutralization indicated that nitric oxide production in patients was restored. Other patients still required continued topical application to maintain nitric oxide production.

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Treatment with modulating agents of the present invention has the benefit of being non-toxic and non-invasive when compared with chemotherapy and surgical options. The only side effect observed was that 1-2 percent of patients developed hypoglycemia. As shown in Table 1, both compositions, namely, insulin and $\text{Na}_2\text{Fe}[(\text{CN})_5\text{NO}]$ in water, were found to be effective against a wide variety of cancers. Insulin was particularly effective in non-Hodgkin's lymphoma, brain cancer, breast cancer with mastectomy, and lung cancer(non-small cell). Whereas the composition comprising $\text{Na}_2\text{Fe}[(\text{CN})_5\text{NO}]$ in water was particularly effective in lung cancer, breast cancer without mastectomy, esophagus, liver cancer, gall bladder cancer, colon cancer, rectum cancer, acute lymphocytic leukemia, acute myeloid leukemia, multiple myeloma, uterine cancer, cervical cancer, Hodgkin's lymphoma, renal cell carcinoma, ovarian cancer, prostate cancer, tongue cancer, pyriform fossa, mandible cancer. Both agents were equally effective against pancreatic cancer and bone cancer.

Treatment methods using agents which modulate the production of nitric oxide is non-invasive and does not produce any discernable side effects such as toxicity that are commonly experienced by patients undergoing surgery or receiving chemotherapy or radiotherapy. In terms of therapeutic approach, the methods of treatment using agents which modulate the production of nitric oxide are more effective than other existing treatments.

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Table 1

Effects of Application of Antineoplastin for 45 Days on
Several Hematological Tests in Patients with Different
Cancer and on the Survival of These Patients Due to
Continued Use of the Agent

5

	Types of Cancer (Diagnosis)	Type of Patient (n)	Hematological Test (Improvement in Hb, TC, DC, or any other test as indicated (n))	Improve- ment in LFT (n)	Obsvd. (T)	Tabu- lated Tau (τ) at 5% Level	Survi- val % (after 2 yrs)
10	Lungs Non Small Cell Carcinoma (Age 45-72)	R (152)	102	98			59
	M F (145) (65)	I			0.0129	-1.645	
15	Diagnosed by histopathology after bronchoscopy, X-ray	NR (58)	3	5			5
20	Lungs Small Cell Carcinoma (Age 30-65)	R (95)	80	79			59
	M F (65) (60)	II			0.0150	-1.645	
25	Diagnosed by histopathology bronchoscopy, X-ray	NR (30)	2	3			2
30	Breast Cancer (without mastectomy) (Age 25-55)	R (520)	470	480			65
	F (600)	II			0.0128	-1.645	
35	Diagnosed by mammography and biopsy as infiltrating ductal cells or invasive	NR					
40	lobular with axillary lymph node metastasis	NR (80)	16	7			22

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5	M F (105) (45) Diagnosed by blood picture and bone marrow test	R (125)	103 improved to normal condition, blast cells not found				70
		II		ND	0.0357	-1.645	
10		NR (25)	2				1
15	M F (45) (35) Diagnosed by bone marrow tests and blood picture	R (65)	50 patients had no immature lymphoid cells, no nucleated RBC & normal Hb, TC, DC				67
		II		ND	0.0404	-1.645	
20	M F (45) (15) Diagnosed by biopsy and blood electro- phoresis	NR (15)	0				1
25	Multiple Myeloma (Age 30-60)	R (45)	38 had Bence Jones protein negative; A2 Macroglobulin within normal limits				72
		II		ND	0.0694	-1.645	
30	Uterus (Age 35-60)	(NR) (35)	2				1
35	F (1020) Diagnosed by biopsy, FNAC, USG	R (815)	600	600			68
		II			0.0121	-1.645	
40	Cervix (Age 35-60)	NR (205)	14	3			8
40	F (998) Diagnosed by biopsy, FNAC, USG	R (761)	625	510			67
		II			0.0114	-1.645	
40		NR (237)	12	10			9

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5	Prostate (Age 35-75)	R (78)	67 (most patients had initial level of PSA (>100ng/ml), it was normalized to 4ng/ml after one month)	52			68
	M (95) Diagnosed by serum PSA, acid phosphatase test, USG and biopsy	II NR (17)	0		0.0396	-1.645	1
10	Glioma (Age 30-72)	R (82)	79				60
15	M F (65) (37) Diagnosed by CT Scan	I NR (20)	1	ND	0.0099	-1.645	1-2

The present invention further provides a method for relieving pain in a patient without causing significant side effects comprising providing an agent which modulates the production of nitric oxide by cells found in the epidermal layer in a patient, and topically delivering the agent to said patient. It is preferred that topical administration of the agent is completed via a dermal patch applied to the skin of said patient, however any other topical administration mode may be used such as ointments, creams, and lotions.

The present invention further provides a useful method for preventing cancer in a human patient comprising topically administering an agent which modulates the production of nitric oxide by cells found in the epidermal layer to a patient. As shown in Table 2, a study of 590 patients with simple radial mastectomy were topically administered an agent which modulates the production of nitric oxide by cells found in the epidermal layer in the patient after the removal of the cancerous tumor tissue.

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480 of the patients favorably responded to the treatment and of the 480 patients who responded to the treatment 40 percent of the patients survived for at least two years as opposed to only seven percent of the patients who did not
5 respond to the treatment. These results strongly suggest that treatment with an agent which modulates the production of nitric oxide by cells found in the epidermal layer actually prevents cancer from progressing in patients.

The present invention further provides a method for
10 improving or reducing the systemic side effects associated with cancer in a patient comprising providing an agent which modulates the production of nitric oxide by cells found in the epidermal layer by topically applying the agent to said patient. As shown in Table 2, the systemic
15 side effects associated with cancer in a patient include, but are not limited to: pain and discomfort, abdominal distention, iron lymphedema, irregular hemoglobin count, irregular serum PSA, hematuria, neurological problems with vision and memory, swelling, dysphagia, irregular white
20 blood cell count, appetite loss, nausea, increased oedema, impaired electrolytic balance, irregular amounts of protein found in patients blood, irregular amounts of A2 macroglobulin found in patients blood, and irregular swelling of lymph nodes.

25 In another aspect, the present invention further provides a method for controlling or preventing diabetes from occurring in patients comprising topically administering an agent which modulates the production of nitric oxide by cells found in the epidermal layer in a
30 patient. In a preferred embodiment the agent which modulates the production of nitric oxide synthase by the red blood cells of a patient comprises insulin or $\text{Na}_2\text{Fe}[(\text{CN})_5\text{NO}]$ in water. In a further preferred embodiment the patient is a type I diabetic, a type II diabetic or is

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a cancer patient.

Table 2
Effect of Antineoplastins on Various Types of Cancer

	Cancer Type	Condition at Presentation		Reduced Pain	Appetite Increase	Hb Stable or Improvement	LFT	Condition Observed After Application of Antineoplastins	% Survival
5	<i>Lungs Non Small Cell Carcinoma</i> (Age 45-72)	Severe chest pain with loss of appetite, cough, fever, respiratory distress, cachexia, anorexia, parenchymal lesion, haemoptysis.	R	102	110	102	98	Reduced pleural effusion, patchopacities, supra-clavicular lymph node metastasis, cough and respiratory distress. Improvement of pain, appetite, weight.	59%
10	M F (135) (65)								
15	Diagnosed by histopathology after bronchoscopy, X-ray		NR	6	5	3	4		5%
20	<i>Lungs Small Cell Carcinoma</i> (Age 30-65)	Severe chest and back pain with loss of appetite, mulemphysematous bullae in cases, cough, severe pleffusion, respiratory distress, haemoptysis, cachexia, anorexia.	R	110	115	80	79	Reduced pleural thickening Infiltration of left lingular lobe, lower lobe of bronchii pain with cough problems, hemoptysis totally reduced (in some cases).	59%
25	M F (90) (60)								
	Diagnosed by histopathology after bronchoscopy X-ray		NR	2	1	3	3		2%

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5	10	<i>Oesophagus</i> (Age 35-50) M F (450) (159) Diagnosed by barium swallow X-ray and GI endoscopy	R	580	570	570	540	Reduced problems in degluti- tion, dysphagis, improvement in cachexia, patients could eat solid food in 60% cases, no sign of malignancy.	80%
			NR	1	1	1	2		3%
15		<i>Stomach</i> (Age 28-60) M F (155) (95) Diagnosed by biopsy, endoscopy, USG	R	210	196	184	185		45%
			NR	2	2	3	2		5%
20	25	<i>Liver</i> (Age 28-60) M F (175) (100) Diagnosed by USG, CT, biopsy	R	220	230	135	128	Nausea decreased, reduction in SGOT, SGPT values, peritoneal accumula- tion sharply reduced. Improved anemic conditions and cachexia.	38%
			NR	2	2	3	3		4%

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5	Pancreas (Age 28-65) M F (98) (25) Diagnosed by biopsy, USG.	Severe jaundice, loss of appetite, extreme cachexia, abdominal disten- tion, in some cases.	R	76	65	81	53	CBD obstruction was totally normalized and hypodense areas could not be located. In cases, high bilirubin counts and abnormal LFT results antineo- plastin was 60% effective. In contrast, patients with severe metastasis and cachexia responded to antineo- plastin.	38%
			NR	2	2	2	3		1%
10 15	Gall Bladder (age 30-65) M F (100) (50) Diagnosed by biopsy/CT/ USG.	In some cases there were CBD obstruc- tion, severe jaundice, extreme cachexia and anorexia.	R	120	135	98	56	Obstructive jaundiced patients lowered bilirubin counts improved cachexia and anorexia. USG results showed a decrease of calculus/ SOL in the gall bladder. No signs of previous metastasis could be located. Patients with previous cholecysc- tectomy and cholelith- iasis showed significant improve- ment.	39%
			NR	1	1	1	3		1%

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5	Colon (Age 35-71) M F (175) (39) Diagnosed by colonoscopy /biopsy	Acute electro- lytic disbal- ance. Severe anorectal pain, bleeding during fecal elimina- tion, cachexia, loss of appetite.	R	192	186	175	184	Anorectal bleeding and pain reduced consider- ably with improvement in cachexia.	69%
			NR	1	1	2	1		3%
10	Rectum (Age 34-68) M F (165) (97) Diagnosed by biopsy	Acute pain, bleeding during fecal eliminati- on, loss of appetite, constipat- ing and cachexia in some cases.	R	209	210	175	184	Improved cachexia, pain no further problem in fecal elimina- tion, were consider- ably improved in cachexia, no bleeding during fecal elimina- tion.	65%
			NR	2	1	2	3		4%
15	ALL (Age 4-70) M F (105) (45) Diagnosed by blood picture and bone marrow test	Severe hepatome- galy, spleno- megaly persis- tant low fever, lack of appetite, cachexia.	R	-	100	130	-	70% of the patients had a sharp improvement in Hb content. Normaliza- tion on WBC count and platelets. In 42 patients, the bone marrow test was normal.	70%
			NR	(ND)	2	1	(ND)		1%
25	AML (Age 5-75) M F (45) (35) Diagnosed by bone marrow tests and blood picture	Severe hepato- spleno- megaly, fever, cachexia, loss of appetite and severe body ache.	R	-	40	70	-	Improved nausea and appetite, normalized hepato- spleno- megaly, reduced body ache and fever.	67%
			NR	(ND)	1	1	(ND)		1%

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5	Multiple Myeloma (Age 30-60) M F (35) (15) Diagnosed by biopsy and blood electro- phoresis	Cachexia, loss of appetite, abnormal blood picture, decrease of sensory response	R	-	40	-	-	Improvement in general conditions, increase of sensory response. Bence Jones protein negative, A2 macroglo- bulin within normal limits.	72%
			NR	(ND)	1	(ND)	(ND)		1%
15	Non Hodgkin's Lymphoma (Age 24-58) M F (158) (100) Diagnosed by biopsy/FNAC	Cachexia, acute pain, regional lymph adeno- pathy, loss of appetite, huge lympho- ma in certain cases.	R	-	240	189	115	Reduction in the size of lymphoma and metastasis of lymph node. In some cases the swollen lymph nodes totally decreased. Improvement of blood picture and LFT.	68%
			NR	(ND)	1	2	4		3%
25	Hodgkin's Lymphoma (Age 17-52) M F (98) (67) Diagnosed by biopsy/FNAC	Swollen lymph node fever, severe weakness, loss of appetite.	R	-	136	120	71	Improved blood picture, LFT, no regional lymphadeno- pathy was evidenced by scan and FNAC reduction in swelling of nodes fever normalized.	62%
			NR	(ND)	1	1	3		2%
30	Uterus (Age 35-60) F (1020) Diagnosed by biopsy, FNAC, USG	Post menopaus- al bleeding P/R with acute pain, cachexia.	R	760	800	600	600	Improved Hb, WBC count, LFT, bleeding and pain reduced. Improved appetite.	68%
			NR	5	6	8	6		8%
35	Cervix (age 35-60) F (998) Diagnosed by biopsy, FNAC, USG	Bleeding P/R with severe pain and cachexia	R	875	960	625	510	Hb, WBC counts and LFT normalized, reduced bleeding and abdominal distention.	67%
			NR	3	1	7	9		9%

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5	Renal Cell Carcinoma (Age 35-70) M F (135) (40) Diagnosed by FNAC/Cystoscopy/ biopsy/USG	Acute pain, frequent micturition, oedema, haematuria, loss of appetite.	R	160	160	140	110	Improved blood picture, LFT, haematuria decreased oedema, electrolytic balance restored, micturition rate reduced. In some cases of bone and prostate metastasis serum PSA level reduced.	69%
			NR	1	1	1	2		2%
10									
15	Ovary (Age 25-60) F (900) Diagnosed by biopsy/ FNAC/USG	Severe bleeding, white discharge, distention of lower abdomen	R	800	620	600	450	Reduced abdominal distention, bleeding post-menopausal bleeding, white discharge, CA-125 values normalized.	68%
			NR	3	8	9	4		8%
20									
25	Prostate (Age 35-75) M (95) Diagnosed by serum PSA, acid phosphatase test, USG and biopsy	Severe pain, haematuria, frequent micturition, oedema of legs, some of them have bone metastasis, some were fixed with a catheter.	R	65	56	67	52	Stabilized Hb levels and improved LFT, serum PSA, cachexia, reduced haematuria, oedema. Catheter could be removed, electrolytic balance could be attained.	68%
			NR	1	1	1	1		1%

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5	Glioma (Age 30-72) M F (65) (37) Evidenced by CT Scan	Loss of vision para- plegia, cachexia, loss of memory.	R	80	75	79	-	Improvement of pain, appetite, TC, DC levels, tumor regression (evidenced by CT Scan) paraplegia, neurologi- cal problems, vision, memory, improved, improvement in generalized cachexia.	50- 60%
			NR	1	1	1	(ND)		1-2%
10	Tongue (Age 30-65) M F (168) (57) Diagnosed by punch biopsy, FNAC	Severe pain, unable to speak, eat, excess saliva, foul odor, open wounds on tongue, can only have liquid/ semisolid diet, most patients reported severe metasta- tis to mandible buccal mucosa, larynx and naso- pharynx.	R	189	178	157	132	Improved hematocrit, LFT, relief from excrutia- ting pain in tongue and throat. Patients markedly improved their general weakness. Foul odor and constant salivation reduced. Subsequent biopsy revealed absence of malignant cells.	58%
			NR	1	1	1	1		2%
15 20	Pyriform Fossa (Age 29-70) M F (139) (39) Diagnosed by biopsy/FNAC	Both right and left, severe pain, excess saliva, consti- pation.	R	145	156	138	146	Hb, LFT improved, ALP, SGOT, SGPT values reduced. Improvement in general weakness, voice normalized, pain reduced, pain killers discontin- ued.	49%
			NR	1	1	1	3		2%

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Mandible (Age 30-65) M F (42) (33)	Severe pain, cachexia, metastasis to naso-pharynx, occipital region of brain some have gone through hamimandibulectomy.	R	62	56	49	54	In cases of hemimandibulectomy and progressive metastasis there was no further spread as evidenced by CT Scan, weakness reduced of voice improved.	51%
		NR	1	1	1	1		1%

5 The present invention is further illustrated by the following non-limiting examples:

Example 1 - Patient Selection

A total of 8,125 patients with different kinds of cancer participated in this study. These patients were
10 divided into two groups. Blood samples were collected from 80 patients designated as Group I. These patients included 45 males between 20-65 years old, and 35 females between 25-55 years old. These patients were newly diagnosed patients with cancer who at the time of blood donation had
15 not taken any medicine at least for 14 days and had not yet undergone any treatment of cancer including radiation or chemotherapy but opted for surgery. None of the patients had overt diabetes mellitus, systemic hypertension or suffered coronary artery disease at presentation. None of
20 these patients had any other diagnosed life threatening condition.

Two categories of patients were included in Group II. The first category included 6,705 patients who had previously undergone all available cancer treatments
25 including surgery, radiation and chemotherapy. At the time of participation in the study these patients had exhausted all therapeutic options and were under the care of private physicians and family members in their home. The second

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category of 1,340 cancer patients in Group II were the patients who for their economic and/or personal reasons did not undergo any conventional treatment for cancer. At the time of the participation in the study all patients, in the
5 Group II in the second category had stopped taking treatments for cancer, such as chemotherapy and radiation, for at least 2 months. The diagnostic procedures and condition of the patients at presentation are described in Table 1. All institutionalized patients in group II were
10 excluded from the study to avoid ambiguity. As in the case of Group I the patients with any other life threatening conditions or severe infection were also excluded from this group.

A control group of 150 patients were selected
15 randomly from the Group II patients. These patients received placebo dermal patches only instead of dermal patches comprising antineoplastin agents. Since the effect of antineoplastins on the neutralization of IANOS antibody *in vivo* in cancer patients (responders) could be noticed by
20 immunoblot technique (end point) in 7 days, the patients in the placebo group received the vehicle for 7 days; the neutralization of the antibody and nitric oxide synthesis were determined. After 7 days these patients began to receive antineoplastins. In this way responder patients
25 were not denied of any beneficial effects of treatment.

Example 2- Agent Identification

Both biologic and non-biologic materials for were screened for their ability to activate human erythrocytes IANOS. The biologic material was a bovine pancreatic
30 protein. Antineoplastin from aqueous extract of Buffer pH 7.4 was purified to homogeneity by the combination of DEAE cellulose chromatography and Sephadex gel filtration

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techniques. The purified protein showed the Mr of about 5 KD in alkaline SDS-Polyacrylamide gel electrophoresis. This purified protein was a potent activator of erythrocyte IANOS.

5 The first antineoplastin agent composition was prepared by dissolving 0.1-0.2 mg antineoplastin protein in 100 ml of 0.9% NaCl containing 0.1 % bovine serum albumin with 4% vol/vol glycerol and adjusted to pH 6.8. Any commercial preparation of insulin or other insulin prepared
10 in the laboratory using bovine pancreas could be substituted.

 The second antineoplastin agent composition was prepared by dissolving 10-20 mg $\text{Na}_2\text{Fe}[(\text{CN})_5\text{NO}]$ in water and adjusting the solution to pH 6.8.

15 **Example 3- Administration of Agents**

 Typically, about 0.2 ml of solution of either of the above identified antineoplastin agents was applied on the absorbent pad in an adhesive bandage. The adhesive bandage was applied to previously cleaned skin, on the lower
20 abdomen, free of hairs, so that the solution soaked pad would tightly adhere to the skin. If required, the antineoplastin patch was replaced by a new one every 24 hours. Although the patch of "antineoplastin" thus prepared was usually applied on the lower abdominal area, any other
25 suitable part of the body can be used. *In vivo* experiments with animals the patch was similarly applied on the skin except that before application the hairs on the abdomen area of the animal were shaved and the skin was cleaned.

 Collection of blood and preparation of plasma and
30 erythrocyte membrane blood was collected from normal healthy volunteers or from Group I cancer patients. The normal volunteers (n= 50) had not taken any medication for at least for 14 days prior to the donation of blood. Only

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age and sex matched volunteers participated in the study.

Example 4- Assay of IANOS of Human Erythrocytes

Insulin activated nitric oxide synthase (IANOS) activity of the erythrocyte suspension was carried out as described by determining the conversion of oxyhemoglobin to methemoglobin. Purification of insulin activated nitric

oxide synthase (from human erythrocyte membranes) was carried out by DEAE cellulose chromatography.

Example 5- Characterization of the Plasma IANOS Inhibitor

The purified inhibitor was immunoblotted with 125I linked anti-human, anti-IgG which in turn was conjugated to protein-A. The immunoblot was performed and quantified.

Example 6- Statistical Analysis

Because of the large number of patients (8,045) with different types of cancer in each cancer category and multi variant parameters, the significance of the effect of the antineoplastins in responders were analyzed by tau test (student "t" test tends to tau large number of 'n'). The acceptance of rejection of the significance in all 26 types of cancer tested by null hypothesis of the 5% level of significance indicated 95% level of acceptance.

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What is Claimed is:

1. A method for preventing and treating cancer without causing significant side effects to a human patient in need thereof, comprising topically administering an agent which
5 modulates the production of nitric oxide by cells found in the epidermal tissue layer of a patient.
2. The method of claim 1, wherein the agent is topically administered via a dermal patch.
3. The method of claim 1, wherein the agent comprises
10 insulin.
4. The method of claim 1, wherein the agent comprises $\text{Na}_2\text{Fe}[(\text{CN})_5\text{NO}]$.
5. A method for relieving pain in a patient without causing significant side effects comprising topically
15 administering an agent which modulates the production of nitric oxide by red blood cells.
6. A method for decreasing the systemic side effects of cancer treatment in a patient comprising topically administering an agent which modulates the production of
20 nitric oxide by cells found in the epidermal tissue layer of a patient.
7. A method for controlling or preventing diabetes in a patient comprising topically administering an agent which modulates the production of nitric oxide by cells

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found in the epidermal tissue layer of a patient.

8. The method of claim 7 wherein the patient is a cancer patient.

9. The method of claim 7 wherein the patient is a type
5 I or type II diabetic.

10. A method for treating cancer and relieving pain in a patient without causing significant side effects comprising topically administering an agent which modulates the production of nitric oxide by red blood cells.