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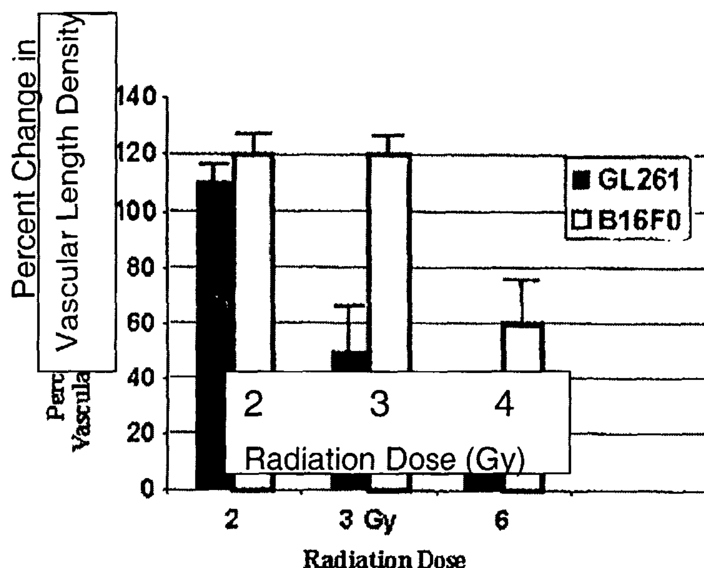

(57) Abstract: A method for increasing radiosensitivity of a tumor in a subject via administration of a VEGF-R2 Inhibitor to a tumor in a subject. Also provided are methods for delaying tumor growth and for inhibiting tumor blood vessel growth via administration of a VEGF-R2 inhibitor.

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Description

INHIBITION OF VEGF RECEPTOR SIGNALING REVERSES TUMOR RESISTANCE TO RADIOTHERAPY

Cross Reference to Related Applications

This application is based on and claims priority to United States Provisional Application Serial Number 60/356,309, filed February 12, 2002, herein incorporated by reference in its entirety.

Grant Statement

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Field of the Invention

The present invention generally relates to compositions and methods for enhancing radiotherapy via inhibition of VEGF signaling. More particularly, the method involves administration of a VEGF-R2 inhibitor to a tumor in a subject, whereby the radiosensitivity of the tumor is increased.

Table of Abbreviations

Abbreviation		Meaning
AAV	-	adeno-associated virus
Ad.ExFlk	-	adenovirus encoding soluble extracellular component of Flk-1
Ad.LacZ	-	adenovirus encoding β-galactosidase
ATCC	-	American Type Culture Collection
B16F0	-	murine melanoma cell line
CEA	-	carcinoembryonic antigen
D54	-	human glioma cell line
Dil	-	1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate
DMEM	-	Dulbecco's Modified Eagle Medium
EPCs	-	endothelial progenitor cells
ExFlk	-	soluble extracellular component of Flk-1
ExFlk.6His	-	soluble extracellular component of Flk-1 tagged with 6 histidine residues
Flk-1	-	fetal liver kinase 1; murine VEGF-R2

Flt-1	-	fms-like tyrosine kinase 1; VEGF-R1
GBM	-	glioblastoma multiforme
GL261	-	murine glioblastoma cell line
Gy	-	gray
HMEC	-	human microdermal endothelial cells
HSPs	-	high scoring sequence pairs
HUVEC	-	human umbilical vein endothelial cells
^{125}I	-	iodine-125
^{131}I	-	iodine-131
IFN-α	-	interferon alpha
IFN-γ	-	interferon gamma
IL-2	-	interleukin 2
IL-4	-	interleukin 4
IL-6	-	interleukin 6
IMRT	-	intensity modulated radiation therapy
ITR	-	inverted terminal repeat
KDR	-	kinase insert domain receptor; human VEGF-R2
K_i	-	inhibition constant
LLC	-	Lewis lung carcinoma cell line
LTR	-	long terminal repeat
p	-	probability
^{32}P	-	phosphorus-32
PBS	-	phosphate buffered saline
PECAM	-	platelet-endothelial cell adhesion molecule-1/CD31
pfu	-	plaque forming unit
SDS	-	sodium dodecyl sulfate
sFlk-1	-	soluble extracellular component of Flk-1
SSC	-	standard saline citrate
SU5416	-	3-[2, 4-dimethyl pyrrol-5-yl) methyl idenyl]-2-indolinone

T_m	-	thermal melting point
TNF	-	tumor necrosis factor
VEGF	-	vascular endothelial growth factor
VEGF-R	-	vascular endothelial growth factor receptor (generally)
VEGF-R1	-	vascular endothelial growth factor receptor type 1; Flt-1
VEGF-R2	-	vascular endothelial growth factor receptor type 2;Flk-1/KDR

Background of the Invention

The inability of local radiation to control tumor growth is a significant clinical outcome leading to unsuccessful cancer therapy. *See e.g.*, Suit, 1996 and Lindegaard *et al.*, 1996. Tumors can be resistant to ionizing radiation, or tumors can recur because it is not possible to deliver a sufficiently high dose of radiation without an unacceptably high risk of damage to non-tumor cells.

One complication in designing radiotherapy is different cellular responses to ionizing radiation observed *in vitro* and *in vivo*. For example, glioblastoma multiforme (GBM) has a universally fatal clinical outcome in both children and adults (Walker *et al.*, 1980; Wallner *et al.*, 1989; Packer, 1999). *In vitro* studies show that numerous human GBM cell lines have radiosensitivity that is in the range of cell lines from more curable human tumors (Allam *et al.*, 1993; Taghian *et al.*, 1993). However, studies of the radiation response in GBM tumors in animal models have shown that the radioresistance of GBM tumors *in vivo* does not correlate with the radiosensitivity of the same cell lines *in vitro* (Baumann *et al.*, 1992; Allam *et al.*, 1993; Taghian *et al.*, 1993; Advani *et al.*, 1998; Staba *et al.*, 1998).

Tumor cells could show radiosensitivity *in vitro* due to the absence of an angiogenic network that appears to contribute to radioresistance *in vivo*. The response of tumor microvasculature to radiation is dependent upon the dose and time interval after treatment (Kallman *et al.*, 1972; Song *et al.*, 1972; Hilmas & Gillette, 1975; Johnson, 1976; Yamaura *et al.*, 1976; Ting *et*

al., 1991). Tumor blood flow decreases when high doses in the range of 20 grays (Gy) to 45 Gy are used (Song *et al.*, 1972). In contrast, blood volume increases when relatively low radiation doses, for example below 500 rads, are administered (Johnson, 1976). In irradiated mouse sarcomas, blood
5 flow was observed to increase during 3 to 7 days following irradiation (Kallman *et al.*, 1972; Hilmas & Gillette, 1975; Johnson, 1976; Yamaura *et al.*, 1976; Gorski *et al.*, 1999).

Increased tumor blood flow following radiation exposure could result from radiation-induced expression of angiogenic factors, such as vascular
10 endothelial growth factor (VEGF) (Gorski *et al.*, 1999). VEGF and vascular endothelial growth factor receptor type 2 (VEGF-R2; also called Flk-1 and KDR) expression levels increase in a time- and dose-dependent manner following irradiation of cultured human endothelial cells or tumor models (Gorski *et al.*, 1999; Kermani *et al.*, 2001). Endothelial cell proliferation and
15 survival after *in vitro* irradiation are enhanced by supplementation of cell cultures with VEGF (Gorski *et al.*, 1999), whereas anti-VEGF antibody enhances cytotoxic effects of irradiation. Binding of VEGF to the Flk-1/KDR receptor contributes to the VEGF-survival phenotype (Millauer *et al.*, 1993; Waltenberger *et al.*, 1994).

20 Levels of VEGF are also correlated with radiation stress and radiotherapy resistance (Shintani *et al.*, 2000). VEGF is observed at increased levels in tumors that are resistant to radiotherapy such as malignant gliomas and melanomas. VEGF expression is also associated with worsened prognosis (Valter *et al.*, 1999).

25 Despite these advances in understanding molecular and cellular bases for radioresistance, there exists a long-felt need in the art for effective therapies for promoting regression of tumors that are resistant to conventional therapies, including radiotherapy. To meet this need, the present invention provides in one embodiment a method for enhancing the
30 radiosensitivity of a tumor via administration of a VEGF-R2 inhibitor. Also provided is a method for inhibiting tumor growth by: (a) administration of a VEGF-R2 inhibitor, whereby the tumor is radiosensitized; and (b) treating the tumor with ionizing radiation.

Summary of the Invention

In one embodiment, the present invention provides a method for increasing radiosensitivity of a tumor in a subject, wherein the method comprises administering a soluble VEGF-R polypeptide to a subject, such as by administering the soluble VEGF-R polypeptide to a tumor in a subject, whereby the radiosensitivity of the tumor is increased.

The present invention provides a method for increasing radiosensitivity of a tumor in a subject comprising administering a VEGF-R2 inhibitor to a tumor in a subject, whereby the radiosensitivity of the tumor is increased.

The present invention also provides a method for delaying tumor growth, the method comprising: (a) administering a VEGF-R2 inhibitor to a subject bearing a tumor; whereby the radiosensitivity of the tumor is increased; and (b) treating the tumor with ionizing radiation, whereby tumor growth is delayed. In one embodiment, the tumor growth delay comprises tumor regression.

The present invention also provides a method for inhibiting tumor blood vessel growth, the method comprising: (a) administering a VEGF-R2 inhibitor to a subject bearing a tumor; whereby the radiosensitivity of the tumor is increased; and (b) treating the tumor with ionizing radiation, whereby tumor blood vessel growth is inhibited. A VEGF-R2 inhibitor can also be administered after irradiation as maintenance therapy for the prevention of vascular regrowth.

The methods of the invention are useful for radiosensitizing tumors, delaying tumor growth, and inhibiting tumor vascularization in mammalian subjects. In one embodiment, a subject is a human subject. The methods are useful for treatment of all tumor types, and are particularly relevant for the treatment of radiation resistant tumors.

In one embodiment, a VEGF-R2 inhibitor comprises SU5416, a small molecule that specifically binds VEGF-R2. In another embodiment, a VEGF-R2 inhibitor comprises a soluble VEGF-R polypeptide.

A soluble VEGF-R polypeptide, including a soluble VEGF-R2 polypeptide and a soluble VEGF-R1 polypeptide, can comprise: (a) a

polypeptide comprising an amino acid sequence of any one of even-numbered SEQ ID NOs:2-8; (b) a polypeptide substantially identical to any one of even-numbered SEQ ID NOs:2-8; (c) a polypeptide encoded by a nucleic acid of any one of odd-numbered SEQ ID NOs:1-7; or (d) a polypeptide encoded by a nucleic acid substantially identical to any one of odd-numbered SEQ ID NOs:1-7.

A soluble VEGF-R polypeptide can also comprise a polypeptide encoded by an isolated nucleic acid segment selected from the group consisting of: (a) an isolated nucleic acid molecule which hybridizes to a nucleic acid sequence of any one of odd-numbered SEQ ID NOs:1-7 under wash stringency conditions represented by a wash solution having less than about 200 mM salt concentration and a wash temperature of greater than about 45°C, and which encodes a VEGF-R polypeptide; and (b) an isolated nucleic acid molecule differing by at least one functionally equivalent codon from the isolated nucleic acid molecule of (a) above in nucleic acid sequence due to the degeneracy of the genetic code, and which encodes a VEGF-R polypeptide encoded by the isolated nucleic acid of (a) above.

A VEGF-R2 inhibitor is administered in one embodiment as a minimally therapeutic dose, although higher doses can be used as well. A subtherapeutic dose or a therapeutic dose of ionizing radiation can be used.

In accordance with the methods of the present invention, a VEGF-R2 inhibitor is provided via administration of a composition comprising: (a) a VEGF-R2 inhibitor; and (b) a pharmaceutically acceptable carrier. In one embodiment of the invention, the carrier comprises a gene therapy construct that encodes a soluble VEGF-R polypeptide.

Accordingly, it is an object of the present invention to provide novel methods for radiosensitizing a tumor to thereby delay tumor growth and to inhibit tumor vascularization. This and other objects are achieved in whole or in part by the present invention.

An object of the invention having been stated above, other objects and advantages of the present invention will become apparent to those of ordinary skill in the art after a study of the following description of the invention and non-limiting Examples.

Brief Description of the Drawings

Figure 1 is a bar graph of the percent change in vascular length density of tumors treated with the radiation doses indicated. Vascular windows were prepared, and the length of tumor blood vessels photographed from each vascular window was determined as described in Example 2. The sum of changes in the quantity of blood vessels over time was compared to that observed at 0 hours. Bars represent the mean percent change in vascular length density and the standard error of the mean determined in 5 experiments. Solid bars, GL261 tumors; open bars, B16F0 tumors.

Figure 2 is a bar graph that depicts the percent change in Power Doppler signal of tumors treated with the radiation doses indicated. GL261 and B16F0 tumor cell lines were pelleted and implanted into the hind limb of C57BL6 mice. Tumors were grown to diameters ranging from 0.7 cm to 1.1 cm and were irradiated. Tumor vasculature was analyzed by Power Doppler sonography as described in Example 3. Changes in Power Doppler signals were compared to signals recorded immediately prior to irradiation. Solid bars, GL261 tumors; open bars, B16F0 tumors.

Figures 3A and 3B are bar graphs that show an enhanced decrease in tumor vasculature following radiotherapy in combination with VEGF-R2 inhibition.

Figure 3A is a bar graph that depicts a decreased percent change in vascular length density in response to adenovirus vector Ad.ExFlk followed by radiotherapy. GL261 and B16F0 tumors were implanted into a dorsal skin fold window, and tumor vasculature was allowed to develop over the course of 1 week. Blood vessels were photographed at the time of treatment (0 hour) and each day following treatment. Changes in treated blood vessels were compared to the same blood vessels prior to treatment (100%). The mean percent change in vascular length density and the standard error of the mean were determined at 72 hours following treatment. Additional methods are described in Example 5. Checkered bars, tumors treated with a control adenovirus vector encoding β-galactosidase (Ad.LacZ) followed by 2 Gy ionizing radiation 24 hours later; striped bars, tumors

treated with 2 Gy ionizing radiation; open bars, tumors treated with an adenovirus vector encoding soluble Flk-1 (Ad.ExFlk); solid bars, tumors treated with 2 Gy ionizing radiation; open bars, tumors treated with an adenovirus vector encoding soluble Flk-1 (Ad.ExFlk) followed by 2 Gy ionizing radiation 24 hours later.

Figure 3B is a bar graph that depicts a decreased percent change in vascular length density in response to SU5416 treatment followed by radiotherapy. GL261 and B16F0 tumors were implanted into a dorsal skin fold window, and tumor vasculature was allowed to develop over the course of 1 week. Blood vessels were photographed at the time of treatment (0 hour) and each day following treatment. Changes in treated blood vessels were compared to the same blood vessels prior to treatment (100%). Additional methods are described in Example 5. The mean percent change in vascular length density and the standard error of the mean were determined at 72 hours and 120 hours following treatment. Solid bars, B16F0 tumors; open bars, GL261 tumors.

Figure 4 is a bar graph depicting a decreased percent change in Power Doppler signal in response to SU5416 followed by radiotherapy. GL261 and B16F0 tumors were implanted into the hind limb of C57BL6 mice. Tumors were grown to the diameter of approximately 1 cm and animals were treated by intraperitoneal administration of SU5416 with or without radiation with 3 Gy ionizing radiation fractions given on 8 days over the course of 11 days. Tumor vascularity was measured by Power Doppler analysis, and changes in tumor vascularity were determined relative to the same tumor at the time of treatment (0 hour, 100%). The mean percent change in Power Doppler signal and the standard error of the mean were determined using 3 mice in each treatment group. Additional methods are described in Example 5. Solid bars, B16F0 tumors; open bars, GL261 tumors.

Figure 5 is a bar graph depicting an increased percentage of endothelial cells undergoing apoptosis in response to VEGF-R2 inhibition followed by radiotherapy. HUVEC cells (human umbilical vein endothelial cells), HMECs (human microdermal endothelial cells), and murine

endothelial 3B11 cells were treated with 50 μM SU5416, 10^8 plaque-forming units (pfu) of Ad.ExFlk, or 10^8 pfu control vector Ad.LacZ. Cells were treated with 6 Gy of radiation and stained 24 hours later. The percentage of cells undergoing apoptosis was determined for each of the treatment groups as
5 described in Example 6. The mean percentage of apoptotic cells and the standard error of the mean were determined using 4 culture plates in each treatment group.

Figures 6A and 6B are line graphs depicting an enhanced decrease in tumor volume in response to SU5416 followed by radiotherapy. GL261 cells
10 were pelleted and implanted into the hind limb of C57BL6 mice. Tumors were grown to diameters ranging from 5 mm to 7 mm at which time treatment was initiated (day 0). Tumor volumes were measured at the indicated time points for each of the treatment groups. Mice were sacrificed when tumor volume exceeded 6 times the original tumor volume. Control
15 mice received no therapy. SU5416 was administered by intraperitoneal injection twice per week for a total of 4 treatments on days 0, 4, 7 and 11. Ad.ExFlk and Ad.LacZ were administered by tail vein injection. Radiation was administered as 3 Gy fractions on 8 occasions on days 0, 1, 2 and 3. Additional methods are described in Example 7.

20 Figure 6A is a line graph depicting the fractional change in tumor volume in response to the following treatments: (■), untreated control; (▲), tumors treated with 3 Gy ionizing radiation; (◆), tumors treated with SU5416; and (●), tumors treated with SU5416 followed by 3 Gy ionizing radiation. The mean fractional change in tumor volume and the standard error of the
25 mean were determined using 6 animals in each treatment group.

Figure 6B is a line graph depicting the fractional change in tumor volume in response to the following treatments: (■), untreated control; (▲), tumors treated with 3 Gy ionizing radiation; (◆), tumors treated with SU5416; and (●), tumors treated with SU5416 and 3 Gy ionizing radiation. The mean
30 fractional change in tumor volume and the standard error of the mean were determined using 10 animals in each treatment group.

Figure 7 is a bar graph depicting inhibition of tumor revascularization via administration of a VEGF-R2 inhibitor. Doppler analysis of blood flow in

four tumor types is shown on 3, 5, and 7 days following radiation treatment. Animals received radiation treatment alone (control) or in combination with SU5416. In control animals, tumor revascularization occurs within 7 days following radiation treatment. Tumor revascularization is repressed in 5 animals receiving SU5416 in combination with radiation treatment. Open bars, B16F0 tumors; solid bars, LLC tumors; stippled bars, GL261 tumors; hatched bars, D54 tumors.

Brief Description of the Sequence Listing

SEQ ID NO: 1 is a nucleic acid sequence encoding a soluble Flk-1 10 receptor from mouse.

SEQ ID NO: 2 is the amino acid sequence of a polypeptide encoded by SEQ ID NO: 1.

SEQ ID NO: 3 is a nucleic acid sequence encoding a soluble human KDR polypeptide.

15 SEQ ID NO: 4 is the amino acid sequence of a polypeptide encoded by SEQ ID NO: 3.

SEQ ID NO: 5 is a nucleic acid sequence encoding a soluble mouse Flt-1 polypeptide.

SEQ ID NO: 6 is the amino acid sequence of a polypeptide encoded 20 by SEQ ID NO: 5.

SEQ ID NO: 7 is a nucleic acid sequence encoding a soluble human Flt-1 polypeptide.

SEQ ID NO: 8 is the amino acid sequence of a polypeptide encoded by SEQ ID NO: 7.

25 Detailed Description of the Invention

I. Tumor Radiosensitivity

In view of the role of angiogenesis in promoting tumor recovery, cancer therapies have been developed that combine radiotherapy and anti-angiogenic agents. *See* Griscelli *et al.*, 2000 and the references cited herein 30 below. In particular, several studies have explored inhibition of the VEGF signaling pathway in combination with radiotherapy. In contrast to the studies cited herein below, the present invention provides a method for

increasing the radiosensitivity of a tumor in a subject by administration of a VEGF-R2 inhibitor.

Two groups have described radiotherapy in combination with administration of an antibody that specifically binds VEGF, e.g. VEGF-A (Gorski *et al.*, 1999; Lee *et al.*, 2000). In contrast to the disclosure of the present invention, these studies did not demonstrate that inhibition of VEGF-R2 can increase tumor radiosensitivity.

In one embodiment, the present invention provides a novel method for increasing the radiosensitivity of a tumor in a subject via administration of a VEGF-R2 inhibitor. The present invention also provides a method for delaying tumor growth in a subject. The method comprises: (a) administering a VEGF-R2 inhibitor to a tumor in a subject, whereby the radiosensitivity of the tumor is increased; and (b) treating the tumor with ionizing radiation, whereby tumor growth is delayed and tumor regression is promoted and sustained.

As used herein, the term "VEGF-R1" refers to the Flt-1 receptor. The terms "VEGF-R1" and "Flt-1" are used interchangeably.

As used herein, the term VEGF-R2 refers to the Flk-1 receptor, which in humans is also called KDR. The terms "VEGF-R2", "Flk-1", and "KDR" are used interchangeably.

The term "radiosensitivity", as used herein to describe a tumor, refers to a quality of susceptibility to treatment using ionizing radiation. Thus, radiotherapy can be used to delay growth of a radiosensitive tumor. Radiosensitivity can be quantified by determining a minimal amount of ionizing radiation that can be used to delay tumor growth. Thus, the term "radiosensitivity" refers to a quantitative range of radiation susceptibility.

The term "delaying tumor growth" refers to a decrease in a duration of time required for a tumor to grow a specified amount. For example, treatment can delay the time required for a tumor to increase in volume 3-fold relative to an initial day of measurement (day 0) or the time required to grow to 1 cm^3.

The terms "radiation resistant tumor" and "radioresistant tumor" each generally refer to a tumor that is substantially unresponsive to radiotherapy

when compared to other tumors. Representative radiation resistant tumor models include glioblastoma multiforme and melanoma.

The term "increase," as used herein to refer to a change in radiosensitivity of a tumor, refers to change that renders a tumor more susceptible to destruction by ionizing radiation. Alternatively stated, an increase in radiosensitivity refers to a decrease in the minimal amount of ionizing radiation that effectively delays tumor growth. An increase in radiosensitivity can also comprise delayed tumor growth when a VEGF-R2 inhibitor is administered with radiation as compared to a same dose of radiation alone. In one embodiment, an increase in radiosensitvity refers to an increase of at least about 2-fold, in another embodiment an increase of at least about 5-fold, and in still another embodiment an increase of at least 10-fold. In yet another embodiment of the invention, an increase in radiosensitivity comprises a transformation of a radioresistant tumor to a radiosensitive tumor.

The term "tumor regression" generally refers to any one of a number of indices that suggest change within the tumor to a less developed form. Such indices include, but are not limited to a destruction of tumor vasculature (for example, a decrease in vascular length density or a decrease in blood flow), a decrease in tumor cell survival, a decrease in tumor volume, and/or a decrease in tumor growth rate. Representative methods for assessing tumor growth delay and tumor regression are described in Examples 2, 3, 6, and 7.

The methods of the present invention are useful for increasing the radiosensitivity of a tumor, for delaying tumor growth, and/or for inhibiting tumor vascularization in any tumor-bearing subject. Thus, the term "subject" as used herein includes any vertebrate species, in one embodiment warm-blooded vertebrates such as mammals and birds. More particularly, the methods of the present invention are contemplated for the treatment of tumors in mammals such as humans, as well as those mammals of importance due to being endangered (such as Siberian tigers), of economical importance (animals raised on farms for consumption by humans) and/or social importance (animals kept as pets or in zoos) to

humans, for instance, carnivores other than humans (such as cats and dogs), swine (pigs, hogs, and wild boars), ruminants and livestock (such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels), and horses. Also contemplated is the treatment of birds, including those kinds of birds
5 that are endangered or kept in zoos, as well as fowl, and more particularly domesticated fowl or poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they are also of economical importance to humans.

The term "tumor" as used herein encompasses both primary and
10 metastasized solid tumors and carcinomas of any tissue in a subject, including, but not limited to breast; colon; rectum; lung; oropharynx; hypopharynx; esophagus; stomach; pancreas; liver; gallbladder; bile ducts; small intestine; urinary tract including kidney, bladder and urothelium; female genital tract including cervix, uterus, ovaries (*e.g.,* choriocarcinoma and
15 gestational trophoblastic disease); male genital tract including prostate, seminal vesicles, testes and germ cell tumors; endocrine glands including thyroid, adrenal, and pituitary; skin (*e.g.*, hemangiomas and melanomas), bone or soft tissues; blood vessels (*e.g.*, Kaposi's sarcoma); brain, nerves, eyes, and meninges (*e.g.*, astrocytomas, gliomas, glioblastomas,
20 retinoblastomas, neuromas, neuroblastomas, Schwannomas and meningiomas). The term "tumor" also encompasses solid tumors arising from hematopoietic malignancies such as leukemias, including chloromas, plasmacytomas, plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia, and lymphomas including both Hodgkin's and non-
25 Hodgkin's lymphomas. The term "tumor" also encompasses radioresistant tumors, including, but not limited to radioresistant variants of the any of the tumor listed above.

The term "about", as used herein when referring to a measurable value such as an amount of weight, time, dose (*e.g.* radiation dose), etc. is meant
30 to encompass variations of in one embodiment ±20% or ±10%, in another embodiment ±5%, in another embodiment ±1%, and in still another embodiment ±0.1% from the specified amount, as such variations are appropriate to perform the disclosed method.

II. VEGF-R2 Inhibitors

The present invention provides methods for increasing radiosensitivity of a tumor via administration of a VEGF-R2 inhibitor. Any suitable VEGF-R2 inhibitor can be used in accordance with the methods of the present invention, wherein the inhibitor has a capacity to increase the radiosensitivity of a tumor. In one embodiment, a VEGF-R2 inhibitor also shows anti-angiogenic activity or angiostatic activity.

The term "VEGF-R2 inhibitor" as used herein refers to a molecule or other chemical entity having a capacity for specifically binding to VEGF-R2 to thereby inhibit a VEGF-R2 biological activity. VEGF-R2 inhibitors include, but are not limited to small molecule inhibitors, soluble VEGF-R polypeptides (*i.e.*, soluble VEGF-R1 and soluble VEGF-R2), and antibodies that specifically bind VEGF-R2. In one embodiment, the term "VEGF-R2 inhibitor" excludes molecules, other than a soluble VEGF-R polypeptide, that specifically bind to VEGF, such as an anti-VEGF antibody.

In accordance with the present invention, however, a soluble VEGF-R polypeptide can specifically bind VEGF. As such, a soluble VEGF-R polypeptide of the present invention can specifically bind one or more of several different isoforms of VEGF including, but not limited to VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E. While applicants do not wish to be bound by any particular theory of operation, a VEGF-R2 inhibitor, such as a soluble VEGF-R polypeptide, could also function as an inhibitor of VEGF-R1 signaling as well by specifically binding one or more of the VEGF isoforms. Thus, for example, as a result of cross-reactivity between the soluble VEGF-R polypeptide and the various isoforms of VEGF, therefore, in certain embodiments the term "VEGF-R2 inhibitor" can encompass molecules that inhibit signaling through the VEGF-R2 receptor, even if the VEGF-R2 inhibitor also inhibits signaling through the VEGF-R1 receptor at the same time.

The term "binding" refers to an affinity between two molecules, for example, an inhibitor and a target molecule. As used herein, "specific binding" means a preferential binding of one molecule for another in a mixture of molecules. The binding of an inhibitor to a target molecule can be

considered specific if the binding affinity is about 1×10^4 M^{-1} to about 1×10^6 M^{-1} or greater.

II.A. Small Molecules

The term "small molecule" as used herein refers to a compound, for example an organic compound, with a molecular weight of in one embodiment less than about 1,000 daltons, in another embodiment less than about 750 daltons, in another embodiment less than about 600 daltons, and in still another embodiment less than about 500 daltons. A small molecule also has a computed log octanol-water partition coefficient in the range of about –4 to about +14 in one embodiment, and in another embodiment in the range of about –2 to about +7.5.

SU5416 is a small molecule inhibitor of Flk-1/KDR that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types (Fong *et al.*, 1999; Shaheen *et al.*, 1999; Vajkoczy *et al.*, 1999). U.S. Patent No. 6,159,443 to Hallahan describes SU5416 as a radiosensitizing agent that becomes more toxic to a cell following exposure of the cell to ionizing radiation.

II.B. Soluble VEGF-R Polypeptides

The term "soluble VEGF-R polypeptide" refers to a VEGF-R polypeptide that inhibits VEGF-R2 signaling. As described above, a soluble VEGF-R polypeptide might also inhibit VEGF-R1 signaling. The soluble VEGF-R polypeptide comprises in one embodiment a truncated VEGF-R polypeptide having an ability to bind VEGF, to heterodimerize with native VEGF-R2, or a combination thereof. The truncated soluble forms of Flk-1 (VEGF-R2) and Flt-1 (VEGF-R1) inhibit VEGF signaling and delay tumor growth. Soluble Flk-1 and Flt-1 bind to and sequester VEGF, thereby limiting the availability of VEGF for binding to native Flk-1 (Leunig *et al.*, 1992; Goldman *et al.*, 1998; Lin *et al.*, 1998). Soluble Flk-1 and soluble Flt-1 also function as dominant negative receptors by forming inactive heterodimers with native Flk-1 (Kendall *et al.*, 1994; Kendall *et al.*, 1996).

In one embodiment of the invention, a soluble VEGF-R polypeptide comprises soluble Flk-1 polypeptide (VEGF-R2) comprising: (a) a polypeptide encoded by a nucleic acid of SEQ ID NO:1 or 3; (b) a

polypeptide encoded by a nucleic acid substantially identical to SEQ ID NO:1 or 3; (c) a polypeptide comprising an amino acid sequence of SEQ ID NO:2 or 4; or (d) a polypeptide substantially identical to SEQ ID NO:2 or 4.

In another embodiment of the invention, a soluble VEGF-R 5 polypeptide comprises soluble Flt-1 polypeptide (VEGF-R1) comprising: (a) a polypeptide encoded by a nucleic acid of SEQ ID NO:5 or 7; (b) a polypeptide encoded by a nucleic acid substantially identical to SEQ ID NO:5 or 7; (c) a polypeptide comprising an amino acid sequence of SEQ ID NO:6 or 8; or (d) a polypeptide substantially identical to SEQ ID NO:6 or 8.

10 Nucleic Acids. The terms "nucleic acid molecule" and "nucleic acid" each refer to deoxyribonucleotides or ribonucleotides and polymers thereof in single-stranded, double-stranded, or triplexed form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar properties as the reference natural 15 nucleic acid. The terms "nucleic acid molecule" and "nucleic acid" can also be used in place of "gene", "cDNA", or "mRNA". Nucleic acids can be synthesized, or can be derived from any biological source, including any organism.

The term "substantially identical", as used herein to describe a degree 20 of similarity between nucleotide sequences, refers to two or more sequences that have in one embodiment at least about least 60%, in another embodiment at least about 70%, in another embodiment at least about 80%, in another embodiment about 90% to about 99%, in another embodiment about 95% to about 99%, and in still another embodiment about 99% 25 nucleotide identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm (described herein below under the heading "Nucleotide and Amino Acid Sequence Comparisons") or by visual inspection. The substantial identity exists in one embodiment in nucleotide sequences of at least about 100 30 residues, in another embodiment in nucleotide sequences of at least about 150 residues, and in yet another embodiment in nucleotide sequences comprising a full length coding sequence. The term "full length", as used herein refers to a complete open reading frame encoding a functional

soluble VEGF-R polypeptide. Representative full-length nucleic acids encoding a soluble VEGF-R polypeptide are set forth as any one of odd-numbered SEQ ID NOs:1-7. Full-length nucleic acids encoding a soluble VEGF-R2 polypeptide include, but are not limited to those set forth as SEQ
5 ID NOs:1 and 3.

In one aspect, substantially identical sequences can comprise polymorphic sequences. The term “polymorphic” refers to the occurrence of two or more genetically determined alternative sequences or alleles in a population. An allelic difference can be as small as one base pair.

10 In another aspect, substantially identical sequences can comprise mutagenized sequences, including sequences comprising silent mutations. A mutation can comprise a single base change.

Another indication that two nucleotide sequences are substantially identical is that the two molecules specifically or substantially hybridize to
15 each other under stringent conditions. In the context of nucleic acid hybridization, two nucleic acid sequences being compared can be designated a “probe” and a “target”. A “probe” is a reference nucleic acid molecule, and a ‘“target” is a test nucleic acid molecule, often found within a heterogeneous population of nucleic acid molecules. A “target sequence” is
20 synonymous with a “test sequence”.

A nucleotide sequence employed for hybridization studies or assays includes probe sequences that in one embodiment are complementary to or mimic at least an about 14 to 40 nucleotide sequence of a nucleic acid molecule of the present invention. In another embodiment, probes comprise
25 14 to 20 nucleotides, or even longer where desired, such as 30, 40, 50, 60, 100, 200, 300, or 500 nucleotides or up to the full length of any one of odd-numbered SEQ ID NOs:1-7. Such probes can be readily prepared by, for example, chemical synthesis of the fragment, by application of nucleic acid amplification technology, or by introducing selected sequences into
30 recombinant vectors for recombinant production.

The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization and wash conditions when that

sequence is present in a complex nucleic acid mixture (*e.g.,* total cellular DNA or RNA).

The phrase “hybridizing substantially to” refers to complementary hybridization between a probe nucleic acid molecule and a target nucleic 5 acid molecule and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired hybridization.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as 10 Southern and Northern blot analysis are both sequence- and environment-dependent. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, 1993. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the 15 specific sequence at a defined ionic strength and pH. Typically, under "stringent conditions” a probe will hybridize specifically to its target subsequence, but to no other sequences.

The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. 20 Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for Southern or Northern Blot analysis of complementary nucleic acids having more than about 100 complementary residues is overnight hybridization in 50% formamide with 1 mg of heparin at 42°C. An example of highly stringent 25 wash conditions is 15 minutes in 0.1X SSC at 65°C. An example of stringent wash conditions is 15 minutes in 0.2X SSC buffer at 65°C. *See* Sambrook & Russell, 2001 for a description of SSC buffer. Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of medium stringency wash conditions for a duplex of more than 30 about 100 nucleotides is 15 minutes in 1X SSC at 45°C. An example of low stringency wash for a duplex of more than about 100 nucleotides is 15 minutes in 4X to 6X SSC at 40°C. For short probes (*e.g.*, about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less

than about 1 M Na^+ ion, typically about 0.01 to 1 M Na^+ ion concentration (or other salts) at pH 7.0-8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2-fold (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

The following are examples of hybridization and wash conditions that can be used to identify nucleotide sequences that are substantially identical to reference nucleotide sequences of the present invention: a probe nucleotide sequence hybridizes to a target nucleotide sequence in one embodiment in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 2X SSC, 0.1% SDS at 50°C; in another embodiment, a probe and target sequence hybridize in 7% SDS, 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 1X SSC, 0.1% SDS at 50°C; in another embodiment, a probe and target sequence hybridize in 7% SDS, 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 0.5X SSC, 0.1% SDS at 50°C; in another embodiment, a probe and target sequence hybridize in 7% SDS, 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 0.1X SSC, 0.1% SDS at 50°C; in still another embodiment, a probe and target sequence hybridize in 7% SDS, 0.5 M $NaPO_4$, 1 mM EDTA at 50°C followed by washing in 0.1X SSC, 0.1% SDS at 65°C.

A further indication that two nucleic acid sequences are substantially identical is that the proteins encoded by the nucleic acids are substantially identical, share an overall three-dimensional structure, or are biologically functional equivalents. These terms are defined further under the heading "Polypeptides" herein below. Nucleic acid molecules that do not hybridize to each other under stringent conditions are still substantially identical if the corresponding proteins are substantially identical. This can occur, for example, when two nucleotide sequences are significantly degenerate as permitted by the genetic code.

The term "conservatively substituted variants" refers to nucleic acid sequences having degenerate codon substitutions wherein the third position of one or more selected (or all) codons is substituted with mixed-base and/or

deoxyinosine residues. *See* Batzer *et al.*, 1991; Ohtsuka *et al.*, 1985; and Rossolini *et al.*, 1994.

The term “subsequence” refers to a sequence of nucleic acids that comprises a part of a longer nucleic acid sequence. An exemplary
5 subsequence is a probe, described herein above, or a primer. The term "primer" as used herein refers to a contiguous sequence comprising in one embodiment about 8 or more deoxyribonucleotides or ribonucleotides, in another embodiment 10-20 nucleotides, and in still another embodiment 20-30 nucleotides of a selected nucleic acid molecule. The primers of the
10 invention encompass oligonucleotides of sufficient length and appropriate sequence so as to provide initiation of polymerization on a nucleic acid molecule of the present invention.

The term “elongated sequence” refers to a sequence comprising additional nucleotides (or other analogous molecules) incorporated into
15 and/or at either end of a nucleic acid. For example, a polymerase (*e.g.*, a DNA polymerase) can add sequences at the 3' terminus of a nucleic acid molecule. In addition, a nucleotide sequence can be combined with other DNA sequences, including, but not limited to promoters, promoter regions, enhancers, polyadenylation signals, intronic sequences, additional restriction
20 enzyme sites, multiple cloning sites, and other coding segments.

The term “complementary sequences”, as used herein, indicates two nucleotide sequences that comprise antiparallel nucleotide sequences capable of pairing with one another upon formation of hydrogen bonds between base pairs. As used herein, the term “complementary sequences”
25 means nucleotide sequences which are substantially complementary, as can be assessed by the same nucleotide comparison set forth above, or is defined as being capable of hybridizing to the nucleic acid segment in question under relatively stringent conditions such as those described herein. An example of a complementary nucleic acid segment is an
30 antisense oligonucleotide.

The term “gene” refers broadly to any segment of DNA associated with a biological function. A gene encompasses sequences including, but not limited to a coding sequence, a promoter region, a cis-regulatory

sequence, a non-expressed DNA segment that is a specific recognition sequence for regulatory proteins, a non-expressed DNA segment that contributes to gene expression, a DNA segment designed to have desired parameters, or combinations thereof. A gene can be obtained by a variety of methods, including, but not limited to cloning from a biological sample, synthesis based on known or predicted sequence information, and recombinant derivation of an existing sequence.

The present invention also encompasses chimeric genes comprising a nucleotide sequence encoding a soluble VEGF-R polypeptide. The term "chimeric gene", as used herein, refers to a gene comprising a heterologous promoter region operatively linked to a nucleotide sequence encoding a soluble VEGF-R polypeptide.

The term "operatively linked", as used herein, refers to a functional combination between a promoter region and a nucleotide sequence such that the transcription of the nucleotide sequence is controlled and regulated by the promoter region. Techniques for operatively linking a promoter region to a nucleotide sequence are known in the art.

The term "heterologous," as used herein to refer to a promoter or any other nucleic acid, refers to a sequence that originates from a source foreign to an intended host cell or, if from the same source, is modified from its original form. Thus, a heterologous nucleic acid in a host cell includes, but is not limited to a gene that is endogenous to the particular host cell but has been modified, for example by mutagenesis or by isolation from native cis-regulatory sequences. The term "heterologous nucleic acid" also includes non-naturally occurring multiple copies of a native nucleotide sequence. The term "heterologous nucleic acid" also encompasses a nucleic acid that is incorporated into a host cell's nucleic acids, however at a position wherein such nucleic acids are not ordinarily found. A representative heterologous nucleic acid comprises a recombinant nucleic acid, as described further herein below.

The term "recombinant" generally refers to an isolated nucleic acid that is replicable in a non-native environment. Thus, a recombinant nucleic acid can comprise a non-replicable nucleic acid in combination with

additional nucleic acids, for example vector nucleic acids, which enable its replication in a host cell.

The term "vector" is used herein to refer to a nucleic acid molecule having nucleotide sequences that enable its replication in a host cell. A vector can also include nucleotide sequences to permit ligation of nucleotide sequences within the vector, wherein such nucleotide sequences are also replicated in a host cell. Representative vectors include plasmids, cosmids, and viral vectors. A vector can also mediate recombinant production of a soluble VEGF-R polypeptide.

The term "construct", as used herein to describe an expression construct, refers to a vector further comprising a nucleotide sequence operatively inserted with the vector, such that the nucleotide sequence is expressed.

The terms "recombinantly expressed" or "recombinantly produced" are used interchangeably to generally refer to the process by which a polypeptide encoded by a recombinant nucleic acid is produced.

The term "heterologous expression system" refers to a host cell comprising a heterologous nucleic acid and the polypeptide encoded by the heterologous nucleic acid. For example, a heterologous expression system can comprise a host cell transfected with a construct comprising a recombinant nucleic acid, or a cell line produced by introduction of heterologous nucleic acids into a host cell genome.

Nucleic acids of the present invention can be cloned, synthesized, recombinantly altered, mutagenized, or combinations thereof. Standard recombinant DNA and molecular cloning techniques used to isolate nucleic acids are known in the art. Site-specific mutagenesis to create base pair changes, deletions, or small insertions are also known in the art as exemplified by publications. *See e.g.,* Sambrook & Russell, 2001; Silhavy *et al.*, 1984; Glover & Hames, 1995; and Ausubel, 1995.

Polypeptides. In one embodiment, the present invention provides an viral construct encoding soluble VEGF-R polypeptides. The present invention also provides a recombinantly expressed and isolated soluble VEGF-R polypeptide. A representative soluble VEGF-R polypeptide

comprises multiple immunoglobulin loops, but lacks a transmembrane domain and a kinase domain as present in native VEGF-R. Representative soluble VEGF-R polypeptides are set forth as any one of even-numbered SEQ ID NOs:2-8. A soluble VEGF-R2 polypeptides is set forth in one
5 embodiment as SEQ ID NO:2 and in another embodiment as SEQ ID NO:4.

The term "substantially identical", as used herein to describe a level of similarity between a soluble VEGF-R polypeptide and a protein substantially identical to a soluble VEGF-R polypeptide, refers to a sequence that is at least 35% identical to any one of even-numbered SEQ ID NOs:2-8, when
10 compared over the full length of a soluble VEGF-R protein. A protein substantially identical to soluble VEGF-R comprises an amino acid sequence that is in one embodiment at least about 35% to about 45% identical to any one of even-numbered SEQ ID NOs:2-8, in another embodiment at least about 45% to about 55% identical to any one of even-
15 numbered SEQ ID NOs:2-8, and in still another embodiment at least about 55% to about 65% identical to any one of even-numbered SEQ ID NOs:2-8 when compared over the full length of a soluble VEGF-R polypeptide. Methods for determining percent identity are defined herein below under the heading "Nucleotide and Amino Acid Sequence Comparisons."

20 Substantially identical polypeptides also encompass two or more polypeptides sharing a conserved three-dimensional structure. Computational methods can be used to compare structural representations, and structural models can be generated and easily tuned to identify similarities around important active sites or ligand binding sites. *See* Saqi *et*
25 *al.*, 1999; Barton, 1998; Henikoff *et al.*, 2000; and Huang *et al.*, 2000.

Substantially identical proteins also include proteins comprising amino acids that are functionally equivalent to amino acids of any one of even-numbered SEQ ID NOs:2-8. The term "functionally equivalent" in the context of amino acid sequences is known in the art and is based on the
30 relative similarity of the amino acid side-chain substituents. *See* Henikoff & Henikoff, 2000. Relevant factors for consideration include side-chain hydrophobicity, hydrophilicity, charge, and size. For example, arginine, lysine, and histidine are all positively charged residues; alanine, glycine, and

serine are all of similar size; and phenylalanine, tryptophan, and tyrosine all have a generally similar shape. By this analysis, described further herein below, arginine, lysine, and histidine; alanine, glycine, and serine; and phenylalanine, tryptophan, and tyrosine; are defined herein as biologically
5 functional equivalents.

In making biologically functional equivalent amino acid substitutions, the hydropathic index of amino acids can be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+ 4.5); valine (+ 4.2);
10 leucine (+ 3.8); phenylalanine (+ 2.8); cysteine (+ 2.5); methionine (+ 1.9); alanine (+ 1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

15 The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte & Doolittle, 1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon
20 the hydropathic index, amino acids whose hydropathic indices are within ±2 of the original value are substituted in one embodiment, those within ±1 of the original value are substituted in another embodiment, and those within ±0.5 of the original value are substituted in yet another embodiment.

It is also understood in the art that the substitution of like amino acids
25 can be made effectively on the basis of hydrophilicity. U.S. Patent No. 4,554,101 describes that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *e.g.*, with a biological property of the protein. It is understood that an amino acid can be
30 substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent protein.

As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+ 3.0); lysine

(+ 3.0); aspartate (+ 3.0±1); glutamate (+ 3.0±1); serine (+ 0.3); asparagine (+ 0.2); glutamine (+ 0.2); glycine (0); threonine (-0.4); proline (-0.5±1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); 5 tryptophan (-3.4).

In making changes based upon similar hydrophilicity values, amino acids whose hydrophilicity values are within ±2 of the original value are substituted in one embodiment, those which are within ±1 of the original value are substituted in another embodiment, and those within ±0.5 of the 10 original value are substituted in yet another embodiment.

The term "substantially identical" also encompasses polypeptides that are biologically functional equivalents. The term "functional" includes activity of a soluble VEGF-R polypeptide in inhibiting VEGF-R2 signaling and increasing radiosensitivity of a tumor, as described herein. Methods for 15 assessing a radiosensitizing function are described in the Examples.

The present invention also provides functional fragments of a soluble VEGF-R polypeptide. Such functional portion need not comprise all or substantially all of the amino acid sequence of an extracellular portion of a native VEGF-R gene product.

20 The present invention also includes functional polypeptide sequences that are longer sequences than that of an extracellular portion of a native soluble VEGF-R polypeptide. For example, one or more amino acids can be added to the N-terminus or C-terminus of a soluble VEGF-R polypeptide. Methods of preparing such proteins are known in the art.

25 Nucleotide and Amino Acid Sequence Comparisons. The terms "identical" or percent "identity" in the context of two or more nucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum 30 correspondence, as measured using one of the sequence comparison algorithms disclosed herein or by visual inspection.

The term "substantially identical" in regards to a nucleotide or polypeptide sequence means that a particular sequence varies from the

sequence of a naturally occurring sequence by one or more deletions, substitutions, or additions, the net effect of which is to retain biological activity of a gene, gene product, or sequence of interest.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer program, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are selected. The sequence comparison algorithm then calculates the percent sequence identity for the designated test sequence(s) relative to the reference sequence, based on the selected program parameters.

Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, 1981, by the homology alignment algorithm of Needleman & Wunsch, 1970, by the search for similarity method of Pearson & Lipman, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA, in the Wisconsin Genetics Software Package, available from Accelrys Inc., San Diego, California, United States of America), or by visual inspection. *See generally,* Ausubel, 1995.

In one embodiment, an algorithm for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described by Altschul *et al.*, 1990. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length **W** in the query sequence, which either match or satisfy some positive-valued threshold score **T** when aligned with a word of the same length in a database sequence. **T** is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters **M** (reward score

for a pair of matching residues; always > 0) and **N** (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by
5 the quantity **X** from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters **W**, **T**, and **X** determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as
10 defaults a wordlength **W** = 11, an expectation **E** = 10, a cutoff of 100, **M** = 5, **N** = -4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (**W**) of 3, an expectation (**E**) of 10, and the BLOSUM62 scoring matrix. *See* Henikoff & Henikoff, 1992.

In addition to calculating percent sequence identity, the BLAST
15 algorithm also performs a statistical analysis of the similarity between two sequences. *See e.g.*, Karlin & Altschul, 1993. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For
20 example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is in one embodiment less than about 0.1, in another embodiment less than about 0.01, and in still another embodiment less than about 0.001.

25 II.C. VEGF-R2 Antibodies

The present invention further provides a VEGF-R2 inhibitor comprising: (a) an antibody that specifically binds VEGF-R2; and (b) a carrier for sustained bioavailability of the antibody at a tumor. The disclosure herein reveals that a prolonged or sustained release of VEGF-R2
30 antagonist is required to optimize the therapeutic effect of combined VEGF-R2 antagonism and radiation.

The term “sustained bioavailability” is used herein to describe a composition comprising a VEGF-R2 inhibitor and a carrier, whereby the

bioavailability of a VEGF-R2 inhibitor at a tumor site is sufficient to achieve radiosensitization of a tumor. The term "sustained bioavailability" also refers to a bioavailability sufficient to inhibit blood vessel growth within the tumor. The term "sustained bioavailability" encompasses factors including but not limited to sustained release of a VEGF-R2 inhibitor from a carrier, metabolic stability of a VEGF-R2 inhibitor, systemic transport of a composition comprising a VEGF-R2 inhibitor, and effective dose of a VEGF-R2 inhibitor. Representative approaches for preparing a sustained bioavailability composition are described herein below under the heading "Carriers."

Kozin *et al.*, 2001 describes a decrease in the dose of radiation required to locally control 50% of human tumor xenografts by combination of radiotherapy with administration of an anti-VEGF-R2 antibody over a period of 6 months. In two tumor models, the combined effect of ionizing radiation and anti-VEGF-R2 antibody was not significantly different from additive. These studies did not include a carrier for sustained bioavailability of a VEGF-R2 antibody at the tumor site, and provision of the antibody failed to confer an increase in radiosensitivity of the tumor. In contrast, the present invention provides that a VEGF-R2 inhibitor comprising a VEGF-R2 antibody further comprises a carrier for sustained bioavailability. In one embodiment, a carrier for sustained bioavailability comprises a gene therapy construct encoding a VEGF-R2 antibody.

As disclosed herein, an immediate response of tumor blood vessels to radiation is a decrease in tumor blood flow. This response can diminish administration of an anti-tumor composition (*e.g.*, a VEGF-R2 inhibitor). Recognizing this response, the disclosure of the present invention provides that sustained bioavailability of a VEGF-R2 inhibitor, for example by selection of a carrier and administration regimen that achieve sustained bioavailability, can improve anti-tumor activity. In one embodiment, a carrier comprises a gene therapy vector encoding a VEGF-R2 inhibitor.

A method comprising a carrier or administration approach for sustained bioavailability can also improve therapies directed toward modulation of other components of the VEGF signaling pathway. For example, treatments that employed direct administration of an anti-VEGF

antibody (in the absence of a carrier or administration regimen for sustained bioavailability of the antibody at the tumor) showed variable anti-tumor efficacy in different tumor types (Gorski *et al.*, 1999; Lee *et al.*, 2000). In addition, therapeutic effects were not observed when sub-therapeutic or
5 minimally therapeutic doses of the anti-VEGF antibody were used. Thus, the present invention further provides an improved method for inhibiting tumor growth, the method comprising administration of a gene therapy vector encoding an inhibitor of VEGF signaling, whereby bioavailability of the inhibitor at a tumor is sustained, and whereby tumor growth delay is
10 improved.

The term "antibody" indicates an immunoglobulin protein, or functional portion thereof, including, but not limited to a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a hybrid antibody, a single chain antibody (*e.g.*, a single chain antibody represented in a phage library), a
15 mutagenized antibody, a humanized antibody, and antibody fragments that comprise an antigen binding site (*e.g.,* Fab and Fv antibody fragments). In one embodiment, an antibody of the invention is a monoclonal antibody.

Techniques for preparing and characterizing antibodies are known in the art. *See e.g.*, Harlow & Lane, 1988 and U.S. Patent Nos. 4,196,265;
20 4,946,778; 5,091,513; 5,132,405; 5,260,203; 5,677,427; 5,892,019; 5,985,279; 6,054,561). Single chain antibodies can be identified by screening a phage antibody library, for example as described by U.S. Patent Nos. 6,174,708; 6,057,098; 5,922,254; 5,840,479; 5,780,225; 5,702,892; and 5,667,988.

25 An antibody of the invention can further be mutagenized or otherwise modified to preferably improve antigen binding and/or antibody stability. For example, to prevent undesirable disulfide bond formation, a nucleotide sequence encoding the variable domain of an antibody or antibody fragment can be modified to eliminate at least one of each pair of codons that encode
30 cysteines for disulfide bond formation. Recombinant expression of the modified nucleotide sequence, for example in a prokaryotic expression system, results in an antibody having improved stability. *See* U.S. Patent No. 5,854,027.

III. Therapeutic Compositions

In accordance with the methods of the present invention, a composition that is administered to increase the radiosensitivity of a tumor in a subject comprises: (a) a VEGF-R2 inhibitor; and (b) a pharmaceutically acceptable
5 carrier. Any suitable carrier that facilitates drug preparation and/or administration can be used.

III.A. Carriers

The carrier can be a viral vector or a non-viral vector. Suitable viral vectors include, but are not limited to adenoviruses, adeno-associated
10 viruses (AAVs), retroviruses, pseudotyped retroviruses, herpes viruses, vaccinia viruses, Semiliki forest virus, and baculoviruses. In one embodiment of the invention, the carrier comprises an adenoviral gene therapy construct that encodes a VEGF-R2 inhibitor.

Suitable non-viral vectors that can be used to deliver a VEGF-R2
15 inhibitor include, but are not limited to a plasmid, a nanosphere (Manome *et al.*, 1994; Saltzman & Fung, 1997), a peptide (U.S. Patent Nos. 6,127,339 and 5,574,172), a glycosaminoglycan (U.S. Patent No. 6,106,866), a fatty acid (U.S. Patent No. 5,994,392), a fatty emulsion (U.S. Patent No. 5,651,991), a lipid or lipid derivative (U.S. Patent No. 5,786,387), collagen
20 (U.S. Patent No. 5,922,356), a polysaccharide or derivative thereof (U.S. Patent No. 5,688,931), a nanosuspension (U.S. Patent No. 5,858,410), a polymeric micelle or conjugate (Goldman *et al.*, 1997 and U.S. Patent Nos. 4,551,482, 5,714,166, 5,510,103, 5,490,840, and 5,855,900), and a polysome (U.S. Patent No. 5,922,545).

25 Where appropriate, two or more types of carriers can be used together. For example, a plasmid vector can be used in conjunction with liposomes. One embodiment of the present invention envisions the use of an adenovirus.

In one embodiment, a composition of the invention comprises a
30 VEGF-R2 inhibitor and a carrier to effect sustained bioavailability of the VEGF-R2 inhibitor following administration to a tumor-bearing subject. The term "sustained bioavailability" is used herein to refer to a bioavailability of a VEGF-R2 inhibitor sufficient to achieve radiosensitization of a tumor. The

term "sustained bioavailability" also refers to a bioavailability of a VEGF-R2 inhibitor sufficient to inhibit blood vessel growth within a tumor. The term "sustained bioavailability" encompasses factors including, but not limited to prolonged release of a VEGF-R2 inhibitor from a carrier, metabolic stability of a VEGF-R2 inhibitor, systemic transport of a composition comprising a VEGF-R2 inhibitor, and effective dose of a VEGF-R2 inhibitor.

Representative compositions for sustained bioavailability of a VEGF-R2 inhibitor include, but are not limited to polymer matrices, including swelling and biodegradable polymer matrices, (U.S. Patent Nos. 6,335,035; 6,312,713; 6,296,842; 6,287,587; 6,267,981; 6,262,127; and 6,221,958), polymer-coated microparticles (U.S. Patent Nos. 6,120,787 and 6,090,925) a polyol:oil suspension (U.S. Patent No. 6,245,740), porous particles (U.S. Patent No. 6,238,705), latex/wax coated granules (U.S. Patent No. 6,238,704), chitosan microcapsules, and microsphere emulsions (U.S. Patent No. 6,190,700).

In one embodiment, a composition for sustained bioavailability of a VEGF-R2 inhibitor comprises a gene therapy construct comprising a gene therapy vectors, for example a gene therapy vector described herein below.

Viral Gene Therapy Vectors. In one embodiment, viral vectors of the invention are disabled, *e.g.* replication-deficient. That is, they lack one or more functional genes required for their replication, which prevents their uncontrolled replication *in vivo* and avoids undesirable side effects of viral infection. In one embodiment, all of the viral genome is removed except for the minimum genomic elements required to package the viral genome incorporating the therapeutic gene into the viral coat or capsid. For example, it is desirable to delete all the viral genome except: (a) the Long Terminal Repeats (LTRs) or Invented Terminal Repeats (ITRs); and (b) a packaging signal. In the case of adenoviruses, deletions are typically made in the E1 region and optionally in one or more of the E2, E3, and/or E4 regions. Other viral vectors can be similarly deleted of genes required for replication. Deletion of sequences can be achieved by recombinant means, for example, involving digestion with appropriate restriction enzymes,

followed by religation. Replication-competent self-limiting or self-destructing viral vectors can also be used.

Nucleic acid constructs of the invention can be incorporated into viral genomes by any suitable means known in the art. Typically, such incorporation is performed by ligating the construct into an appropriate restriction site in the genome of the virus. Viral genomes can then be packaged into viral coats or capsids using any suitable procedure. In particular, any suitable packaging cell line can be used to generate viral vectors of the invention. These packaging lines complement the replication-deficient viral genomes of the invention, as they include, for example by incorporation into their genomes, the genes which have been deleted from the replication-deficient genome. Thus, the use of packaging lines allows viral vectors of the invention to be generated in culture.

Suitable packaging lines for retroviruses include derivatives of PA317 cells, ψ-2 cells, CRE cells, CRIP cells, E-86-GP cells, and 293GP cells. In one embodiment, 293 cells are employed for use with adenoviruses and adeno-associated viruses.

Plasmid Gene Therapy Vectors. A soluble VEGF-R receptor can also be encoded by a plasmid. Advantages of a plasmid carrier include low toxicity and easy large-scale production. A polymer-coated plasmid can be delivered using electroporation as described by Fewell *et al.*, 2001. Alternatively, a plasmid can be combined with an additional carrier, for example a cationic polyamine, a dendrimer, or a lipid, that facilitates delivery. *See e.g.*, Baher *et al.*, 1999; Maruyama-Tabata *et al.*, 2000; and Tam *et al.*, 2000.

Liposomes. A VEGF-R2 inhibitor of the present invention can also be delivered using a liposome. For example, a recombinantly produced soluble VEGF-R polypeptide can be encapsulated in liposomes. Liposomes can be prepared by any of a variety of techniques that are known in the art. *See e.g.*, Dracopoli *et al.*, 1994; Lasic & Martin, 1995; Janoff, 1999; Gregoriadis, 1993; Betageri *et al.*, 1993; and U.S. Patent Nos. 4,235,871; 4,551,482; 6,197,333; and 6,132,766. Temperature-sensitive liposomes can also be used, for example THERMOSOMES™ as disclosed in U.S. Patent No.

6,200,598. Entrapment of a VEGF-R2 inhibitor within liposomes of the present invention can be carried out using any conventional method in the art. In preparing liposome compositions, stabilizers such as antioxidants and other additives can be used.

5 Other lipid carriers can also be used in accordance with the claimed invention, such as lipid microparticles, micelles, lipid suspensions, and lipid emulsions. *See e.g.*, Labat-Moleur *et al.*, 1996; and U.S. Patent Nos. 5,011,634; 6,056,938; 6,217,886; 5,948,767; and 6,210,707.

III.B. Targeting Ligands

10 As desired, a composition of the invention can include one or more ligands having affinity for a specific cellular marker to thereby enhance delivery of a VEGF-R2 inhibitor to a tumor *in vivo*. Ligands include, but are not limited to antibodies, cell surface markers, peptides, and the like, which act to home the VEGF-R2 inibitor to a tumor, including the tumor 15 vasculature.

The terms "targeting" and "homing", as used herein to describe the *in vivo* activity of a ligand following administration to a subject, each refer to the preferential movement and/or accumulation of a ligand in a target tissue (*e.g.*, a tumor) as compared with a control tissue.

20 The term "target tissue" as used herein refers to an intended site for accumulation of a ligand following administration to a subject. For example, the methods of the present invention employ a target tissue comprising a tumor.

The term "control tissue" as used herein refers to a site suspected to 25 substantially lack binding and/or accumulation of an administered ligand. For example, in accordance with the methods of the present invention, a non-cancerous tissue is a control tissue.

The terms "selective targeting" of "selective homing" as used herein each refer to a preferential localization of a ligand that results in an amount 30 of ligand in a target tissue that is in one embodiment about 2-fold greater than an amount of ligand in a control tissue, in another embodiment an amount that is about 5-fold or greater, and in still another embodiment an amount that is about 10-fold or greater. The terms "selective targeting" and

"selective homing" also refer to binding or accumulation of a ligand in a target tissue concomitant with an absence of targeting to a control tissue, in one embodiment the absence of targeting to all control tissues.

The terms "targeting ligand" and "targeting molecule" as used herein each refer to a ligand that displays targeting activity. In one embodiment, a targeting ligand displays selective targeting. Representative targeting ligands include peptides and antibodies.

The term "peptide" encompasses any of a variety of forms of peptide derivatives, that include, but are not limited to amides, conjugates with proteins, cyclized peptides, polymerized peptides, conservatively substituted variants, analogs, fragments, peptoids, chemically modified peptides, and peptide mimetics. Representative peptide ligands that show tumor-binding activity include, for example, those described in U.S. Patent Nos. 6,180,084 and 6,296,832.

The term "antibody" indicates an immunoglobulin protein, or functional portion thereof, including a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a hybrid antibody, a single chain antibody (*e.g.*, a single chain antibody represented in a phage library), a mutagenized antibody, a humanized antibody, and antibody fragments that comprise an antigen binding site (*e.g.,* Fab and Fv antibody fragments). Representative antibody ligands that can be used in accordance with the methods of the present invention include antibodies that bind the tumor-specific antigens Her2/neu (v-erb-b2 avian erythroblastic leukemia viral oncogene homologue 2; Kirpotin *et al.*, 1997; Becerril *et al.*, 1999) and antibodies that bind to CEA (carcinoembryonic antigen; Ito *et al.*, 1991). *See also* U.S. Patent Nos. 5,111,867; 5,632,991; 5,849,877; 5,948,647; 6,054,561 and PCT International Publication No. WO 98/10795.

In an effort to identify ligands that are capable of targeting to multiple tumor types, targeting ligands have been developed that bind to target molecules present on tumor vasculature (Baillie *et al.*, 1995; Pasqualini & Ruoslahti, 1996; Arap *et al.*, 1998; Burg *et al.*, 1999; Ellerby *et al.*, 1999).

A targeting ligand can also comprise a ligand that specifically binds to a radiation induced target molecule. Ionizing radiation induces proteins in

tumor vascular endothelium through transcriptional induction and/or posttranslational modification of cell adhesion molecules such as integrins (Hallahan *et al.*, 1995; Hallahan *et al.*, 1996; Hallahan *et al.*, 1998; Hallahan & Virudachalam, 1999). For example, radiation induces activation of the
5 integrin $\alpha_{2b}\beta_3$, also called the fibrinogen receptor, on platelets. The induced molecules can serve as binding sites for targeting ligands. A representative peptide ligand that binds to irradiated tumors comprises Biapcitide (ACUTECT® available from Diatide, Inc., Londonberry, New Hampshire, United States of America), which specifically binds to GP-IIb/IIIa receptors
10 on activated platelets (Hawiger *et al.*, 1989; Hawiger & Timmons, 1992; Hallahan *et al.*, 2001).

Antibodies, peptides, or other ligands can be coupled to drugs (*e.g.*, a VEGF-R2 inhibitor) or drug carriers using methods known in the art, including but not limited to carbodiimide conjugation, esterification, sodium
15 periodate oxidation followed by reductive alkylation, and glutaraldehyde crosslinking. *See e.g.*, Bauminger & Wilchek, 1980; Goldman *et al.*, 1997; Kirpotin *et al.*, 1997; Dracopoli *et al.*, 1994; Neri *et al.*, 1997; Park *et al.*, 1997; and Pasqualini *et al.*, 1997; U.S. Patent No. 6,071,890; and European Patent No. 0 439 095. Alternatively, pseudotyping of a retrovirus can be
20 used to target a virus towards a particular cell (Marin *et al.*, 1997).

III.C. Formulation

In one embodiment, a composition of the present invention comprises a VEGF-R2 inhibitor and a pharmaceutically acceptable carrier. Suitable formulations include aqueous and non-aqueous sterile injection solutions
25 which can contain anti-oxidants, buffers, bacteriostats, bactericidal antibiotics and solutes which render the formulation isotonic with the bodily fluids of the intended recipient; and aqueous and non-aqueous sterile suspensions which can include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers, for
30 example sealed ampoules and vials, and can be stored in a frozen or freeze-dried (lyophilized) condition requiring only the addition of sterile liquid carrier, for example water for injections, immediately prior to use. Some exemplary ingredients are SDS, for example in the range of in one embodiment about

0.1 to 10 mg/ml, in another embodiment about 2.0 mg/ml; and/or mannitol or another sugar, for example in the range of in one embodiment 10 to 100 mg/ml, in another embodiment about 30 mg/ml; phosphate-buffered saline (PBS), and any other formulation agents conventional in the art.

5 The therapeutic regimens and pharmaceutical compositions of the invention can be used with additional adjuvants or biological response modifiers including, but not limited to, the cytokines interferon alpha (IFN-α), interferon gamma (IFN-γ), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), tumor necrosis factor (TNF), or other cytokine affecting immune 10 cells.

IV. Therapeutic Methods

The disclosed VEGF-R2 inhibitors are useful as radiosensitizing agents. Thus, the present invention provides a method for treating a tumor comprising: (a) administering a VEGF-R2 inhibitor to a subject bearing a 15 tumor; whereby the radiosensitivity of the tumor is increased; and (b) treating the tumor with ionizing radiation, whereby tumor growth is delayed. Also provided is a method for inhibiting tumor blood vessel growth and/or delaying tumor growth via administration of a VEGF-R2 inhibitor. In one embodiment, the performance of the method also promotes tumor 20 regression.

While applicants do not intend to be bound by any particular theory of operation, a VEGF-R2 inhibitor may effectively delay tumor growth by blocking reprofusion of an irradiated tumor. Specifically, a VEGF-R2 inhibitor can block processes that require VEGF, including endothelial cell 25 infiltration and budding of tumor blood vessels.

IV.A. Administration of a VEGF-R2 Inhibitor

Suitable methods for administration of a composition of the present invention include but are not limited to intravascular, subcutaneous, or intratumoral administration. For delivery of compositions to pulmonary 30 pathways, compositions can be administered as an aerosol or coarse spray. A delivery method is selected based on considerations including, but not limited to the type of VEGF-R2 inhibitor, the type of carrier or vector, toxicity

of the VEGF-R2 inhibitor, therapeutic efficacy of the VEGF-R2 inhibitor, and the condition of the tumor to be treated. In one embodiment of the invention, intravascular administration is employed.

In one embodiment, a therapeutically effective amount of a composition of the invention is administered to a subject. A "therapeutically effective amount" is an amount of a composition comprising a VEGF-R2 inhibitor sufficient to produce a measurable anti-tumor response (*e.g.*, an anti-angiogenic response, a cytotoxic response, and/or tumor regression). Actual dosage levels of active ingredients in a therapeutic composition of the invention can be varied so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular subject. The selected dosage level will depend upon a variety of factors including the activity of the therapeutic composition, formulation, the route of administration, combination with other drugs or treatments, tumor size and longevity, and the physical condition and prior medical history of the subject being treated. Determination and adjustment of a therapeutically effective dose, as well as evaluation of when and how to make such adjustments, are known to those of ordinary skill in the art of medicine.

In one embodiment of the invention, a minimally therapeutic dose of a VEGF-R2 inhibitor is administered. The term "minimally therapeutic dose" refers to the smallest dose, or smallest range of doses, determined to be a therapeutically effective amount as defined herein above.

For local administration of viral vectors, previous clinical studies have demonstrated that up to 10^{13} pfu of virus can be injected with minimal toxicity. In human patients, 1×10^{9} – 1×10^{13} pfu are routinely used. *See* Habib *et al.*, 1999. To determine an appropriate dose within this range, preliminary treatments can begin with 1×10^{9} pfu, and the dose level can be escalated in the absence of dose-limiting toxicity. Toxicity can be assessed using criteria set forth by the National Cancer Institute and is reasonably defined as any grade 4 toxicity or any grade 3 toxicity persisting more than 1 week. Dose is also modified to maximize anti-tumor and/or anti-angiogenic activity. Representative criteria and methods for assessing anti-tumor and/or anti-angiogenic activity are described herein below.

For small molecule VEGF-R2 inhibitors and soluble formulations of VEGF-R2 inhibitors, conventional methods of extrapolating human dosage based on doses administered to a murine animal model can be carried out using the conversion factor for converting the mouse dosage to human dosage: Dose Human per kg = Dose Mouse per kg × 12 (Freireich *et al.*, 1966). Drug doses are also given in milligrams per square meter of body surface area because this method rather than body weight achieves a good correlation to certain metabolic and excretionary functions. Moreover, body surface area can be used as a common denominator for drug dosage in adults and children as well as in different animal species as described by Freireich *et al.*, 1966. Briefly, to express a mg/kg dose in any given species as the equivalent mg/m^2 dose, the dose is multiplied by the appropriate km factor. In adult humans, 100 mg/kg is equivalent to 100 mg/kg × 37 kg/m^2 = 3700 mg/m^2.

Additional formulation and dose techniques have been described in the art, see for example, those described in U.S. Patent Nos. 5,326,902 and 5,234,933, and PCT International Publication No. WO 93/25521.

IV.B. Radiation Treatment

For treatment of a radiosensitized tumor, the tumor is irradiated concurrent with, or subsequent to, administration of a composition comprising a VEGF-R2 receptor. In one embodiment of the present invention, the tumor is irradiated daily for 2 weeks to 7 weeks (for a total of 10 treatments to 35 treatments). Alternatively, tumors can be irradiated with brachytherapy utilizing high dose rate or low dose rate brachytherapy internal emitters.

The duration for administration of a VEGF-R2 inhibitor comprises in one embodiment a period of several months coincident with radiotherapy, but in another embodiment can extend to a period of 1 year to 3 years as needed to effect tumor control. A VEGF-R2 inhibitor comprising a small compound (*e.g.*, SU5416) can be administered about one hour before each fraction of radiation. A gene therapy vector encoding a VEGF-R2 inhibitor (*e.g.*, Ad.ExFlk) can be administered prior to an initial radiation treatment and then at desired intervals during the course of radiation treatment (*e.g.*,

weekly, monthly, or as required). An initial administration of a VEGF-R2 inhibitor comprising a gene therapy vector or a sustained release drug carrier can comprise administering the VEGF-R2 inhibitor to a tumor during placement of a brachytherapy afterloading device.

5 Subtherapeutic or therapeutic doses of radiation can be used for treatment of a radiosensitized tumor as disclosed herein. In one embodiment, a subtherapeutic or minimally therapeutic dose (when administered alone) of ionizing radiation is used. For example, the dose of radiation can comprise in one embodiment at least about 2 Gy ionizing 10 radiation, in another embodiment about 2 Gy to about 6 Gy ionizing radiation, and in still another embodiment about 2 Gy to about 3 Gy ionizing radiation. When radiosurgery is used, representative doses of radiation include about 10 Gy to about 20 Gy administered as a single dose during radiosurgery or about 7 Gy administered daily for 3 days (about 21 Gy total). 15 When high dose rate brachytherapy is used, a representative radiation dose comprises about 7 Gy daily for 3 days (about 21 Gy total). For low dose rate brachytherapy, radiation doses typically comprise about 12 Gy administered twice over the course of 1 month. ^{125}I seeds can be implanted into a tumor can be used to deliver very high doses of about 110 Gy to about 140 Gy in a 20 single administration.

Radiation can be localized to a tumor using conformal irradiation, brachytherapy, stereotactic irradiation, or intensity modulated radiation therapy (IMRT). The threshold dose for treatment can thereby be exceeded in the target tissue but avoided in surrounding normal tissues. For treatment 25 of a subject having two or more tumors, local irradiation enables differential drug administration and/or radiotherapy at each of the two or more tumors. Alternatively, whole body irradiation can be used, as permitted by the low doses of radiation required following radiosensitization of the tumor.

Radiation can also comprise administration of internal emitters, for 30 example ^{131}I for treatment of thyroid cancer, NETASTRON™ and QUADRAGEN® pharmaceutical compositions (Cytogen Corp., Princeton, New Jersey, United States of America) for treatment of bone metastases, ^{32}P for treatment of ovarian cancer. Other internal emitters include ^{125}I,

iridium, and cesium. Internal emitters can be encapsulated for administration or can be loaded into a brachytherapy device.

Radiotherapy methods suitable for use in the practice of this invention can be found in Leibel & Phillips, 1998, among other sources.

5

Examples

The following Examples have been included to illustrate modes of the invention. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated by the present inventor to work well in the practice of the invention. These Examples illustrate 10 standard laboratory practices of the inventor. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the invention.

15

Example 1

Tumor Models

The B16F0 cell line was obtained from American Type Culture Collection (ATCC; Manassas, Virginia, United States of America). B16F0 cells were maintained in HYCLONE® minimal essential culture medium 20 (Hyclone Laboratories, Logan, Utah, United States of America), supplemented with 10% fetal calf serum and 0.5% penicillin-streptomycin. GL261 cells were maintained in a 1:1 mixture of Dulbecco's Modified Eagle Medium (DMEM) and F-12 nutrient mixture 1:1, supplemented with 7% fetal calf serum, 0.5% penicillin/streptomycin, and 1% sodium pyruvate. 25 HYCLONE® culture medium was purchased from VWR, West Chester, Pennsylvania, United States of America. DMEM, F-12 nutrient mixture, fetal calf serum, and penicillin/streptomycin were purchased from GIBCO™ Invitrogen Corporation of Carlsbad, California, United States of America.

The GL261 cell line was obtained from Yancie Gillespie (University of 30 Alabama, Birmingham, Alabama, United States of America) and is described by Hallahan *et al.*, 1998; Staba *et al.*, 1998; and Baumann *et al.*, 1992. GL261 cells form tumors in C57BL6J mice following subcutaneous injection

into hind limb (Hallahan *et al.*, 1998) and into a dorsal skin fold window chamber. Cells were trypsinized and counted using a hemocytometer. Cells were washed in complete medium and 10^6 cells were injected subcutaneously into the hind limb or into the dorsal skin fold window.

5 The GL216 glioblastoma model shows high levels of VEGF expression and radiation resistance *in vivo*. *See* Gorski *et al.*, 1999.

Example 2

Tumor Vascular Window Model

Penicillin/streptomycin solution (200 µl; available from GIBCO™ 10 Invitrogen Corporation, Carlsbad, California, United States of America) was injected into the hind limb of the mouse prior to preparation of the window chamber. The dorsal ventral window chamber was prepared using a 3-gram plastic frame, which was applied to the skin of a test animal and remained attached for the duration of the study. Briefly, the chamber was screwed 15 together, and the epidermis was cut and left open with a plastic covering.

To prepare a test animal for application of the plastic frame, the dorsal midline was found along the animal's back, and a clip was placed to hold the skin in position. A template, equivalent to the outer diameter of the chamber was traced, producing the outline of the incision. A circular cut was 20 made tracing the perimeter of the outline (a circular area about 7 mm in diameter) followed by a crisscross cut through the center of the circular area, thus producing four skin flaps. The epidermis of the four flaps was removed using a scalpel with an effort to follow the hypodermis superior to the fascia. The area was then trimmed and manicured with a pair of fine forceps and iris 25 scissors. During surgery, the area was kept moist by applying moist drops of phosphate buffered saline with 1% penicillin/streptomycin solution.

The template was removed and the top piece of the chamber was fixed with screws. The bottom portion of the chamber was put in place and the top portion of the chamber was carefully positioned on the cut side, so 30 that the window and the circular incision were fitted. Antibiotic ointment was applied to keep the area clear of infection. The three screws that hold the

chamber together, were then put into the chamber holes and tightened so that the skin was not pinched, thus avoiding diminished circulation.

GL261 and B16F0 cells were implanted into the dorsal skin fold window chamber. Blood vessels developed over 1 week. Tumor vasculature was observed in response to various treatments as described below. To document changes in tumor blood vessels, the window frame was marked with coordinates, which were used to photograph the same microscopic field each day beginning at day 0 through day 7. Vascular windows were photographed using a 4X objective to obtain a 40X total magnification. Color slides were scanned into PHOTOSHOP® software (Adobe Systems, Inc., San Jose, California, United States of America). Scanned slides were analyzed using OPTIMAS® image analysis software (Bioscan, Inc., Edmonds, Washington, United States of America; available from Media Cybernetics, Silver Spring, Maryland, United States of America) or IMAGE-PRO® software (Media Cybernetics, Silver Spring, Maryland, United States of America) as described below.

Vascular center lines were positioned by OPTIMAS® software or IMAGE-PRO® software and verified by an observer blinded to the treatment groups. Tumor blood vessels were quantified using OPTIMAS® software, which quantified the vascular length density of blood vessel within the microscopic field. The mean vascular length density and the standard error of the mean were calculated for each treatment group. Variance was analyzed using the Kruskal-Wallis method. *See* Kruskal & Wallis, 1952 and Hallahan *et al.*, 1995.

The vascular window model allowed direct measurement of the vascular response to ionizing radiation. Window models were prepared using the GL261 and B16F0 tumor cell lines. Animals were treated with 2 Gy ionizing radiation, 3 Gy ionizing radiation, or 6 Gy ionizing radiation. Photographs of blood vessels taken prior to and each day subsequent to radiation treatment were used for analysis.

A dose- and time-dependent reduction of tumor vasculature was observed in response to radiation treatment. B16F0 tumor blood vessels showed a slight increase in vascular length density following treatment with

2 Gy ionizing radiation. Treatment with 3 Gy reduced tumor vascular length density within 48 hours (probability $p<0.05$), and 6 Gy eliminated vasculature within 24 hours. Blood vessels within the GL261 glioma showed increased vascular length density in response to 3 Gy ionizing radiation, and only a minimal reduction in vascular length density in response to 6 Gy ionizing radiation ($p=0.04$, measured at 72 hours following radiation treatment). Minimal variation in radiation response was observed among members within a treatment group. *See* Figure 1.

Example 3

Power Doppler Sonography

Power Doppler sonography was used to study the response of tumor blood vessels to ionizing radiation. This method utilizes amplitude to measure flow in microvasculature. Tumors were imaged with a 10-5 MHz ENTOS® linear probe (Advanced Technology Laboratories, Inc., Bothell, Washington, United States of America) attached to an HDI® 5000 diagnostic ultrasound system (Advanced Technology Laboratories, Inc., Bothell, Washington, United States of America).

Power Doppler sonography images were obtained with the power gain set to 82%. Care was taken to minimize motion artifact. A 20-frame cineloop sweep of the entire tumor was obtained with the probe perpendicular to the long axis of the lower extremity along the entire length of the tumor.

The images were analyzed using HDI® software (Advanced Technology Laboratories, Inc., Bothell, Washington, United States of America). This software allows direct evaluation of Power Doppler cineloop raw data. Color area was recorded for the entire tumor. Five mice were included in each treatment group. Values for color area were averaged for each tumor set and treated groups were compared to controls using the unpaired student t-test.

B16F0 melanoma and GL261 glioma tumors were implanted in the hind limb of C57BL6J mice. Tumors were grown to a diameter of about 0.7 cm to about 1.1 cm and then irradiated with 2 Gy ionizing radiation, 3 Gy ionizing radiation, 6 Gy ionizing radiation, or 10 Gy ionizing radiation. Power

Doppler analysis of tumor blood flow was measured on day 0, day 3, and day 7 after radiation treatment. *See* Figure 2.

Blood flow increased following low dose irradiation (3 Gy), and blood flow decreased following high dose irradiation. The flow in each pixel (Power weighted pixel density) was summed to compare dose-dependent and tumor-dependent changes in tumor blood flow over time. Blood flow throughout the entire tumor was measured after treatment with 6 Gy ionizing radiation. Blood flow decreased by day 3 ($p=0.045$). At day 7, blood flow in B16F0 tumors was recovered, and blood flow in GL261 tumors was greater than prior to treatment.

Example 4

VEGF-R2 Inhibitors

Flk-1 is a receptor tyrosine kinase that is specifically inhibited by the dominant negative function of a soluble and truncated form of the receptor, which is called soluble Flk-1 receptor. A soluble Flk-1 receptor was constructed by fusing a sequence encoding the extracellular domain of murine Flk-1 to a sequence encoding a 6-histidine tag at the carboxyl terminus. An adenoviral construct encoding the fusion protein is referred to as "Ad.ExFlk.6His," and the encoded fusion protein is referred to as "ExFlk.6His."

ExFlk.6His blocks activation of Flk-1 and formed heterodimers with endogenous cell surface Flk-1 in the presence of VEGF. ExFlk.6His also inhibits VEGF-induced DNA synthesis and migration in HUVEC. *See* Lin *et al.*, 1998.

SU5416 is a potent and selective inhibitor of Flk-1/KDR that blocks tyrosine kinase catalysis and inhibits tumor vascularization and growth (Fong *et al.*, 1999; Shaheen *et al.*, 1999; Vajkoczy *et al.*, 1999). The chemical formula is 3-[2,4-dimethyl pyrrol-5-yl) methyl idenyl]-2-indolinone, and the compound has an inhibition constant (K_i) of 0.16 μM for Flk-1. The plasma half-life of SU5416 is relatively short (1-2 hours), but the biological effectiveness of this agent is durable and capable to inhibit VEGF-R2 for greater than 3 days. Thus, for combined Flk-1 inhibition and radiation

treatments (Examples 5 and 7), SU5416 was administered at a frequency less than the standard radiation course.

Example 5

Flk-1 Antagonists Eliminate Radioresistance

5 To determine whether the Flk-1 receptor inhibition alters the radiation response in tumor blood vessels, window model analysis and Power Doppler blood flow analysis were performed in animals treated with VEGF-R2 inhibitors followed by radiotherapy.

To determine whether the Flk-1 receptor inhibition alters the radiation 10 response in tumor blood vessels, GL261 and B16F0 tumors were established in C57BL6 mice, and vascular length density and blood flow were studied following administration of a Flk-1 inhibitor in combination with radiotherapy. Ad.ExFlk.6His and control construct Ad.LacZ were administered to tumor bearing mice at a minimally effective dose of $2x10^8$ 15 pfu by tail vein injection, and a subtherapeutic dose of radiation (2 Gy) was administered 24 hours later. To determine whether Flk-1 kinase inhibitors enhance radiation cytotoxicity, a minimal effective dose of SU5416 (0.75 mg) was administered in combination with a subtherapeutic dose of radiation (2 Gy).

20 Following treatment of animals by administration of Ad.ExFlk in combination with radiotherapy, vascular regression was studied as described in Example 2. No vascular regression was achieved following treatment with 2 Gy and control construct Ad.lacZ. GL261 and B16F0 tumors treated with 2 Gy alone showed no vascular response to radiation. 25 Administration of Ad.ExFlk.6His at $2x10^8$ pfu, in the absence of radiation treatment, achieved minimal regression of tumor blood vessels. When Ad.ExFlk.6His was administered 16 hours prior to exposure to 2 Gy ionizing radiation, tumor blood vessels regressed at a rate typically observed following treatment with 6 Gy. Enhanced anti-angiogenic effects following 30 combined Ad.ExFlk.6His and radiation treatments were observed in both tumor models. *See* Figure 3A.

Similarly, administration of 0.75 mg SU5416, in the absence of ionizing radiation, resulted in a minimal regression of the tumor blood

vessels and rapid restoration of vasculature. SU5416 administered 1 hour prior to treatment with 2 Gy ionizing radiation completely destroyed tumor blood vessels within 24 hours of treatment (p=0.02, as compared to radiation alone). Enhanced anti-angiogenic effects following combined SU5416 and 5 radiation treatments were observed in both tumor models. *See* Figure 3B.

To determine whether inhibition of Flk-1-mediated signal transduction also enhances the cytotoxic effects of radiotherapy in established tumors, Power Doppler sonography was used to quantify blood flow in 1 cm diameter tumors treated with Flk-1 inhibitors and ionizing radiation. Tumor bearing 10 mice were treated with a minimally effective dose of SU5416 (0.75 mg) administered by intraperitoneal injection at 1 hour prior to administration of 2 Gy ionizing radiation. SU5416 was injected every other fraction for a total of 3 doses. Radiation was delivered in fraction doses of 3 Gy per day for 7 days. Blood flow was quantified by Power Doppler sonography on days 4 15 and 7 according to methods described in Example 3. Treatment with radiation alone and SU5416 alone achieved minimal reduction in GL261 and B16F0 tumor blood flow. Tumors treated with SU5416 one hour prior to irradiation showed a significant reduction in tumor blood flow as compared to either treatment alone (p=0.004), consistent with observations of enhanced 20 effect of combined treatment when using the window model. *See* Figure 4.

Example 6

Flk-1 Inhibition Abrogates Endothelial Cell Survival

VEGF prolongs the survival of human endothelial cells, which is associated with increased expression of the anti-apoptotic protein Bcl-2 25 (Gerber *et al.*, 1998; Nor *et al.*, 1999). Enhanced endothelial cell survival was associated with a dose-dependent increase in Bcl-2 expression and decrease in expression of the processed forms of caspase-3 (Nor *et al.*, 1999). Over-expression of Bcl-2 in HUVEC prevented apoptotic cell death in the absence of VEGF (Gerber *et al.*, 1998). Although it is not applicant's 30 intention to be bound by any particular theory of operation, Flk-1 inhibitors appear to modify a subsequent response to radiotherapy at least in part, although not necessarily exclusively, via regulation of apoptosis.

To determine whether Flk-1 inhibitors enhance radiation-induced intrinsic cell death mechanisms, radiation-induced apoptosis in HUVEC human primary culture endothelial cells, 3B11 murine endothelial cells, and human microdermal endothelial cells (HMEC) was studied following Flk-1 inhibition. HMEC cells and 3B11 cells were treated with 50 μm SU5416 or with 10^8 pfu Ad.ExFlk or control vector Ad.LacZ. Cells were then treated with 6 Gy of ionizing radiation. Cultures were stained to reveal apoptotic cells 24 hours later. Endothelial cell death was quantified by counting the number of apoptotic bodies in 100 cells.

Endothelial cells showed minimal radiation-induced apoptosis in complete medium. When soluble Flk-1 receptor (Ad.ExFlk) or SU5416 were included in the medium, endothelial cells showed radiation-induced apoptosis. The percentage of HUVEC undergoing apoptosis following exposure to ionizing radiation was 2%. No apoptotic cells were observed in the population of control cells not exposed to ionizing radiation. Cells exposed to SU5416 or to Ad.ExFlk, but not exposed to ionizing radiation, induced apoptosis in 4% of cells and 5% of cells, respectively. When Flk-1 inhibitors were added to the culture medium prior to exposure of cells to ionizing radiation, increased apoptosis was observed. HUVEC cultures showed 14% apoptotic cells in response to SU5416 treatment following radiation treatment ($p=0.005$ compared to radiation alone). Similarly, HUVEC cultures treated with Ad.ExFlk following by ionizing radiation showed 12% apoptotic cells ($p=0.01$ compared to radiation alone). 3B11 and HMEC cells treated with Flk-1 inhibitors and radiation showed a similar enhancement of radiation-induced apoptosis. *See* Figure 5.

Example 7

Tumor Volume Assessment

GL261 tumors were generated by subcutaneous injection of 10^6 viable GL261 cells suspended in 0.2 ml of a 0.6% agarose solution into the right thigh of C57BL6 mice. Mice were stratified into four groups so that the mean tumor volume of each group was comparable. An equal number of large and intermediate size tumors were present in each group. The mean tumor volumes in each group were as follows: radiation, 0.52 cm^3; SU5416,

0.56 cm^3; SU5416 followed by radiation, 0.59 cm^3; untreated control, 0.49 cm^3.

GL261 tumor bearing mice were treated with 3 Gy ionizing radiation on each of 3 days per week for two weeks. SU5416 was administered at 0.75 mg by intraperitoneal injection given twice per week for a total of 4 treatments on days 0, 4, 7, and 11. Radiation was administered as 3 Gy fractions on 8 occasions on days 0, 1, 3, 4, 7, 8, 10, and 11. Control mice received identical doses and schedule of either SU5416 alone or radiation alone.

Twice each week, tumor volumes were measured using skin calipers as previously described by Advani *et al.*, 1998; Hallahan *et al.*, 1995; and Staba *et al.*, 1998. Tumor volumes were calculated using a formula:

$$a \times b \times c/2,$$

where a = maximal width dimension, b = maximal length dimension, and c = maximal depth dimension, which was derived from the formula for an ellipsoid ($\pi d^3/6$). Data were calculated as the percent of original (day 0) tumor volume and graphed as fractional tumor volume $\pm$ 1 standard deviation for each treatment group. Variance was analyzed by the Kruskal-Wallis method (Kruskal & Wallis, 1952).

Growth delay was determined as the number of days required for a tumor to reach a predetermined volume, for example three-fold the original volume, or a volume of 1 cm^3. GL261 tumors treated with radiation alone showed minimal growth delay. No growth delay was observed in tumors treated with SU5416 alone. However, the combination of SU5416 followed by radiation significantly reduced tumor volumes when compared to radiation alone ($p=0.01$) or SU5416 alone ($p<0.009$). *See* Figure 6A.

In a second experiment, mice were randomly assigned to each of the four treatment groups for a total of 10 mice in each group. The mean tumor volume on day 1 for mice in each treatment group was as follows: radiation, 0.90 cm^3; SU5416, 0.99 cm^3; SU5416 followed by radiation, 0.90 cm^3; untreated control, 0.91 cm^3. Flk-1 inhibition and radiation treatments were initiated fourteen days after the tumor cells had been injected and were

performed as described herein above. Tumors treated with SU5416 and radiation showed significantly reduced tumor volumes when compared to radiation alone (p= 0.01) and to SU5416 alone (p=0.006). *See* Figure 6B.

Example 8

Restoration of Tumor Blood Flow After Radiotherapy

Tumor-bearing mice were generated as described in Example 1. Mice were treated with SU5416 in combination with radiation treatment as described in Example 5. Control animals were treated with radiation alone. Doppler analysis of tumor blood flow was measured daily for 7 days using the methods described in Example 3. In control animals receiving only radiation treatment, restoration of tumor blood flow was observed by day 7. In animals receiving SU5416 following radiation treatment, tumor blood flow was repressed (Figure 7).

While applicants do not intend to be bound to any particular theory of operation, the mechanisms of tumor blood flow restoration appears to involve infiltration of a tumor by endothelial progenitor cells (EPCs). EPCs were isolated from peripheral circulation, fixed with paraformaldehyde, and incubated with antibodies that specifically bind to Flk or to CD31. A fluorescently-labeled secondary antibody was used to detect Flk and CD31 (PECAM; platelet-endothelial cell adhesion molecule 1) proteins on the surface of EPCs.

Mice bearing Gl261 gliomas, generated as described in Example 1, received radiation treatment of the tumor. EPCs were isolated from peripheral circulation and fluorescently labeled with Dil (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; available from Molecular Probes, Inc., Eugene, Oregon, United States of America). Labeled EPCs were then administered via tail vein injection to tumor-bearing mice immediately following tumor irradiation. Tumor sections were obtained on days 1, 4, and 7 following irradiation. In control mice, EPCs were detected in the perivascular space at day 4, and then proliferated and migrated into to the tumor by day 7. Mice treated with SU5416 and radiation, extravasation of EPCs into the tumor was prevented.

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The references listed below as well as all references cited in the specification are incorporated herein by reference to the extent that they supplement, explain, provide a background for or teach methodology,
5 techniques and/or compositions employed herein.

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It will be understood that various details of the invention can be changed without departing from the scope of the invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation--the invention being defined by the claims appended hereto.

CLAIMS

What is claimed is:

1. A method for increasing radiosensitivity of a tumor in a subject comprising administering a soluble VEGF-R polypeptide to a tumor in a subject, whereby the radiosensitivity of the tumor is increased.

2. The method of claim 1, wherein the administering comprises administering a composition comprising:

   (a) a soluble VEGF-R polypeptide; and

   (b) a pharmaceutically acceptable carrier.

3. The method of claim 2, wherein the pharmaceutically acceptable carrier comprises a gene therapy construct that encodes the soluble VEGF-R polypeptide.

4. The method of claim 1, wherein the administering comprises administering a minimally therapeutic dose of a soluble VEGF-R polypeptide.

5. The method of claim 1, wherein the soluble VEGF-R polypeptide comprises:

   (a) a polypeptide encoded by a nucleic acid of SEQ ID NO:1 or 3;

   (b) a polypeptide encoded by a nucleic acid substantially identical to SEQ ID NO:1 or 3;

   (c) a polypeptide comprising an amino acid sequence of SEQ ID NO:2 or 4; or

   (d) a polypeptide substantially identical to SEQ ID NO:2 or 4.

6. The method of claim 1, wherein the soluble VEGF-R polypeptide comprises a polypeptide encoded by an isolated nucleic acid segment selected from the group consisting of:

   (a) an isolated nucleic acid molecule which hybridizes to a nucleic acid sequence of SEQ ID NO:1 or 3 under wash stringency conditions represented by a wash solution having less than about 200 mM salt concentration and a wash temperature of greater than about 45°C, and which encodes a VEGF-R polypeptide; and

(b) an isolated nucleic acid molecule differing by at least one functionally equivalent codon from the isolated nucleic acid molecule of (a) above in nucleic acid sequence due to the degeneracy of the genetic code, and which encodes a VEGF-R
5 polypeptide encoded by the isolated nucleic acid of (a) above.

7. The method of claim 1, wherein the soluble VEGF-R polypeptide comprises:

(a) a polypeptide encoded by a nucleic acid of SEQ ID NO:5 or 7;

(b) a polypeptide encoded by a nucleic acid substantially identical
10 to SEQ ID NO:5 or 7;

(c) a polypeptide comprising an amino acid sequence of SEQ ID NO:6 or 8; or

(d) a polypeptide substantially identical to SEQ ID NO:6 or 8.

8. The method of claim 1, wherein the soluble VEGF-R
15 polypeptide comprises a polypeptide encoded by an isolated nucleic acid segment selected from the group consisting of:

(a) an isolated nucleic acid molecule which hybridizes to a nucleic acid sequence of SEQ ID NO:5 or 7 under wash stringency conditions represented by a wash solution having less than
20 about 200 mM salt concentration and a wash temperature of greater than about 45°C, and which encodes a VEGF-R polypeptide; and

(b) an isolated nucleic acid molecule differing by at least one functionally equivalent codon from the isolated nucleic acid
25 molecule of (a) above in nucleic acid sequence due to the degeneracy of the genetic code, and which encodes a VEGF-R polypeptide encoded by the isolated nucleic acid of (a) above.

9. The method of claim 1, wherein the tumor comprises a radiation resistant tumor.

30 10. The method of claim 1, wherein the subject comprises a mammal.

11. The method of claim 10, wherein the mammal comprises a human.

12. A method for delaying tumor growth, the method comprising:

(a) administering a VEGF-R2 inhibitor to a subject bearing a tumor; whereby the radiosensitivity of the tumor is increased; and

5 (b) treating the tumor with ionizing radiation, whereby tumor growth is delayed.

13. The method of claim 12, wherein the administering a VEGF-R2 inhibitor comprises administering a composition comprising:

(a) a VEGF-R2 inhibitor; and

10 (b) a pharmaceutically acceptable carrier.

14. The method of claim 13, wherein the VEGF-R2 inhibitor comprises SU5416.

15. The method of claim 13, wherein the VEGF-R2 inhibitor comprises an antibody that specifically binds to VEGF-R2.

15 16. The method of claim 13, wherein the VEGF-R2 inhibitor comprises a soluble VEGF-R polypeptide.

17. The method of claim 16, wherein the soluble VEGF-R polypeptide comprises:

(a) a polypeptide encoded by a nucleic acid of SEQ ID NO:1 or 3;

20 (b) a polypeptide encoded by a nucleic acid substantially identical to SEQ ID NO:1 or 3;

(c) a polypeptide comprising an amino acid sequence of SEQ ID NO:2 or 4; or

(d) a polypeptide substantially identical to SEQ ID NO:2 or 4.

25 18. The method of claim 16, wherein the soluble VEGF-R polypeptide comprises a polypeptide encoded by an isolated nucleic acid segment selected from the group consisting of:

(a) an isolated nucleic acid molecule which hybridizes to a nucleic acid sequence of SEQ ID NO:1 or 3 under wash stringency

30 conditions represented by a wash solution having less than about 200 mM salt concentration and a wash temperature of greater than about 45°C, and which encodes a VEGF-R polypeptide; and

(b) an isolated nucleic acid molecule differing by at least one functionally equivalent codon from the isolated nucleic acid molecule of (a) above in nucleic acid sequence due to the degeneracy of the genetic code, and which encodes a VEGF-R
5 polypeptide encoded by the isolated nucleic acid of (a) above.

19. The method of claim 16, wherein the soluble VEGF-R polypeptide comprises:

(a) a polypeptide encoded by a nucleic acid of SEQ ID NO:5 or 7;

(b) a polypeptide encoded by a nucleic acid substantially identical
10 to SEQ ID NO: 5 or 7;

(c) a polypeptide comprising an amino acid sequence of SEQ ID NO:6 or 8; or

(d) a polypeptide substantially identical to SEQ ID NO:6 or 8.

20. The method of claim 16, wherein the soluble VEGF-R
15 polypeptide comprises a polypeptide encoded by an isolated nucleic acid segment selected from the group consisting of:

(a) an isolated nucleic acid molecule which hybridizes to a nucleic acid sequence of SEQ ID NO: 5 or 7 under wash stringency conditions represented by a wash solution having less than
20 about 200 mM salt concentration and a wash temperature of greater than about 45°C, and which encodes a VEGF-R polypeptide; and

(b) an isolated nucleic acid molecule differing by at least one functionally equivalent codon from the isolated nucleic acid
25 molecule of (a) above in nucleic acid sequence due to the degeneracy of the genetic code, and which encodes a VEGF-R polypeptide encoded by the isolated nucleic acid of (a) above.

21. The method of claim 13, wherein the pharmaceutically acceptable carrier comprises a gene therapy vector.

30 22. The method of claim 12, wherein the administering a VEGF-R2 inhibitor comprises administering a minimally therapeutic dose of a VEGF-R2 inhibitor.

23. The method of claim 12, wherein the tumor comprises a radiation resistant tumor.

24. The method of claim 12, wherein the subject comprises a mammal.

5 25. The method of claim 24, wherein the mammal comprises a human.

26. The method of claim 12, wherein the treating the tumor with ionizing radiation comprises treating the tumor with a subtherapeutic dose of ionizing radiation.

10 27. The method of claim 12, further comprising promoting tumor regression.

28. A method for inhibiting tumor blood vessel growth, the method comprising:

(a) administering a VEGF-R2 inhibitor to a subject bearing a
15 tumor; whereby the radiosensitivity of the tumor is increased; and

(b) treating the tumor with ionizing radiation, whereby tumor blood vessel growth is inhibited.

29. The method of claim 28, wherein the administering a VEGF-R2
20 inhibitor comprises administering a composition comprising:

(a) a VEGF-R2 inhibitor; and

(b) a pharmaceutically acceptable carrier.

30. The method of claim 29, wherein the VEGF-R2 inhibitor comprises SU5416.

25 31. The method of claim 29, wherein the VEGF-R2 inhibitor comprises an antibody that specifically binds to VEGF-R2.

32. The method of claim 29, wherein the VEGF-R2 inhibitor comprises a soluble VEGF-R polypeptide.

33. The method of claim 32, wherein the soluble VEGF-R
30 polypeptide comprises:

(a) a polypeptide encoded by a nucleic acid of SEQ ID NO:1 or 3;

(b) a polypeptide encoded by a nucleic acid substantially identical to SEQ ID NO:1 or 3;

(c) a polypeptide comprising an amino acid sequence of SEQ ID NO:2 or 4; or

(d) a polypeptide substantially identical to SEQ ID NO:2 or 4.

34. The method of claim 32, wherein the soluble VEGF-R
5 polypeptide comprises a polypeptide encoded by an isolated nucleic acid segment selected from the group consisting of:

(a) an isolated nucleic acid molecule which hybridizes to a nucleic acid sequence of SEQ ID NO:1 or 3 under wash stringency conditions represented by a wash solution having less than
10 about 200 mM salt concentration and a wash temperature of greater than about 45°C, and which encodes a VEGF-R polypeptide; and

(b) an isolated nucleic acid molecule differing by at least one functionally equivalent codon from the isolated nucleic acid
15 molecule of (a) above in nucleic acid sequence due to the degeneracy of the genetic code, and which encodes a VEGF-R polypeptide encoded by the isolated nucleic acid of (a) above.

35. The method of claim 32, wherein the soluble VEGF-R polypeptide comprises:

20 (a) a polypeptide encoded by a nucleic acid of SEQ ID NO:5 or 7;

(b) a polypeptide encoded by a nucleic acid substantially identical to SEQ ID NO: 5 or 7;

(c) a polypeptide comprising an amino acid sequence of SEQ ID NO:6 or 8; or

25 (d) a polypeptide substantially identical to SEQ ID NO:6 or 8.

36. The method of claim 32, wherein the soluble VEGF-R polypeptide comprises a polypeptide encoded by an isolated nucleic acid segment selected from the group consisting of:

(a) an isolated nucleic acid molecule which hybridizes to a nucleic
30 acid sequence of SEQ ID NO: 5 or 7 under wash stringency conditions represented by a wash solution having less than about 200 mM salt concentration and a wash temperature of

greater than about 45°C, and which encodes a VEGF-R polypeptide; and

(b) an isolated nucleic acid molecule differing by at least one functionally equivalent codon from the isolated nucleic acid molecule of (a) above in nucleic acid sequence due to the degeneracy of the genetic code, and which encodes a VEGF-R polypeptide encoded by the isolated nucleic acid of (a) above.

37. The method of claim 28, wherein the pharmaceutically acceptable carrier comprises a gene therapy construct that encodes the VEGF-R2 inhibitor.

38. The method of claim 28, wherein the administering a VEGF-R2 inhibitor comprises administering a minimally therapeutic dose of a VEGF-R2 inhibitor.

39. The method of claim 28, wherein the tumor comprises a radiation resistant tumor.

40. The method of claim 28, wherein the subject comprises a mammal.

41. The method of claim 40, wherein the mammal comprises a human.

42. The method of claim 28, wherein the treating the tumor with ionizing radiation comprises treating the tumor with a subtherapeutic dose of ionizing radiation.

43. The method of claim 28, further comprising delaying tumor growth.

44. The method of claim 43, further comprising promoting tumor regression.

FIG. 1

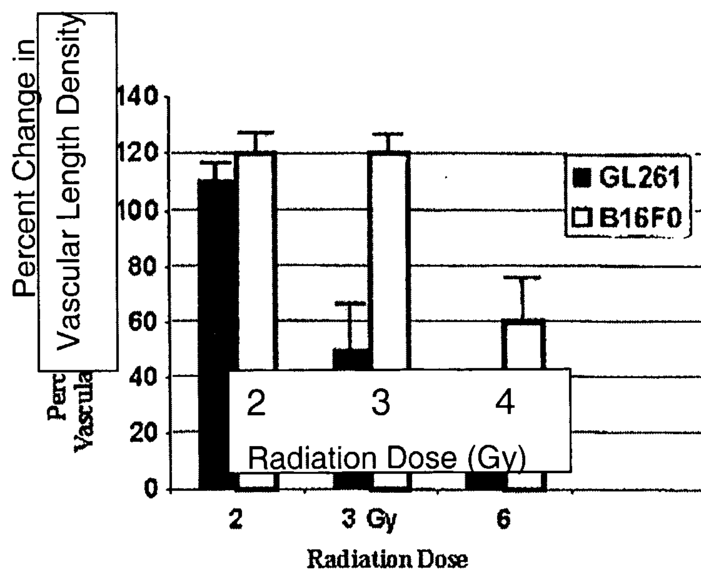


FIG. 2

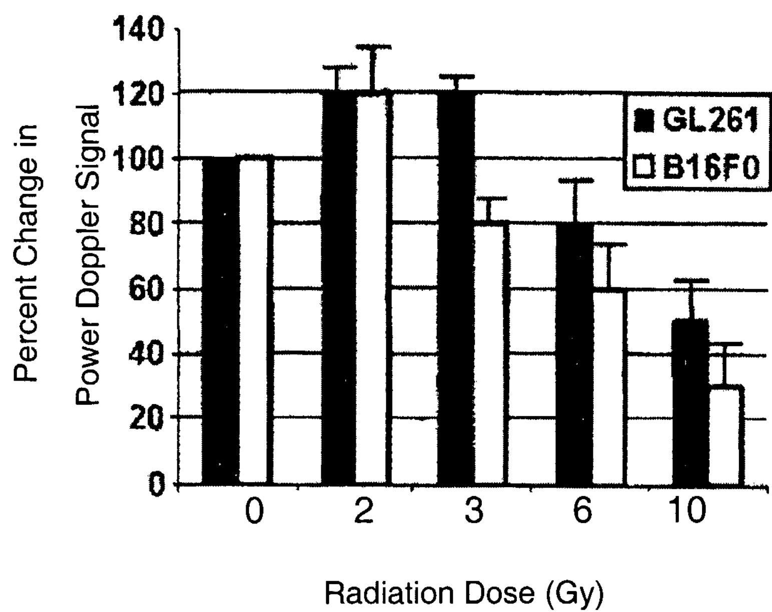


FIG. 3A

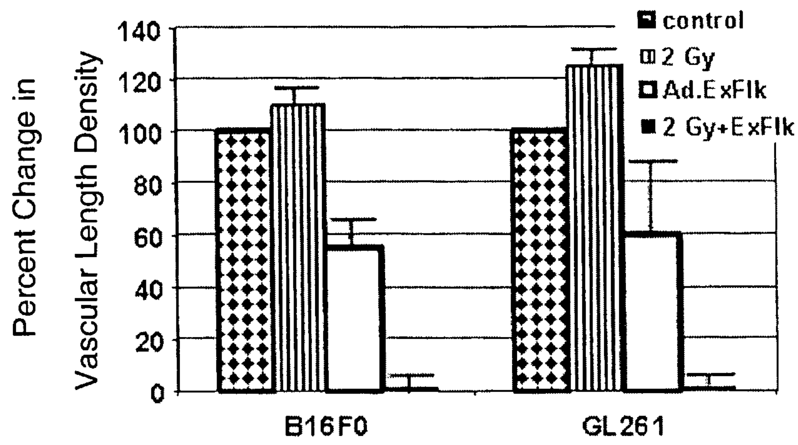


FIG. 3B

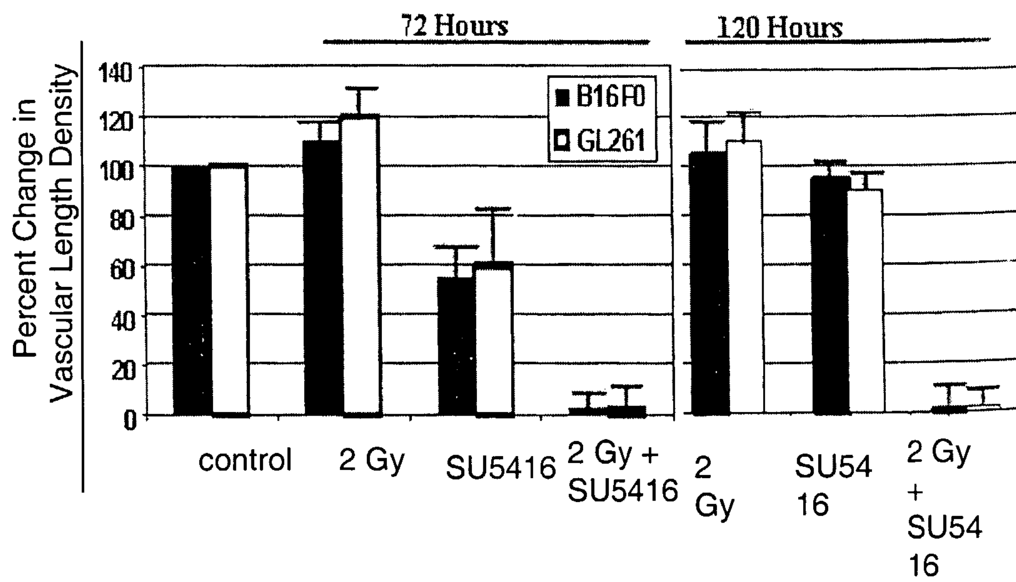


FIG. 4

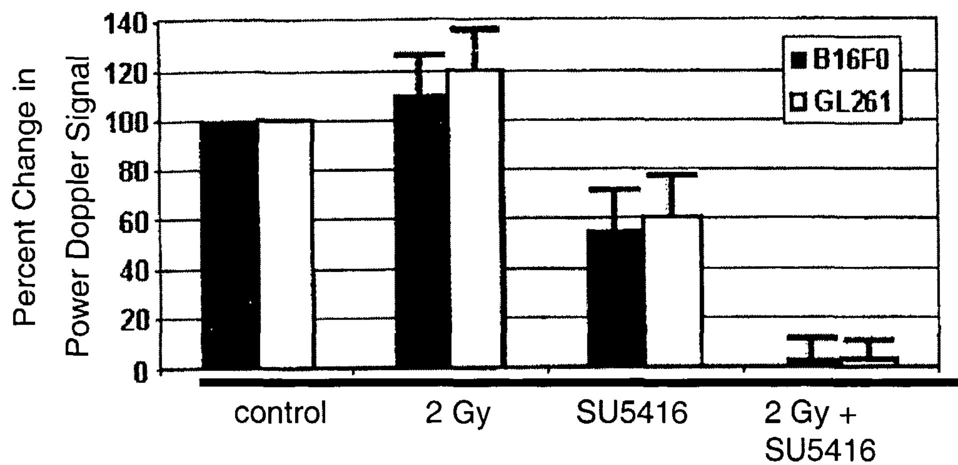


FIG. 5

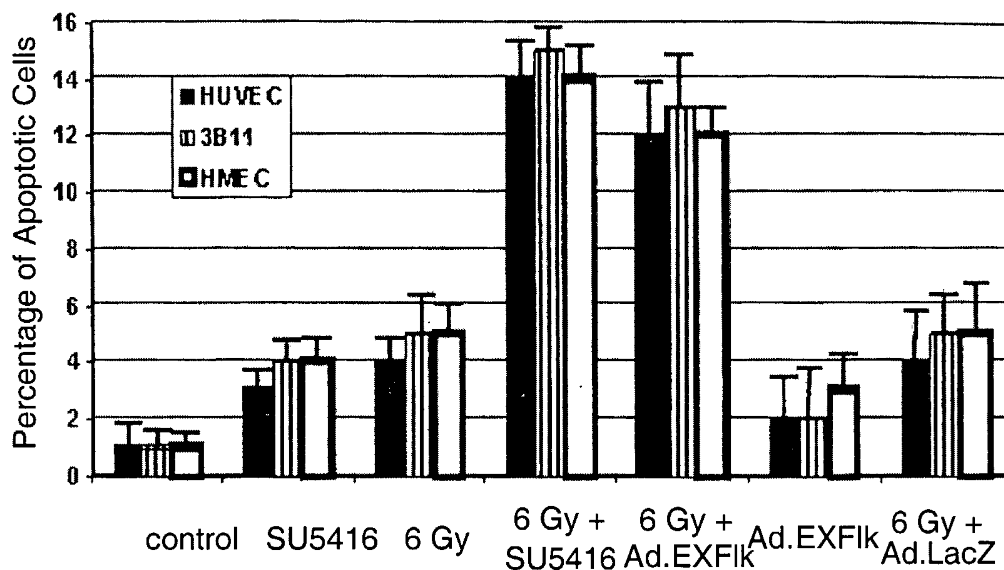


FIG. 6A

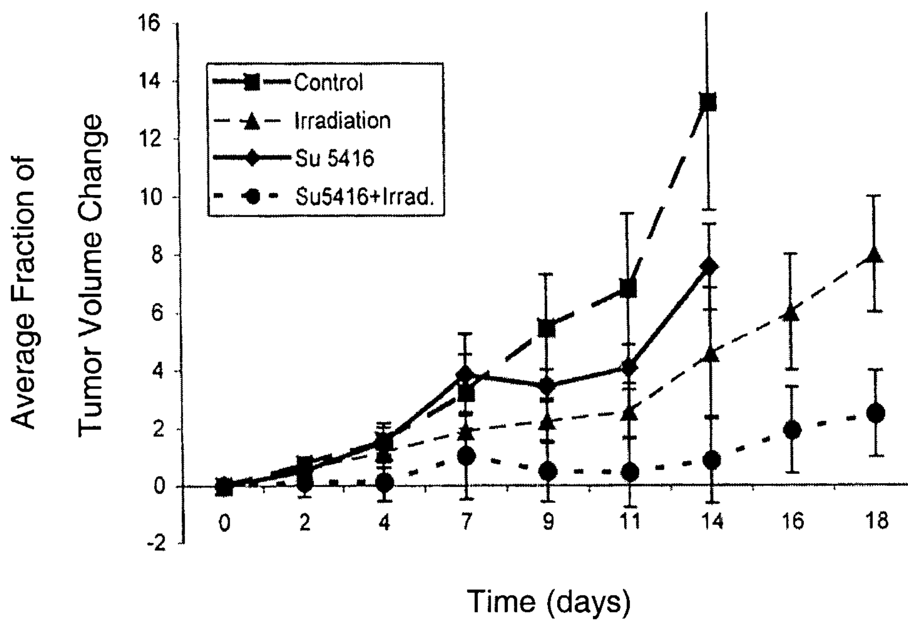


FIG. 6B

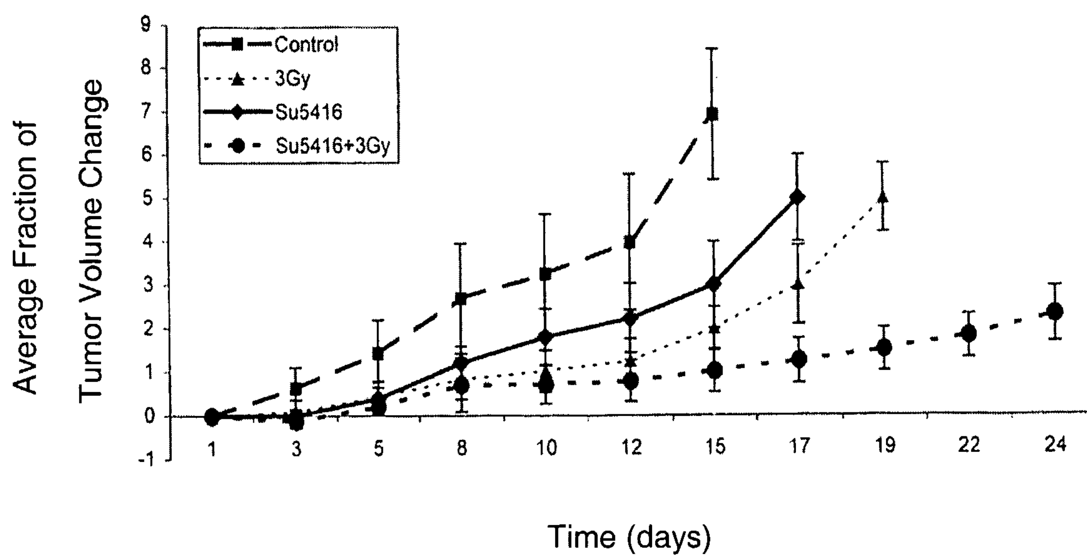
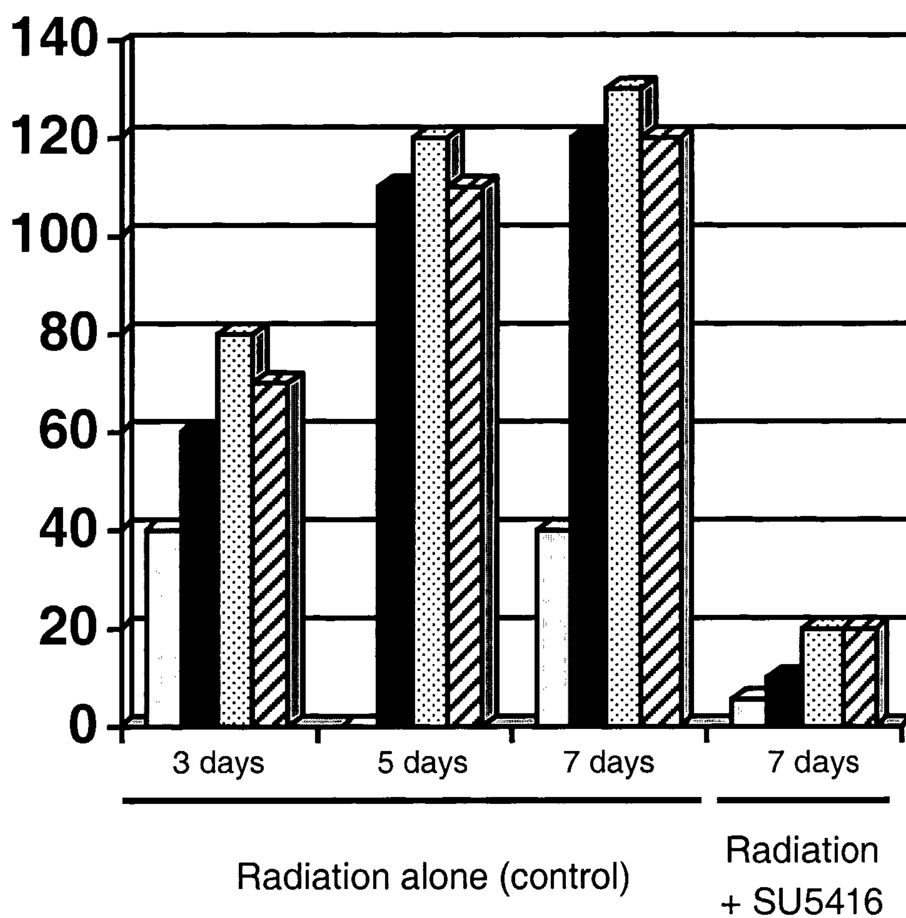


FIG. 7



SEQUENCE LISTING

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Ser Gln Val Arg Ile Pro Val Lys Tyr Leu Ser Tyr Pro Ala Pro Asp
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Asn Arg Ile Ser Trp Asp Ser Glu Ile Gly Phe Thr Leu Pro Ser Tyr
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Glu Thr Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg
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Ile Tyr Asp Val Ile Leu Ser Pro Pro His Glu Ile Glu Leu Ser Ala
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Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val
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Gly Leu Asp Phe Thr Trp His Ser Pro Pro Ser Lys Ser His His Lys
            260                 265                 270

Lys Ile Val Asn Arg Asp Val Lys Pro Phe Pro Gly Thr Val Ala Lys
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Met Phe Leu Ser Thr Leu Thr Ile Glu Ser Val Thr Lys Ser Asp Gln
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Gly Glu Tyr Thr Cys Val Ala Ser Ser Gly Arg Met Ile Lys Arg Asn
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Arg Thr Phe Val Arg Val His Thr Lys Pro Phe Ile Ala Phe Gly Ser
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Gly Met Lys Ser Leu Val Glu Ala Thr Val Gly Ser Gln Val Arg Ile
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Pro Val Lys Tyr Leu Ser Tyr Pro Ala Pro Asp Ile Lys Trp Tyr Arg
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Pro Met Asp Ser Tyr Gln Tyr Gly Thr Met Gln Thr Leu Thr Cys Thr
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Val Tyr Ala Asn Pro Pro Leu His His Ile Gln Trp Tyr Trp Gln Leu
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Glu Glu Ala Cys Ser Tyr Arg Pro Gly Gln Thr Ser Pro Tyr Ala Cys
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Lys Glu Trp Arg His Val Glu Asp Phe Gln Gly Gly Asn Lys Ile Glu
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Val Thr Lys Asn Gln Tyr Ala Leu Ile Glu Gly Lys Asn Lys Thr Val
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Val Ile Arg Gly Pro Glu Ile Thr Val Gln Pro Ala Ala Gln Pro Thr
545                 550                 555                 560

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Glu Asn Leu Thr Trp Tyr Lys Leu Gly Ser Gln Ala Thr Ser Val His
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Met Gly Glu Ser Leu Thr Pro Val Cys Lys Asn Leu Asp Ala Leu Trp
        595                 600                 605

Lys Leu Asn Gly Thr Met Phe Ser Asn Ser Thr Asn Asp Ile Leu Ile
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Asn Gln Thr Thr Thr Ile Gly Glu Thr Ile Glu Val Thr Cys Pro Ala
        675                 680                 685

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Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
        35                  40                  45

ctt caa att act tgc agg gga cag agg gac ttg gac tgg ctt tgg ccc       192
Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
    50                  55                  60

aat aat cag agt ggc agt gag caa agg gtg gag gtg act gag tgc agc       240
Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65                  70                  75                  80

gat ggc ctc ttc tgt aag aca ctc aca att cca aaa gtg atc gga aat       288
Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
                85                  90                  95
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gac act gga gcc tac aag tgc ttc tac cgg gaa act gac ttg gcc tcg      336
Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
            100                 105                 110

gtc att tat gtc tat gtt caa gat tac aga tct cca ttt att gct tct      384
Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
        115                 120                 125

gtt agt gac caa cat gga gtc gtg tac att act gag aac aaa aac aaa      432
Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
    130                 135                 140

act gtg gtg att cca tgt ctc ggg tcc att tca aat ctc aac gtg tca      480
Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145                 150                 155                 160

ctt tgt gca aga tac cca gaa aag aga ttt gtt cct gat ggt aac aga      528
Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
                165                 170                 175

att tcc tgg gac agc aag aag ggc ttt act att ccc agc tac atg atc      576
Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
            180                 185                 190

agc tat gct ggc atg gtc ttc tgt gaa gca aaa att aat gat gaa agt      624
Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
        195                 200                 205

tac cag tct att atg tac ata gtt gtc gtt gta ggg tat agg att tat      672
Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
    210                 215                 220

gat gtg gtt ctg agt ccg tct cat gga att gaa cta tct gtt gga gaa      720
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225                 230                 235                 240

aag ctt gtc tta aat tgt aca gca aga act gaa cta aat gtg ggg att      768
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
                245                 250                 255

gac ttc aac tgg gaa tac cct tct tcg aag cat cag cat aag aaa ctt      816
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
            260                 265                 270

gta aac cga gac cta aaa acc cag tct ggg agt gag atg aag aaa ttt      864
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
        275                 280                 285

ttg agc acc tta act ata gat ggt gta acc cgg agt gac caa gga ttg      912
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
    290                 295                 300

tac acc tgt gca gca tcc agt ggg ctg atg acc aag aag aac agc aca      960
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305                 310                 315                 320

ttt gtc agg gtc cat gaa aaa cct ttt gtt gct ttt gga agt ggc atg     1008
Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
                325                 330                 335

gaa tct ctg gtg gaa gcc acg gtg ggg gag cgt gtc aga atc cct gcg     1056
Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
            340                 345                 350

aag tac ctt ggt tac cca ccc cca gaa ata aaa tgg tat aaa aat gga     1104
Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
        355                 360                 365

ata ccc ctt gag tcc aat cac aca att aaa gcg ggg cat gta ctg acg     1152
Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
    370                 375                 380

att atg gaa gtg agt gaa aga gac aca gga aat tac act gtc atc ctt     1200
Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385                 390                 395                 400
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     acc aat ccc att tca aag gag aag cag agc cat gtg gtc tct ctg gtt      1248
     Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
                     405                 410                 415

 5   gtg tat gtc cca ccc cag att ggt gag aaa tct cta atc tct cct gtg      1296
     Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
                 420                 425                 430

     gat tcc tac cag tac ggc acc act caa acg ctg aca tgt acg gtc tat      1344
10   Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
             435                 440                 445

     gcc att cct ccc ccg cat cac atc cac tgg tat tgg cag ttg gag gaa      1392
     Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
15       450                 455                 460

     gag tgc gcc aac gag ccc agc caa gct gtc tca gtg aca aac cca tac      1440
     Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
     465                 470                 475                 480
20
     cct tgt gaa gaa tgg aga agt gtg gag gac ttc cag gga gga aat aaa      1488
     Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
                     485                 490                 495

25   att gaa gtt aat aaa aat caa ttt gct cta att gaa gga aaa aac aaa      1536
     Ile Glu Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
                 500                 505                 510

     act gta agt acc ctt gtt atc caa gcg gca aat gtg tca gct ttg tac      1584
30   Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
             515                 520                 525

     aaa tgt gaa gcg gtc aac aaa gtc ggg aga gga gag agg gtg atc tcc      1632
     Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
35       530                 535                 540

     ttc cac gtg acc agg ggt cct gaa att act ttg caa cct gac atg cag      1680
     Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
     545                 550                 555                 560
40
     ccc act gag cag gag agc gtg tct ttg tgg tgc act gca gac aga tct      1728
     Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
                     565                 570                 575

45   acg ttt gag aac ctc aca tgg tac aag ctt ggc cca cag cct ctg cca      1776
     Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
                 580                 585                 590

     atc cat gtg gga gag ttg ccc aca cct gtt tgc aag aac ttg gat act      1824
50   Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
             595                 600                 605

     ctt tgg aaa ttg aat gcc acc atg ttc tct aat agc aca aat gac att      1872
     Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile
55       610                 615                 620

     ttg atc atg gag ctt aag aat gca tcc ttg cag gac caa gga gac tat      1920
     Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
     625                 630                 635                 640
60
     gtc tgc ctt gct caa gac agg aag acc aag aaa aga cat tgc gtg gtc      1968
     Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
                     645                 650                 655

65   agg cag ctc aca gtc cta gag cgt gtg gca ccc acg atc aca gga aac      2016
     Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
                 660                 665                 670

     ctg gag aat cag acg aca agt att ggg gaa agc atc gaa gtc tca tgc      2064
70   Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
             675                 680                 685

     acg gca tct ggg aat ccc cct cca cag atc atg tgg ttt aaa gat aat      2112
     Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
75       690                 695                 700
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gag acc ctt gta gaa gac tca ggc att gta ttg aag gat ggg aac cgg      2160
Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
705                 710                 715                 720

aac ctc act atc cgc aga gtg agg aag gag gac gaa ggc ctc tac acc      2208
Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Thr
                725                 730                 735

tgc cag gca tgc agt gtt ctt ggc tgt gca aaa gtg gag gca ttt ttc      2256
Cys Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe
            740                 745                 750

ata ata gaa ggt gcc cag gaa aag acg aac ttg gaa atc att att cta      2304
Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu
        755                 760                 765

gta ggc acg gcg gtg a                                                2320
Val Gly Thr Ala Val
    770


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Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
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Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
            20                  25                  30

Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
        35                  40                  45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
    50                  55                  60

Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65                  70                  75                  80

Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
                85                  90                  95

Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
            100                 105                 110

Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
        115                 120                 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
    130                 135                 140

Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145                 150                 155                 160

Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
                165                 170                 175

Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
            180                 185                 190

Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
        195                 200                 205

Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
    210                 215                 220

Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225                 230                 235                 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
                245                 250                 255

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
            260                 265                 270
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Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
        275                 280                 285

Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
    290                 295                 300

Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305                 310                 315                 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
                325                 330                 335

Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
            340                 345                 350

Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
        355                 360                 365

Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
    370                 375                 380

Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385                 390                 395                 400

Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
                405                 410                 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
            420                 425                 430

Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
        435                 440                 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
    450                 455                 460

Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
465                 470                 475                 480

Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
                485                 490                 495

Ile Glu Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
            500                 505                 510

Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
        515                 520                 525

Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
    530                 535                 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
545                 550                 555                 560

Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
                565                 570                 575

Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
            580                 585                 590

Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
        595                 600                 605

Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile
    610                 615                 620

Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
625                 630                 635                 640

Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
                645                 650                 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
            660                 665                 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
        675                 680                 685
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Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
    690                 695                 700

Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
705                 710                 715                 720

Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Thr
                725                 730                 735

Cys Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe
            740                 745                 750

Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu
        755                 760                 765

Val Gly Thr Ala Val
    770


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Met Val Ser Cys Trp Asp Thr Ala Val Leu Pro Tyr Ala Leu Leu Gly
1               5                   10                  15

tgt ctg ctt ctc aca gga tat ggc tca ggg tcg aag tta aaa gtg cct      96
Cys Leu Leu Leu Thr Gly Tyr Gly Ser Gly Ser Lys Leu Lys Val Pro
            20                  25                  30

gaa ctg agt tta aaa ggc acc cag cat gtc atg caa gca ggc cag act     144
Glu Leu Ser Leu Lys Gly Thr Gln His Val Met Gln Ala Gly Gln Thr
        35                  40                  45

ctc ttt ctc aag tgc aga ggg gag gca gcc cac tca tgg tct ctg ccc     192
Leu Phe Leu Lys Cys Arg Gly Glu Ala Ala His Ser Trp Ser Leu Pro
    50                  55                  60

acg acc gtg agc cag gag gac aaa agg ctg agc atc act ccc cca tcg     240
Thr Thr Val Ser Gln Glu Asp Lys Arg Leu Ser Ile Thr Pro Pro Ser
65                  70                  75                  80

gcc tgt ggg agg gat aac agg caa ttc tgc agc acc ttg acc ttg gac     288
Ala Cys Gly Arg Asp Asn Arg Gln Phe Cys Ser Thr Leu Thr Leu Asp
                85                  90                  95

acg gcg cag gcc aac cac acg ggc ctc tac acc tgt aga tac ctc cct     336
Thr Ala Gln Ala Asn His Thr Gly Leu Tyr Thr Cys Arg Tyr Leu Pro
            100                 105                 110

aca tct act tcg aag aaa aag aaa gcg gaa tct tca atc tac ata ttt     384
Thr Ser Thr Ser Lys Lys Lys Lys Ala Glu Ser Ser Ile Tyr Ile Phe
        115                 120                 125

gtt agt gat gca ggg agt cct ttc ata gag atg cac act gac ata ccc     432
Val Ser Asp Ala Gly Ser Pro Phe Ile Glu Met His Thr Asp Ile Pro
    130                 135                 140

aaa ctt gtg cac atg acg gaa gga aga cag ctc atc atc ccc tgc cgg     480
Lys Leu Val His Met Thr Glu Gly Arg Gln Leu Ile Ile Pro Cys Arg
145                 150                 155                 160

gtg acg tca ccc aac gtc aca gtc acc cta aaa aag ttt cca ttt gat     528
Val Thr Ser Pro Asn Val Thr Val Thr Leu Lys Lys Phe Pro Phe Asp
                165                 170                 175

act ctt acc cct gat ggg caa aga ata aca tgg gac agt agg aga ggc     576
Thr Leu Thr Pro Asp Gly Gln Arg Ile Thr Trp Asp Ser Arg Arg Gly
            180                 185                 190
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ttt ata ata gca aat gca acg tac aaa gag ata gga ctg ctg aac tgc      624
Phe Ile Ile Ala Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Asn Cys
        195                 200                 205

gaa gcc acc gtc aac ggg cac ctg tac cag aca aac tat ctg acc cat      672
Glu Ala Thr Val Asn Gly His Leu Tyr Gln Thr Asn Tyr Leu Thr His
    210                 215                 220

cgg cag acc aat aca atc cta gat gtc caa ata cgc ccg ccg agc cca      720
Arg Gln Thr Asn Thr Ile Leu Asp Val Gln Ile Arg Pro Pro Ser Pro
225                 230                 235                 240

gtg aga ctg ctc cac ggg cag act ctt gtc ctc aac tgc acc gcc acc      768
Val Arg Leu Leu His Gly Gln Thr Leu Val Leu Asn Cys Thr Ala Thr
                245                 250                 255

acg gag ctc aat acg agg gtg caa atg agc tgg aat tac cct ggt aaa      816
Thr Glu Leu Asn Thr Arg Val Gln Met Ser Trp Asn Tyr Pro Gly Lys
            260                 265                 270

gca act aag aga gca tct ata agg cag cgg att gac cgg agc cat tcc      864
Ala Thr Lys Arg Ala Ser Ile Arg Gln Arg Ile Asp Arg Ser His Ser
        275                 280                 285

cac aac aat gtg ttc cac agt gtt ctt aag atc aac aat gtg gag agc      912
His Asn Asn Val Phe His Ser Val Leu Lys Ile Asn Asn Val Glu Ser
    290                 295                 300

cga gac aag ggg ctc tac acc tgt cgc gtg aag agt ggg tcc tcg ttc      960
Arg Asp Lys Gly Leu Tyr Thr Cys Arg Val Lys Ser Gly Ser Ser Phe
305                 310                 315                 320

cag tct ttc aac acc tcc gtg cat gtg tat gaa aaa gga ttc atc agt     1008
Gln Ser Phe Asn Thr Ser Val His Val Tyr Glu Lys Gly Phe Ile Ser
                325                 330                 335

gtg aaa cat cgg aag cag ccg gtg cag gaa acc aca gca gga aga cgg     1056
Val Lys His Arg Lys Gln Pro Val Gln Glu Thr Thr Ala Gly Arg Arg
            340                 345                 350

tcc tat cgg ctg tcc atg aaa gtg aag gcc ttc ccc tcc cca gaa atc     1104
Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Ile
        355                 360                 365

gta tgg tta aaa gat ggc tcg cct gca aca ttg aag tct gct cgc tat     1152
Val Trp Leu Lys Asp Gly Ser Pro Ala Thr Leu Lys Ser Ala Arg Tyr
    370                 375                 380

ttg gta cat ggc tac tca tta att atc aaa gat gtg aca acc gag gat     1200
Leu Val His Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Thr Glu Asp
385                 390                 395                 400

gca ggg gac tat acg atc ttg ctg ggc ata aag cag tca agg cta ttt     1248
Ala Gly Asp Tyr Thr Ile Leu Leu Gly Ile Lys Gln Ser Arg Leu Phe
                405                 410                 415

aaa aac ctc act gcc act ctc att gta aac gtg aaa cct cag atc tac     1296
Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr
            420                 425                 430

gaa aag tcc gtg tcc tcg ctt cca agc cca cct ctc tat ccg ctg ggc     1344
Glu Lys Ser Val Ser Ser Leu Pro Ser Pro Pro Leu Tyr Pro Leu Gly
        435                 440                 445

agc aga caa gtc ctc act tgc acc gtg tat ggc atc cct cgg cca aca     1392
Ser Arg Gln Val Leu Thr Cys Thr Val Tyr Gly Ile Pro Arg Pro Thr
    450                 455                 460

atc acg tgg ctc tgg cac ccc tgt cac cac aat cac tcc aaa gaa agg     1440
Ile Thr Trp Leu Trp His Pro Cys His His Asn His Ser Lys Glu Arg
465                 470                 475                 480

tat gac ttc tgc act gag aat gaa gaa tcc ttt atc ctg gat ccc agc     1488
Tyr Asp Phe Cys Thr Glu Asn Glu Glu Ser Phe Ile Leu Asp Pro Ser
                485                 490                 495
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agc aac tta gga aac aga att gag agc atc tct cag cgc atg acg gtc      1536
Ser Asn Leu Gly Asn Arg Ile Glu Ser Ile Ser Gln Arg Met Thr Val
            500                 505                 510

ata gaa gga aca aat aag acg gtt agc aca ttg gtg gtg gct gac tct      1584
Ile Glu Gly Thr Asn Lys Thr Val Ser Thr Leu Val Val Ala Asp Ser
        515                 520                 525

cag acc cct gga atc tac agc tgc cgg gcc ttc aat aaa ata ggg act      1632
Gln Thr Pro Gly Ile Tyr Ser Cys Arg Ala Phe Asn Lys Ile Gly Thr
    530                 535                 540

gtg gaa aga aac ata aaa ttt tac gtc aca gat gtg ccg aat ggc ttt      1680
Val Glu Arg Asn Ile Lys Phe Tyr Val Thr Asp Val Pro Asn Gly Phe
545                 550                 555                 560

cac gtt tcc ttg gaa aag atg cca gcc gaa gga gag gac ctg aaa ctg      1728
His Val Ser Leu Glu Lys Met Pro Ala Glu Gly Glu Asp Leu Lys Leu
                565                 570                 575

tcc tgt gtg gtc aat aaa ttc ctg tac aga gac att acc tgg att ctg      1776
Ser Cys Val Val Asn Lys Phe Leu Tyr Arg Asp Ile Thr Trp Ile Leu
            580                 585                 590

cta cgg aca gtt aac aac aga acc atg cac cat agt atc agc aag caa      1824
Leu Arg Thr Val Asn Asn Arg Thr Met His His Ser Ile Ser Lys Gln
        595                 600                 605

aaa atg gcc acc act caa gat tac tcc atc act ctg aac ctt gtc atc      1872
Lys Met Ala Thr Thr Gln Asp Tyr Ser Ile Thr Leu Asn Leu Val Ile
    610                 615                 620

aag aac gtg tct cta gaa gac tcg ggc acc tat gcg tgc aga gcc agg      1920
Lys Asn Val Ser Leu Glu Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg
625                 630                 635                 640

aac ata tac aca ggg gaa gac atc ctt cgg aag aca gaa gtt ctc gtt      1968
Asn Ile Tyr Thr Gly Glu Asp Ile Leu Arg Lys Thr Glu Val Leu Val
                645                 650                 655

aga ggt gag cac tgc ggc aaa aag gcc att ttc tct cgg atc tcc aaa      2016
Arg Gly Glu His Cys Gly Lys Lys Ala Ile Phe Ser Arg Ile Ser Lys
            660                 665                 670

ttt aaa agc agg agg aat gat tgt acc aca caa agt cat gtc aaa cat      2064
Phe Lys Ser Arg Arg Asn Asp Cys Thr Thr Gln Ser His Val Lys His
        675                 680                 685

taa                                                                  2067


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1               5                   10                  15

Cys Leu Leu Leu Thr Gly Tyr Gly Ser Gly Ser Lys Leu Lys Val Pro
            20                  25                  30

Glu Leu Ser Leu Lys Gly Thr Gln His Val Met Gln Ala Gly Gln Thr
        35                  40                  45

Leu Phe Leu Lys Cys Arg Gly Glu Ala Ala His Ser Trp Ser Leu Pro
    50                  55                  60

Thr Thr Val Ser Gln Glu Asp Lys Arg Leu Ser Ile Thr Pro Pro Ser
65                  70                  75                  80

Ala Cys Gly Arg Asp Asn Arg Gln Phe Cys Ser Thr Leu Thr Leu Asp
                85                  90                  95

Thr Ala Gln Ala Asn His Thr Gly Leu Tyr Thr Cys Arg Tyr Leu Pro
            100                 105                 110
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Thr Ser Thr Ser Lys Lys Lys Lys Ala Glu Ser Ser Ile Tyr Ile Phe
        115                 120                 125

Val Ser Asp Ala Gly Ser Pro Phe Ile Glu Met His Thr Asp Ile Pro
    130                 135                 140

Lys Leu Val His Met Thr Glu Gly Arg Gln Leu Ile Ile Pro Cys Arg
145                 150                 155                 160

Val Thr Ser Pro Asn Val Thr Val Thr Leu Lys Lys Phe Pro Phe Asp
                165                 170                 175

Thr Leu Thr Pro Asp Gly Gln Arg Ile Thr Trp Asp Ser Arg Arg Gly
            180                 185                 190

Phe Ile Ile Ala Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Asn Cys
        195                 200                 205

Glu Ala Thr Val Asn Gly His Leu Tyr Gln Thr Asn Tyr Leu Thr His
    210                 215                 220

Arg Gln Thr Asn Thr Ile Leu Asp Val Gln Ile Arg Pro Pro Ser Pro
225                 230                 235                 240

Val Arg Leu Leu His Gly Gln Thr Leu Val Leu Asn Cys Thr Ala Thr
                245                 250                 255

Thr Glu Leu Asn Thr Arg Val Gln Met Ser Trp Asn Tyr Pro Gly Lys
            260                 265                 270

Ala Thr Lys Arg Ala Ser Ile Arg Gln Arg Ile Asp Arg Ser His Ser
        275                 280                 285

His Asn Asn Val Phe His Ser Val Leu Lys Ile Asn Asn Val Glu Ser
    290                 295                 300

Arg Asp Lys Gly Leu Tyr Thr Cys Arg Val Lys Ser Gly Ser Ser Phe
305                 310                 315                 320

Gln Ser Phe Asn Thr Ser Val His Val Tyr Glu Lys Gly Phe Ile Ser
                325                 330                 335

Val Lys His Arg Lys Gln Pro Val Gln Glu Thr Thr Ala Gly Arg Arg
            340                 345                 350

Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Ile
        355                 360                 365

Val Trp Leu Lys Asp Gly Ser Pro Ala Thr Leu Lys Ser Ala Arg Tyr
    370                 375                 380

Leu Val His Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Thr Glu Asp
385                 390                 395                 400

Ala Gly Asp Tyr Thr Ile Leu Leu Gly Ile Lys Gln Ser Arg Leu Phe
                405                 410                 415

Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr
            420                 425                 430

Glu Lys Ser Val Ser Ser Leu Pro Ser Pro Pro Leu Tyr Pro Leu Gly
        435                 440                 445

Ser Arg Gln Val Leu Thr Cys Thr Val Tyr Gly Ile Pro Arg Pro Thr
    450                 455                 460

Ile Thr Trp Leu Trp His Pro Cys His His Asn His Ser Lys Glu Arg
465                 470                 475                 480

Tyr Asp Phe Cys Thr Glu Asn Glu Glu Ser Phe Ile Leu Asp Pro Ser
                485                 490                 495

Ser Asn Leu Gly Asn Arg Ile Glu Ser Ile Ser Gln Arg Met Thr Val
            500                 505                 510

Ile Glu Gly Thr Asn Lys Thr Val Ser Thr Leu Val Val Ala Asp Ser
        515                 520                 525
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     Gln Thr Pro Gly Ile Tyr Ser Cys Arg Ala Phe Asn Lys Ile Gly Thr
         530                 535                 540

     Val Glu Arg Asn Ile Lys Phe Tyr Val Thr Asp Val Pro Asn Gly Phe
5    545                 550                 555                 560

     His Val Ser Leu Glu Lys Met Pro Ala Glu Gly Glu Asp Leu Lys Leu
                     565                 570                 575

10   Ser Cys Val Val Asn Lys Phe Leu Tyr Arg Asp Ile Thr Trp Ile Leu
                 580                 585                 590

     Leu Arg Thr Val Asn Asn Arg Thr Met His His Ser Ile Ser Lys Gln
             595                 600                 605
15
     Lys Met Ala Thr Thr Gln Asp Tyr Ser Ile Thr Leu Asn Leu Val Ile
         610                 615                 620

     Lys Asn Val Ser Leu Glu Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg
20   625                 630                 635                 640

     Asn Ile Tyr Thr Gly Glu Asp Ile Leu Arg Lys Thr Glu Val Leu Val
                     645                 650                 655

25   Arg Gly Glu His Cys Gly Lys Lys Ala Ile Phe Ser Arg Ile Ser Lys
                 660                 665                 670

     Phe Lys Ser Arg Arg Asn Asp Cys Thr Thr Gln Ser His Val Lys His
             675                 680                 685
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     atg gtc agc tac tgg gac acc ggg gtc ctg ctg tgc gcg ctg ctc agc       48
     Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
     1               5                   10                  15
45
     tgt ctg ctt ctc aca gga tct agt tca ggt tca aaa tta aaa gat cct       96
     Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
                 20                  25                  30

50   gaa ctg agt tta aaa ggc acc cag cac atc atg caa gca ggc cag aca      144
     Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
             35                  40                  45

     ctg cat ctc caa tgc agg ggg gaa gca gcc cat aaa tgg tct ttg cct      192
55   Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
         50                  55                  60

     gaa atg gtg agt aag gaa agc gaa agg ctg agc ata act aaa tct gcc      240
     Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
60   65                  70                  75                  80

     tgt gga aga aat ggc aaa caa ttc tgc agt act tta acc ttg aac aca      288
     Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
                     85                  90                  95
65
     gct caa gca aac cac act ggc ttc tac agc tgc aaa tat cta gct gta      336
     Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
                 100                 105                 110

70   cct act tca aag aag aag gaa aca gaa tct gca atc tat ata ttt att      384
     Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
             115                 120                 125

     agt gat aca ggt aga cct ttc gta gag atg tac agt gaa atc ccc gaa      432
75   Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
         130                 135                 140
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att ata cac atg act gaa gga agg gag ctc gtc att ccc tgc cgg gtt      480
Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145                 150                 155                 160

acg tca cct aac atc act gtt act tta aaa aag ttt cca ctt gac act      528
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
                165                 170                 175

ttg atc cct gat gga aaa cgc ata atc tgg gac agt aga aag ggc ttc      576
Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
            180                 185                 190

atc ata tca aat gca acg tac aaa gaa ata ggg ctt ctg acc tgt gaa      624
Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
        195                 200                 205

gca aca gtc aat ggg cat ttg tat aag aca aac tat ctc aca cat cga      672
Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
    210                 215                 220

caa acc aat aca atc ata gat gtc caa ata agc aca cca cgc cca gtc      720
Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225                 230                 235                 240

aaa tta ctt aga ggc cat act ctt gtc ctc aat tgt act gct acc act      768
Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
                245                 250                 255

ccc ttg aac acg aga gtt caa atg acc tgg agt tac cct gat gaa aaa      816
Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
            260                 265                 270

aat aag aga gct tcc gta agg cga cga att gac caa agc aat tcc cat      864
Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
        275                 280                 285

gcc aac ata ttc tac agt gtt ctt act att gac aaa atg cag aac aaa      912
Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
    290                 295                 300

gac aaa gga ctt tat act tgt cgt gta agg agt gga cca tca ttc aaa      960
Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305                 310                 315                 320

tct gtt aac acc tca gtg cat ata tat gat aaa gca ttc atc act gtg     1008
Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
                325                 330                 335

aaa cat cga aaa cag cag gtg ctt gaa acc gta gct ggc aag cgg tct     1056
Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
            340                 345                 350

tac cgg ctc tct atg aaa gtg aag gca ttt ccc tcg ccg gaa gtt gta     1104
Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
        355                 360                 365

tgg tta aaa gat ggg tta cct gcg act gag aaa tct gct cgc tat ttg     1152
Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
    370                 375                 380

act cgt ggc tac tcg tta att atc aag gac gta act gaa gag gat gca     1200
Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385                 390                 395                 400

ggg aat tat aca atc ttg ctg agc ata aaa cag tca aat gtg ttt aaa     1248
Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
                405                 410                 415

aac ctc act gcc act cta att gtc aat gtg aaa ccc cag att tac gaa     1296
Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
            420                 425                 430

aag gcc gtg tca tcg ttt cca gac ccg gct ctc tac cca ctg ggc agc     1344
Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
        435                 440                 445
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aga caa atc ctg act tgt acc gca tat ggt atc cct caa cct aca atc       1392
Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
    450                 455                 460

aag tgg ttc tgg cac ccc tgt aac cat aat cat tcc gaa gca agg tgt       1440
Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465                 470                 475                 480

gac ttt tgt tcc aat aat gaa gag tcc ttt atc ctg gat gct gac agc       1488
Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
                485                 490                 495

aac atg gga aac aga att gag agc atc act cag cgc atg gca ata ata       1536
Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
            500                 505                 510

gaa gga aag aat aag atg gct agc acc ttg gtt gtg gct gac tct aga       1584
Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
        515                 520                 525

att tct gga atc tac att tgc ata gct tcc aat aaa gtt ggg act gtg       1632
Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
    530                 535                 540

gga aga aac ata agc ttt tat atc aca gat gtg cca aat ggg ttt cat       1680
Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545                 550                 555                 560

gtt aac ttg gaa aaa atg ccg acg gaa gga gag gac ctg aaa ctg tct       1728
Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
                565                 570                 575

tgc aca gtt aac aag ttc tta tac aga gac gtt act tgg att tta ctg       1776
Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
            580                 585                 590

cgg aca gtt aat aac aga aca atg cac tac agt att agc aag caa aaa       1824
Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
        595                 600                 605

atg gcc atc act aag gag cac tcc atc act ctt aat ctt acc atc atg       1872
Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
    610                 615                 620

aat gtt tcc ctg caa gat tca ggc acc tat gcc tgc aga gcc agg aat       1920
Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625                 630                 635                 640

gta tac aca ggg gaa gaa atc ctc cag aag aaa gaa att aca atc aga       1968
Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
                645                 650                 655

gat cag gaa gca cca tac ctc ctg cga aac ctc agt gat cac aca gtg       2016
Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val
            660                 665                 670

gcc atc agc agt tcc acc act tta gac tgt cat gct aat ggt gtc           2061
Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val
        675                 680                 685


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Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1               5                   10                  15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
            20                  25                  30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
        35                  40                  45
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Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
    50                  55                  60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65                  70                  75                  80

Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
                85                  90                  95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
            100                 105                 110

Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
        115                 120                 125

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
    130                 135                 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145                 150                 155                 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
                165                 170                 175

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
            180                 185                 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
        195                 200                 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
    210                 215                 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225                 230                 235                 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
                245                 250                 255

Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
            260                 265                 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
        275                 280                 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
    290                 295                 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305                 310                 315                 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
                325                 330                 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
            340                 345                 350

Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
        355                 360                 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
    370                 375                 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385                 390                 395                 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
                405                 410                 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
            420                 425                 430

Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
        435                 440                 445

Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
    450                 455                 460
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Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465                 470                 475                 480

Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
                485                 490                 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
            500                 505                 510

Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
        515                 520                 525

Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
    530                 535                 540

Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545                 550                 555                 560

Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
                565                 570                 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
            580                 585                 590

Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
        595                 600                 605

Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
    610                 615                 620

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625                 630                 635                 640

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
                645                 650                 655

Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val
            660                 665                 670

Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val
        675                 680                 685
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