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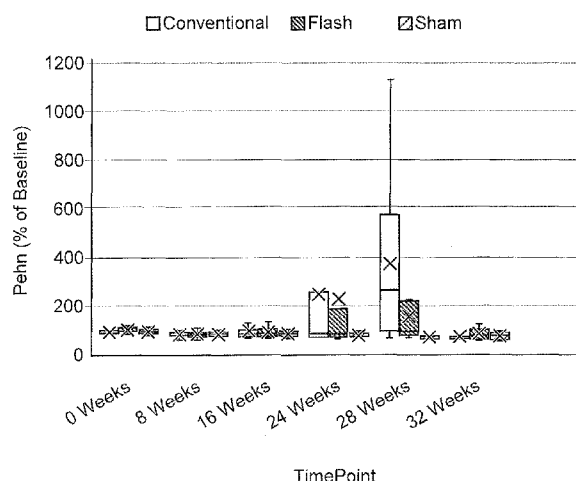


FIG. 6

(57) **Abstract:** Methods for treating tumors by administering FLASH radiation and a therapeutic agent to a patient with cancer are disclosed. The methods provide the dual benefits of anti-tumor efficacy plus normal tissue protection when combining therapeutic agents with FLASH radiation to treat cancer patients. The methods described herein also allow for the classification of patients into groups for receiving optimized radiation treatment in combination with a therapeutic agent based on patient-specific biomarker signatures. Also provided are radiation treatment planning methods and systems incorporating FLASH radiation and therapeutic agents.



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METHODS OF USE OF ULTRA-HIGH DOSE RATE RADIATION AND THERAPEUTIC AGENTS

CROSS-REFERENCES TO RELATED APPLICATIONS

- 5 **[0001]** The present application claims priority from U.S. Provisional Application No. 62/700,783, entitled “Methods of Use of Ultra-High Dose Rate Radiation and Therapeutic Agents” filed July 19, 2019, the entire contents of which is herein incorporated by reference for all purposes.

BACKGROUND OF THE INVENTION

- 10 **[0002]** Radiation therapy is a key therapeutic modality for patients with cancer. Radiation can be delivered to the tumor with submillimeter precision while mostly sparing normal tissue, ultimately leading to tumor cell killing. However, the tumor cell’s ability to escape the cell killing effects of radiation and/or to develop resistance mechanisms can counteract the tumor cell killing action of radiotherapy, potentially limiting the therapeutic effect of radiotherapy to treat
15 cancer. Furthermore, the potential for normal tissue toxicity can impact the therapeutic window of radiation therapy as a treatment paradigm.

- [0003]** Radiation-induced tumor cell death leads to release of tumor antigens from lysed cells, increased MHC-1 expression on antigen presenting cells, and enhanced diversity of the intratumoral T-cell population. These factors and others are key to initiate activation of the
20 body’s own immune systems to eradicate cancer cells. Immune modulators are being explored to activate the body’s own immune system, but are known to have limitations as monotherapy (*e.g.*, response rate in patients). The response rate of immune modulators when used as monotherapy is in the range of 20-30% of the targeted patient population. Combination approaches such as using two immune modulators or an immune modulator with a targeted anti-
25 cancer drug have limitations due to systemic normal tissue toxicity.

BRIEF SUMMARY OF THE INVENTION

[0004] Treating tumors with ultra-high-dose-rate radiation (e.g., FLASH RT) may improve the therapeutic window by decreasing the normal tissue side effects while maintaining tumor toxicity when compared to conventional RT. It is possible to decrease normal tissue side effects by either
5 increasing the dose rate or by limiting the time normal tissue is exposed to radiation. It is possible to enforce the increased therapeutic window by means of multiple discrete fields or continuous rotational delivery of ionizing radiation. Reduced side effects to the normal tissue could also allow for dose escalation in the tumor leading to enhanced tumor kill and control.

[0005] Conventional radiation induces stromal, immunological, and vascular changes that can
10 counteract the tumor cell killing effects of radiation. It was unexpectedly discovered that FLASH irradiation impacts the microenvironment differently, ultimately leading to a more or heightened immune competent tumor environment. As a result, FLASH irradiation combined with a therapeutic agent can increase tumor cell killing while minimizing normal tissue toxicity. In some embodiments, the therapeutic agent is an immune modulator, a senolytic agent, a
15 radiosensitizer, and/or a nanoparticle.

[0006] Several mechanisms are employed to foster immune suppression in the tumor microenvironment including, but not limited to, the recruitment of regulatory T cells (Tregs), tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). In addition, anti-inflammatory elements, such as transforming (or tumor) growth factor beta (TGF-
20 beta) and IL-10, inhibit cytolytic activity of cytotoxic T-cells (CTLs). TAMs and MDSCs modify the metabolic milieu of the tumor microenvironment by producing arginase and nitric oxide that depletes L-arginine, an essential nutrient for T-cell function. Further, abnormal tumor angiogenesis results in hypoxia, which initiates the recruitment of immune-suppressive myeloid cells. Suppressive myeloid cells generate reactive oxygen and nitrogen species that modify
25 receptors on cytotoxic lymphocytes (CTLs) both in the lymphoid organs and in the tumor itself, impacting the ability of CTL's to home to tumors and kill tumor cells.

[0007] When treating a patient with FLASH RT, the immune-suppressive microenvironment does not have an opportunity to fully develop. With a severely limited immune-suppressive tumor microenvironment, there will be an increased immunological response to the dying tumor
30 cells that ultimately will improve tumor cell killing.

[0008] Other factors that play roles in the immune-suppressive microenvironment include tumor-infiltrating macrophages that can develop an M1-M2 phenotype switch that can impact the killing of tumor cells. These effects are not seen with FLASH RT. Another benefit of FLASH RT is that it spares circulating immune cells from radiation induced toxicity, leading to improved immune-related tumor cell killing effects.

[0009] The methods described herein provide the dual benefits of anti-tumor efficacy and normal tissue protection when combining a therapeutic agent with FLASH radiation to treat cancer patients. Methods described herein can be used to treat local and metastatic cancers by FLASH radiation therapy to deliver a highly conformal dose to the tumor, and an immune modulator. This combination therapy has the potential to improve both the efficacy of radiation therapy both locally and systemically, and the efficacy of the immune modulators while minimizing toxicity to normal tissues.

[0010] Protons deposit their energy most densely at the end of their path, a mechanism that has been used to its advantage in cancer radiotherapy (Jones et al., British Journal of Radiotherapy 2006, Jakel Radiat Protection Dosimetry 2009). The characteristic plot of absorbed dose as a function of penetration depth has a maximum at the location just before the particle stops, enabling the therapeutic targeting of tumors at specific depths. In contrast, dose deposition for electron/photon beam radiotherapy is maximal near the entrance surface of the tissue, e.g., the skin, followed by an exponential decrease with tissue depth. Due to these physical characteristics, proton therapy allows for high dose deposition in deep seated tumors.

[0011] The inventors surprisingly determined that an unexpected advantage of proton energy deposition is not only dependent on the absolute dose delivered to the target, but also on the dose rate (speed) used to deliver the dose. It was unexpected to observe a proton dose rate-dependent biology, e.g. different biological pathways are activated when protons are delivered at different dose rates. The dose rate dependent characteristics of proton deposition have major implications for cancer therapy and the development of novel treatment options, including dose rate dependent combination therapies.

[0012] Thus, in one aspect, provided herein is a method for treating a tumor in a subject with cancer comprising administering an effective amount of ultra-high-dose-rate (FLASH) radiation and a therapeutic agent to the tumor. In some embodiments, the method reduces damage to

normal tissue when compared to administering conventional radiation (e.g., a dose of 0.5 Gy/sec) to the tumor. In some embodiments, dermatitis, lung fibrosis or lymphocyte apoptosis are decreased compare to administering conventional radiation.

[0013] In some embodiments, the FLASH radiation is administered at a dose rate equal to or greater than 40 Gy/sec, or the dose is administered in 1 second or less. In some embodiments, the FLASH radiation is administered in a single pulse or in multiple pulses. In some embodiments, the FLASH radiation comprises or consists of protons. In some embodiments, the he FLASH radiation does not comprise electrons.

[0014] In some embodiments, the therapeutic agent is an immune modulator, a radiosensitizer, or a nanoparticle.

[0015] In some embodiments, the therapeutic agent is a mitotic spindle inhibitor, a DNA damage repair and response inhibitor, a MAPK pathway inhibitor, an epithelial to mesenchymal (EMT) inhibitor, an activator of T helper type 1 (TH1) lymphocytes, an activator of the PTEN pathway, and inhibitor of the TGF-beta pathway, an activator of the type-1 interferon signaling pathway, an activator of dendritic cell maturation, an inhibitor of CD47/SIRP-alpha, or an inhibitor of the Aryl Hydrocarbon Receptor (ahR).

[0016] In some embodiments, the mitotic spindle inhibitor is selected from a CDK4/6 inhibitor, an AURKA inhibitor, a TPX2-AURKA complex inhibitor, or a taxane.

[0017] In some embodiments, the DNA damage repair and response inhibitor is selected from a PARP inhibitor, a RAD51 inhibitor, or an inhibitor of a DNA damage response kinase selected from CHCK1, ATM, or ATR.

[0018] In some embodiments, the MAPK pathway inhibitor is an inhibitor of EGFR, MEK, BRAF, or ERK.

[0019] In some embodiments, the EMT inhibitor is a TGFβ-pathway inhibitor selected from a compound, small molecule, antibodies or fragments thereof that bind TGF-beta.

[0020] In some embodiments, the activator of T helper type 1 (TH1) lymphocytes is a cytokine, a toll-like receptor agonist, a STAT3 modulator, compounds derived from inactivated bacteria/ or parasites or their derivatives that trigger interferon gamma or IL-12 production,

included but not limited to *Listeria monocytogenes*, *Leishmania major* and *Toxoplasma gondii*, *Mycobacterium tuberculosis*, staphylococcus enterotoxin B and unmethylated CpG nucleotides that activate a Th1 response in the body, or gene therapy systems including bacterial or virus based gene expression systems that lead to production of IL2, IL-12 and IFN-gamma when

5 injected at the tumor site.

[0021] In some embodiments, the activator of the PTEN pathway is selected from an mTOR inhibitor selected from rapamycin, temsirolimus, everolimus, sirolimus or AP-2357; Ublituximab, Rituximab, Sunitinib, (Induces PTEN), Trastuzumab and Pertuzumab (Increases PTEN through Src inhibition), Resistin (p38 MAPK modulator, increases PTEN), Simvastatin 10 (NF0-kB inhibitor), Lovastatin and Rosiglitazone (PPAR-gamma modulators), NVP-AEW541 (IGF-1R modulator that increases PTEN), and PP1 Herbimycin (Src inhibitors) (see, e.g., Boosani et al Expert Opin Ther Pat. 2013 May; 23(5): 569-580.)

[0022] In some embodiments, the inhibitor of the TGF-beta pathway is selected from a compound, small molecule, antibodies or fragments thereof that bind TGF-beta.

15 [0023] In some embodiments, the activator of the type-1 interferon signaling pathway is a STING agonist, an Toll-like receptor (TLR) agonist, or a MAVS agonist.

[0024] In some embodiments, the activator of dendritic cell maturation is a synthetic peptide vaccine. In some embodiments, the inhibitor of CD47/SIRP-alpha is selected from an antibody or fragment thereof, or a small molecule compound that inhibits the CD47/DSIRP-alpha 20 interaction.

[0025] In some embodiments, the nanoparticle has a high effective atomic number, or comprises gold or gadolinium.

[0026] In some embodiments, the ahR inhibitor is SR1, CH-223191, UM729, or Galangin.

[0027] In some aspects, provided herein is a method for treating a tumor in a subject with 25 cancer comprising administering ionizing FLASH radiation and an immune modulator to the tumor, wherein the immune modulator is selected from the group consisting of an inhibitor to an inhibitory checkpoint molecule, an activator of a stimulatory checkpoint molecule, a chemokine inhibitor, an inhibitor of macrophage migration inhibitory factor (MIF), a growth factor, a

cytokine, an interleukin, an interferon, an antibody that binds to an immune system cell, a cellular immune modulator, a vaccine, an oncolytic virus, and any combination thereof. Administration of the immune modulator was unexpectedly found to increase the anti-tumor response when combined with FLASH radiation.

5 **[0028]** In some embodiments, the inhibitor to the inhibitory checkpoint molecule is a small molecule drug, or an antibody or a fragment thereof that specifically binds to the inhibitory checkpoint molecule and inhibits its activity, wherein the inhibitory checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, BTLA, A2aR, B7-H2, B7-H3, B7-H4, B7-H6, CD47, CD48, CD160, CD244 (2B4), CHK1, CHK2, CGEN-15049, ILT-2, 10 ILT-4, LAG-3, VISTA, gp49B, PIR-B, TIGIT, TIM1, TIM2, TIM3, TIM4, and KIR, and ligands thereof. In some embodiments, the activator of the stimulatory checkpoint molecule is a small molecule drug, polypeptide-based activator, or polynucleotide-based activator that specifically binds to the stimulatory checkpoint molecule and increases its activity, wherein the stimulatory checkpoint molecule is selected from the group consisting of B7-1 (CD80), B7-2 (CD86), 4-1BB (CD137), OX40 (CD134), HVEM, inducible costimulator (ICOS), glucocorticoid-induced tumor necrosis factor receptor (GITR), CD27, CD28, CD40, and ligands thereof. In some instances, the chemokine inhibitor is a small molecule drug, or antibody or fragment thereof that specifically binds to the chemokine (or its receptor) and inhibits chemokine activity. In some 15 embodiments, the chemokine is selected from the group consisting of CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL5, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL5, and CXCL16. In some embodiments, the chemokine inhibitor binds to a chemokine receptor selected from the group consisting of CCR1, CCR2, 25 CCR3, CCR, 4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7. In some cases, the inhibitor of MIF is a small molecule drug, or antibody or fragment thereof that specifically binds to MIF and inhibits MIF activity.

[0029] In some aspects, provided herein is a method for treating a tumor in a subject with cancer comprising administering FLASH radiation and an immune modulator to the tumor. In 30 some embodiments, the immune modulator can be an antibody or antibody fragment targeting

CD44, MMP9, ALDH1A1, Vimentin, hyaluronan, beta-catenin, MFG-E8, CD68, TGF β , a TGF β -pathway related biomarker, or any combination thereof.

[0030] Also provided herein are improved methods for treating a tumor that include administering an immune modulator and FLASH radiation to the subject with cancer. This combination therapy can elicit an increased anti-cancer response compared to immune modulator monotherapy or conventional radiation monotherapy.

[0031] Immunomodulatory agents to be combined with FLASH include, but are not limited, to checkpoint inhibitors, co-stimulators, broad immune modulators modulating the adenosine pathway or STING (Stimulating Interferon Genes) pathways, bispecific antibodies targeting both immune cell antigens and cancer antigens, and cell therapy approaches (e.g., chimeric antigen receptor (CAR) therapies).

[0032] In some embodiments, the positive effects of FLASH RT are enhanced by combining FLASH RT with delivery of an immunotherapy agent as a concomitant, adjuvant, or neo-adjuvant procedure. Thus, the immunomodulating properties of FLASH RT described herein can be enhanced and provide further benefit to a patient or subject in need of treatment when used in combination with immunomodulatory agents. The combination of FLASH RT and immunotherapy can be incorporated into radiation treatment planning as well as in the treatments themselves.

[0033] The combination of FLASH RT and an immune modulator described herein can also be combined with personalized medicine treatment protocols. Thus, in some embodiments, the methods include detecting the expression of one or more biomarkers in the subject. In some embodiments, the one or more biomarkers are selected from CD44, MMP9, ALDH1A1, Vimentin, hyaluronan, beta-catenin, MFG-E8, CD68, TGF β , a TGF β -pathway related biomarker, or any combination thereof. In some embodiments, methods include radiomics information such as tumor phenotype. In some embodiments, methods include functional imaging information, including but not limited to PET, SPECT, and fMRI.

[0034] In some aspects, provided herein is a method for treating a tumor in a subject with cancer comprising administering ionizing FLASH radiation and an immune modulator to the tumor. The method comprises (a) determining an expression level of one or more biomarkers in

a tumor sample from the subject, wherein the one or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), and any combination thereof; (b) comparing the expression level of the one or more biomarkers to an expression level of the one or more biomarkers in a normal tissue sample; and (c) administering to the tumor in the subject a treatment comprising ionizing FLASH radiation and an immune modulator if the expression level of the one or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. The biomarker can be CD44, MMP9, ALDH1A1, Vimentin, hyaluronan, beta-catenin, MFG-E8, CD68, TGF β , a TGF β -pathway related biomarker, or any combination thereof.

[0035] In certain aspects, provided herein is a method, for example an in-vitro method, of identifying a subject with cancer as a candidate for treatment comprising ionizing FLASH radiation and an immune modulator. The method includes: (a) determining an expression level of one or more biomarkers in a tumor sample from the subject, wherein the one or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), imaging marker(s), and any combination thereof; (b) comparing the expression level of the one or more biomarkers to an expression level of the one or more biomarkers in a normal tissue sample; and (c) classifying the subject as a candidate for treatment comprising ionizing FLASH radiation and the immune modulator if the expression level of the one or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. The biomarker can be CD44, MMP9, ALDH1A1, Vimentin, hyaluronan, beta-catenin, MFG-E8, CD68, TGF β , a TGF β -pathway related biomarker, or any combination thereof.

[0036] In other aspects, provided herein is a method, for example an in-vitro method, of selecting a treatment for a subject with cancer. The method comprises: (a) determining an expression level of one or more biomarkers in a tumor sample from the subject, wherein the one or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), and any combination thereof; (b) comparing the expression level of the one or more biomarkers to an expression level of the one or more biomarkers in a normal tissue sample; and (c) selecting a treatment comprising ionizing FLASH radiation and an immune modulator if the expression level of the one or more biomarkers in the tumor sample is

modified compared to the expression level in the normal tissue sample. The biomarker can be CD44, MMP9, ALDH1A1, Vimentin, hyaluronan, beta-catenin, MFG-E8, CD68, TGF β , a TGF β -pathway related biomarker, or any combination thereof.

[0037] In some embodiments, the subject is administered ionizing FLASH radiation and/or combination therapy comprising ionizing FLASH radiation and an immune modulator if the expression level of CD44 is increased and/or the expression level of MFG-E8 is decreased relative to the expression level in a normal or control sample. In some embodiments, the amount of ionizing FLASH radiation and/or the amount of an immune modulator administered to the subject is increased if the expression level of CD44 is increased and/or the expression level of MFG-E8 is decreased relative to the expression level in a normal or control sample. On the other hand, the amount of ionizing FLASH radiation and/or the amount of an immune modulator administered to the subject can be decreased if the expression level of CD44 is decreased and/or the expression level of MFG-E8 is increased relative to the expression level in a normal or control tissue sample.

[0038] In some embodiments, (a) treatment comprising ionizing FLASH radiation and an immune modulator is selected if the expression level of CD44 is increased and/or the expression level of MFG-E8 is decreased relative to the expression level in a normal or control sample; (b) the amount of ionizing FLASH radiation and/or the amount of an immune modulator selected is increased if the expression level of CD44 is increased and/or the expression level of MFG-E8 is decreased relative to the expression level in a normal or control sample; and (c) the amount of ionizing FLASH radiation and/or the amount of an immune modulator selected is decreased if the expression level of CD44 is decreased and/or the expression level of MFG-E8 is increased relative to the expression level in a normal or control tissue sample.

[0039] In some embodiments, the subject is administered ionizing FLASH radiation and/or combination therapy comprising ionizing FLASH radiation and an immune modulator if the expression level of CD68 is increased relative to the expression level in a normal or control tissue sample. In some embodiments, the amount of ionizing FLASH radiation and/or the amount of an immune modulator administered to the subject is increased if the expression level of CD68 is increased relative to the expression level in a normal or control tissue sample. On the other hand, the amount of ionizing FLASH radiation and/or the amount of an immune modulator

administered to the subject can be decreased if the expression level of CD68 is decreased relative to the expression level in a normal or control tissue sample.

[0040] In some embodiments, (a) treatment comprising ionizing FLASH radiation and an immune modulator is selected if the expression level of CD68 is increased relative to the expression level in a normal or control tissue sample; (b) the amount of ionizing FLASH radiation and/or the amount of an immune modulator selected is increased if the expression level of CD68 is increased relative to the expression level in a normal or control tissue sample; (c) the amount of ionizing FLASH radiation and/or the amount of an immune modulator selected can be decreased if the expression level of CD68 is decreased relative to the expression level in a normal or control tissue sample.

[0041] In some aspects, provided herein is use of FLASH radiation and a therapeutic agent for treating a tumor in a subject. In some embodiments, the use comprises a combination of ionizing FLASH radiation and a therapeutic agent selected from an immune modulator described herein, a senolytic agent, and/or a radiosensitizer described herein.

[0042] In another aspect, the disclosure provides a therapeutic agent for use in a method of treating a tumor in a subject with cancer, characterized in that the method comprises administering FLASH radiation and the therapeutic agent to the tumor. In some embodiments, a therapeutic agent in combination with ultra-high dose rate (FLASH) radiation for use in the treatment of cancer or a tumor is provided.

[0043] In another aspect, the disclosure provides a therapeutic agent for use in a method of treating a tumor in a subject with cancer, characterized in that the therapeutic agent is administered in combination with FLASH radiation.

[0044] In another aspect, the disclosure provides a therapeutic agent for use in a method of treating a tumor in a subject with cancer, wherein the therapeutic agent is administered to a heightened immune competent tumor environment caused by said FLASH radiation.

[0045] In another aspect, the disclosure provides a hedgehog antagonist for use in a method of treating a tumor in a subject with cancer, characterized in that the hedgehog antagonist is administered in combination with FLASH radiation.

[0046] In another aspect, provided herein is a therapeutic agent for use in a method of treating a tumor in a subject with cancer, characterized in that the method comprises:

(a) determining an expression level of one or more biomarkers in a tumor sample from the subject, wherein the one or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), and any combination thereof;

(b) comparing the expression level of the one or more biomarkers to an expression level of the one or more biomarkers in a normal tissue sample; and

(c) administering to the tumor in the subject a treatment comprising ionizing FLASH radiation and the therapeutic agent if the expression level of the one or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample.

[0047] In another aspect, a method for treating a tumor in a subject is described, the method comprising:

i) detecting expression of a biomarker described herein in the tumor microenvironment or a tumor sample from a subject, and

ii) administering an effective amount of FLASH radiation and a therapeutic agent to the subject, thereby treating the tumor.

[0048] In some embodiments, the expression level of the biomarker(s) described herein is compared to the expression level detected in a normal or control (e.g., non-tumor) tissue in the subject.

[0049] In another aspect, a method for treating a tumor in a subject is described, the method comprising:

i) contacting a tumor sample from the subject with an antibody that binds to a biomarker described herein;

ii) detecting modified expression of the biomarker in the tumor sample, and

iii) administering an effective amount of FLASH radiation and a therapeutic agent to the subject, thereby treating the tumor.

[0050] In some embodiments, the modified biomarker expression comprises increased and/or decreased expression levels of the biomarker. In some embodiments, the biomarker is selected

from the group consisting of CD44, MMP9, ALDH1A1, Vimentin, hyaluronan, beta-catenin, MFG-E8 and CD68. In some embodiments, the expression level of biomarker CD68 is increased in the tumor environment or in the tumor sample. In some embodiments, the expression level of biomarker CD44 is increased in the tumor environment or in the tumor sample. In some
5 embodiments, the expression level of biomarker CD44 is increased and the expression level of MFG-E8 is decreased in the tumor environment or in the tumor sample.

[0051] In some embodiments, the method further comprises detecting the expression level of a biomarker described herein in a normal tissue sample. In some embodiments, the expression level of the biomarker is detected with an antibody that binds to the biomarker(s).

10 **[0052]** In another aspect, a radiation treatment system is provided. In some embodiments, the radiation treatment system provides radiation treatment for a subject or patient in need thereof. In some embodiments, the radiation treatment system further comprises an immunotherapy system that treats the patient with a therapeutic agent in coordination with the radiation treatment. In some embodiments, the radiation treatment is FLASH RT.

15 **[0053]** In another aspect, a radiation treatment planning system is described, the radiation treatment planning system operable for generating a radiation treatment plan that also includes immunotherapy performed in coordination with the radiation treatment. In some embodiments, the radiation treatment is FLASH RT.

20 **[0054]** In another aspect, a non-transitory computer-readable storage medium having computer-executable instructions for causing a computing system to perform a method of ultra-high-dose-rate (FLASH) radiation treatment planning for treating a tumor in combination with a therapeutic agent is described. In some embodiments, said method comprises adjusting beam parameters using information about treatment of the tumor with a therapeutic agent.

25 **[0055]** In some embodiments, provided is a non-transitory computer-readable storage medium having computer-executable instructions for causing a computing system to perform a method of ultra-high-dose-rate (FLASH) radiation treatment planning for treating a tumor in combination with a therapeutic agent, the method comprising:

accessing values of parameters from memory of the computing system, wherein the parameters comprise directions of beams to be directed into sub-volumes in a target and beam energies for the beams;

accessing information that specifies limits for the radiation treatment plan,
5 wherein the limits are based on a dose threshold and comprise a limit on irradiation time for each sub-volume outside the target;

wherein the information that specifies limits comprises information about treatment of the tumor with a therapeutic agent; and
adjusting the values of the parameters that affect a calculated amount of dose to be delivered by
10 the beams until differences between respective total values for the sub-volumes satisfy a threshold value.

[0056] In some embodiments, each portion of the beams that is in the target is represented as a respective set of longitudinal beam regions, and wherein the method further comprises:

for each of the beam regions, computing an amount of dose to be delivered by a
15 beam region and assigning a value to the beam region corresponding to the amount; and

for each of the sub-volumes, computing a total value for the sub-volume by adding together the value for each beam region of each beam that reaches the sub-volume; wherein said adjusting further comprises adjusting the parameters that affect the calculated amounts of dose to be delivered by the beam regions until differences between respective total
20 values for the sub-volumes satisfy the threshold value.

[0057] In some embodiments, said adjusting further comprises:

determining whether a beam overlaps any other beams outside the target; and weighting beam intensities for beam segments of the beam according to how many other beams are overlapped by the beam outside the target.

25 **[0058]** In some embodiments, the method further comprises performing a dose calculation for an outside-the-target sub-volume, wherein said performing a dose calculation comprises:

accessing a value for a dose calculation factor for the outside-the-target sub-volume, wherein the value for the dose calculation factor is determined according to how many beams reach the outside-the-target sub-volume;

calculating a dose for the outside-the-target sub-volume; and
applying the value of the dose calculation factor to the dose calculated for the outside-the-target sub-volume.

[0059] In some embodiments, the dose calculation factor reduces the dose calculated for the outside-the-target sub-volume if only one beam reaches the outside-target sub-volume.

[0060] In some embodiments, the limits are selected from the group consisting of: a limit on irradiation time for each sub-volume in the target; a limit on dose rate for each sub-volume in the target; and a limit on dose rate for each sub-volume outside the target.

[0061] In some embodiments, the dose threshold is further dependent on tissue type.

In some embodiments, the beams comprise a type of beam selected from the group consisting of: proton; electron; photon; atom nuclei; and ion.

[0062] In another aspect, a computer-implemented method of radiation treatment planning for treating a tumor in combination with a therapeutic agent is described. In some embodiments, the method comprises determining beam parameters for ultra-high-dose-rate (FLASH) radiation, the method using a prescribed dose based on response of the tumor to a therapeutic agent.

[0063] In some embodiments, the computer-implemented method of radiation treatment planning comprises:

determining a prescribed dose of ultra-high-dose-rate (FLASH) radiation to be delivered into and across a tumor target, wherein the prescribed dose is determined based on a response of the tumor to a therapeutic agent;

accessing values of parameters comprising a number of beams in a plurality of beams to be directed into sub-volumes in the target, directions of the plurality of beams, and beam energies for the plurality of beams, wherein each of the beams comprises a plurality of beam segments;

identifying any overlapping beams in the plurality of beams that have respective beam paths that overlap outside the target;

for each beam in the plurality of beams, determining a maximum beam energy for the beam and determining beam energies for the beam segments of the beam as a percentage of the maximum beam energy for the beam; and

for each overlapping beam of the overlapping beams that overlap outside the target, reducing the beam intensities for the beam segments of the overlapping beam by a dose calculation factor, wherein the beam intensities for the beam segments for the plurality of beams are determined such that a cumulative dose delivered to the target satisfies the prescribed dose.

5 **[0064]** In some embodiments, the method comprises:

representing each of the beams in the target as a respective set of longitudinal beam regions, wherein each beam region in the set has a value corresponding to a calculated amount of dose to be delivered by the beam region;

10 for each sub-volume in the target, adding together the value for each beam region of each beam that reaches the sub-volume to determine a total value for the sub-volume, to produce respective total values for the sub-volumes in the target; and
adjusting the values of the parameters that affect the calculated amounts of dose to be delivered by the beam regions until differences between the total values for the sub-volumes satisfy a threshold value.

15 **[0065]** In some embodiments, the method comprises:

accessing a value for the dose calculation factor for an outside-the-target sub-volume, wherein the value for the dose calculation factor is determined according to how many beams reach the outside-the-target sub-volume;

20 calculating a dose for the outside-the-target sub-volume; and
applying the value of the dose calculation factor to the dose calculated for the outside-the-target sub-volume, wherein the dose calculation factor reduces the dose calculated for the outside-the-target sub-volume if only one beam reaches the outside-target sub-volume.

[0066] In some embodiments, the method comprises using a dose threshold to specify limits for the radiation treatment plan, wherein the limits are selected from the group consisting of: a
25 limit on irradiation time for each sub-volume in the target; a limit on irradiation time for each sub-volume outside the target; a limit on dose rate for each sub-volume in the target; and a limit on dose rate for each sub-volume outside the target.

[0067] In some embodiments, the dose threshold is dependent on a plurality of biological factors including but not limited to tissue type and/or immunological profile.

[0068] In some embodiments, the beams comprise a type of beam selected from the group consisting of: proton; electron; photon; atom nuclei; and ion.

[0069] In another aspect, use of ultra-high-dose-rate (FLASH) radiation in combination with a therapeutic agent for treating cancer or a tumor in a subject in need thereof is provided.

5 **[0070]** In another aspect, ultra-high-dose-rate (FLASH) radiation in combination with a therapeutic agent for use in the treatment of cancer or a tumor is provided.

[0071] In any of the aspects and embodiments described herein, the therapeutic agent can be an immune modulator, a senolytic agent, a radiosensitizer, and/or a nanoparticle.

[0072] In another aspect, a method for reducing activation of the hedgehog signaling pathway
10 in a subject is provided. In some embodiments, the method comprises administering a therapeutically effective amount of ultra-high-dose-rate (FLASH) radiation to the subject, where activation of the hedgehog signaling pathway is reduced compared to administering conventional proton radiation therapy to the subject. In some embodiments, the subject has cancer or a tumor. In some embodiments, the method comprises administering a therapeutically effective amount of
15 ultra-high-dose-rate (FLASH) radiation to a tumor in the subject. In some embodiments, FLASH radiation is administered as proton therapy, or the FLASH radiation comprises or consists of protons.

[0073] In some embodiments, the expression of genes activated by the hedgehog signaling pathway is decreased when FLASH radiation is administered to the subject compared to
20 administering conventional proton radiation therapy. In some embodiments, the expression of one or more genes selected from CELSR1, TLE3, OPHN1, GPR56, PTCH1, TLE1, MYH9, RASA1, HEY1, ETS2, HEY2, LDB1, UNC5C, NF1, CDK6, VLDLR, NRP2, DPYSL2, and NRP1 is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy. In some embodiments, the expression of
25 one or more proteins encoded by genes selected from CELSR1, TLE3, OPHN1, GPR56, PTCH1, TLE1, MYH9, RASA1, HEY1, ETS2, HEY2, LDB1, UNC5C, NF1, CDK6, VLDLR, NRP2, DPYSL2, and NRP1 is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy. In some embodiments, the expression of one or more genes encoding proteins having an amino acid sequence selected from

SEQ ID Nos: 15-70 is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy. In some embodiments, the expression of one or more proteins having an amino acid sequence selected from SEQ ID Nos: 15-70 is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy. In some embodiments, the expression of one or more genes encoding a protein in Table 7 is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy. In some embodiments, the expression of one or more proteins in Table 7 is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy.

[0074] In some embodiments, the method further comprises administering a therapeutically effective amount of a hedgehog antagonist to the subject. In some embodiments, the hedgehog antagonist is selected from a Smo antagonist, a PTCH1 inhibitor, Cyclopamine, Vismodegib, LDE 225, saridegib, BMS 833923, LEQ 506, PF- 04449913 , PF-5274857, GANT61, SANT-1, Glasdegib (PF-04449913), Taladegib (LY2940680) or TAK-441. In some embodiments, the subject has cancer or a tumor. In some embodiments, the method comprises administering a therapeutically effective amount of ultra-high-dose-rate (FLASH) radiation and a therapeutically effective amount of a hedgehog antagonist, to a tumor in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0075] FIG. 1. illustrates a cross-sectional view of a sample beam geometry in an embodiment described herein.

[0076] FIG. 2. shows a representative dose surface plot as an example of the variables used to determine dose threshold in an embodiment described herein.

[0077] FIG. 3. is a block diagram of an example of a computing system upon which the embodiments described herein may be implemented.

[0078] FIG. 4. is a block diagram illustrating an example of an automated radiation treatment planning system in an embodiment described herein.

[0079] FIG. 5 shows the experimental design for normal tissue toxicity studies in control mice (sham treated) and mice treated with conventional, FLASH, and split-Flash radiation.

[0080] FIG. 6 shows improved lung function for FLASH when compared to Conventional radiation treatment.

5 [0081] FIG. 7 shows an improved median survival for FLASH over Conventional radiation treatment groups: 20.0 Gy: 14% increase; 17.5 Gy: 18% increase.

[0082] FIG. 8 shows an improved median survival for FLASH over Conventional radiation treatment groups and the potential for a therapeutic window that is dose rate dependent: 17.5 Gy FLASH > 17.5 Gy CON; 17.5 Gy FLASH = 20.0 Gy CON.

10 [0083] FIG. 9 shows a reduction in average dermatitis for FLASH when compared to Conventional radiation: FLASH: 34% reduction; Split-FLASH: 52% reduction.

[0084] FIG. 10 shows a 23% reduction in average lung fibrosis severity for FLASH over Conventional group at 17.5 Gy.

15 [0085] FIG. 11 shows an increased average lung weight for Conventional when compared to FLASH treatment – 0.46 g vs. 0.40 g (M); 0.37 g vs. 0.32 g (F)).

[0086] FIG. 12A-D shows Venn Diagrams of Differentially Expressed genes at each time point. **A)** DE genes at 24 hours **B)** 8 weeks **C)** 16 weeks **D)** 24 weeks with adjusted p-values less than or greater than 0.05.)

20 [0087] FIG. 13A-D shows overlap of GSEA Hallmark pathways between three treatment groups. **A)** GSEA at 24 hours **B)** 8 weeks **C)** 16 weeks **D)** 24 weeks with FDR-q values < 0.25 and adjusted p-values < 0.05. Underlined text denotes downregulated while italicized text denotes upregulated pathways relative to Sham treated samples.

25 [0088] FIG. 14A-D shows Hallmark Mitotic Spindle enrichment plots and core enrichment genes for Split Flash-Sham and Flash-Sham GSEA analysis. Enrichment plot for Hallmark Mitotic Spindle for **A)** Split-Flash and **B)** Flash treatment group gene expression heatmaps of core enrichment genes for Split-Flash and Flash. **C)** Overlap between split flash and flash treatment groups. **D)** List of genes from Fig.14C, **BOLD** genes are key players in Mitotic Spindle assembly.

[0089] FIG. 15A and 15B show Flash Specific DNA repair signature and core enrichment genes: A) (Top left): Enrichment plot of DNA repair signature (Bottom Left): Enrichment statistics for DNA Repair Signature. B) Gene expression heatmap of core Enrichment genes in the Hallmark DNA repair signature.

5 **[0090]** FIG. 16A-C shows Flash Specific KRAS Signaling Up 8 Weeks-24 Weeks: (Top): Enrichment plot of KRAS signaling Up signature & enrichment statistics for A) 8 Weeks B) 16 weeks and C) 24 weeks for flash treated mice.

[0091] FIG. 17A-C shows EMT Signature and Core enrichment gene analysis: EMT Enrichment plot and statistics for 24 week A) Conventional B) Flash treated cells. C) Overlap of
10 Core enrichment genes between Conventional and Flash, highlighting TGF Beta genes. See Table 5 for details core enrichment genes and full list of overlap.

[0092] FIG. 18 shows a pie chart representing various canonical pathways found to be regulated by different radiation regimens at 24 Hr. Analysis was done on filtered list of genes having p value of < 0.05 . samples. Pathways presented here and listed in Table 6 have p value < 0.05 , FDR < 0.1 and Z score of > 1.5 . Underlined text denotes downregulated while italicized text denotes upregulated pathways relative to Sham treated samples.
15

[0093] FIG. 19 shows a heat map of comparative analysis of major canonical pathways regulated by both Flash and Conventional radiation treatment as analyzed by IPA. P value 0.05 and Z score of > 1.5 . pathways found to be differentially regulated between these treatment
20 regimens were mostly involved in inflammation and immune regulation.

[0094] FIG. 20 shows a pie chart representing various canonical pathways found to be regulated by different radiation regimens at 16 weeks. Analysis was done on filtered list of genes having p value of < 0.05 . samples. Pathways presented here and listed in Table 4 have p value < 0.05 , FDR < 0.1 and Z score of > 1.5 . Underlined text denotes downregulated while italicized text denotes upregulated pathways relative to Sham treated samples.)
25

[0095] FIG. 21 shows a heat map of comparative analysis of major canonical pathways regulated by both Flash and Conventional radiation treatment at 16 weeks as analyzed by IPA. P

value 0.05 and Z score of >1.5 . pathways found to be differentially regulated between these treatment regimens were mostly involved in inflammation and immune regulation.

[0096] FIG. 22 shows the result of QuPath cell detection applied to a DAPI image.

[0097] FIG. 23A and B shows (A) the original FITC image, and (B) the segmented TUNEL positive cells. The Fiji distribution of ImageJ was used to quantify the number of TUNEL positive cells in each field of view. A gaussian blur was first applied to the image, and then thresholding was used to segment the objects.

[0098] FIG. 24 is a graph showing the TUNEL results. The graph shows the average number of counts per animal for the roughly 20 animals per group (males and females pooled).

[0099] Fig. 25 shows differential gene expression and pathway analysis of FLASH versus Conventional samples 24 hours post irradiation. Fig. 25A shows a volcano plot of top differentially expressed genes. Red dots represents genes with FDR values less than 5%, for clarity labeled genes represent genes with greater than a 1.3 log fold change in expression. Fig. 25B shows a table of the top GSEA Hallmarks enriched between FLASH and Conventional radiation. Fig. 25C shows an enrichment plot and the top 50 leading edge genes for GSEA's Hallmark Inflammatory Response. Fig. 25D shows IPA result for the top enriched pathways in a microarray dataset between FLASH and Convectional radiation treated samples.

DEFINITIONS

[0100] The term “treating” refers to administering a treatment to a tumor or the subject diagnosed with a tumor. The treatment can be administered in an amount or therapeutic dose that is sufficient or effective to kill tumor cells (i.e., a therapeutically effective amount), slow the growth of the tumor, reduce the size of the tumor, or eliminate the tumor from the subject entirely. Examples of treatments include ionizing radiation, such as FLASH radiation therapy, a therapeutic agent, an immune modulator agent, a senolytic agent, a radiosensitizer, a nanoparticle or combinations thereof. The term also includes selecting a treatment or treatment plan, and providing treatment options to a healthcare provider or the subject.

[0101] The term “therapeutic agent” refers to an agent such as a small molecule drug or biologic drug that can be used to treat a tumor, and can include an immune modulator, a

senolytic agent, a radiosensitizer, or a nanoparticle. The therapeutic agent can be an agent approved by a regulatory agency for treating tumors or cancer, undergoing clinical trials prior to regulatory approval, or that is under investigation for treating tumors or cancer. The therapeutic agent can be combined with radiation therapy, such as conventional or FLASH radiation therapy, to treat a tumor. In some embodiments, the therapeutic agent is combined with FLASH radiation therapy to treat a tumor.

[0102] The term “ionizing radiation” refers to radiation comprising particles having enough kinetic energy to discharge an electron from an atom or molecule, thereby producing an ion. The term includes both directly ionizing radiation, such as that caused by atomic particles such as alpha particles (helium nuclei), beta particles (electrons), and protons, and indirectly ionizing radiation, such as photons, including gamma rays and x-rays. Examples of ionizing radiation used in radiation therapy include high energy x-rays, electron beams, ion beams, and proton beams.

[0103] The terms “FLASH”, “FLASH radiation” or “FLASH Radiation Therapy (FLASH RT)” refer to ultra-high-dose-rate radiation that is administered to a subject or patient as therapy. In some embodiments, the dose can be administered to a subject or patient in need of treatment in one to many short pulses at an ultra-high dose rate. In some embodiments, the entire radiation dose is delivered in a total “beam-on” time of no more than one second.

[0104] FLASH refers to a mode of administering ionizing radiation at a dose rate which ensures all normal tissue irradiation occurs in 1 second or less for the delivery of the entire dose prescription. For example, a dose prescription of 20 Gray (Gy) would require a dose rate of at least 20 Gy per second for a single irradiation direction, 10 Gy per second for 2 irradiation directions, 5 Gy per second for 4 irradiation directions, and so on. The number of fields can reduce the dose rate to satisfy the FLASH irradiation conditions so long as either the fields do not overlap or overlapping fields are delivered in the 1 second or less time frame.

[0105] Pulsed FLASH is a mode of administering ionizing radiation at a dose rate which results in the total active delivery time to normal tissue for a prescribed dose of ionizing radiation in 1 second or less, with allowance for repeated irradiation of the same normal tissue volume in a single treatment session or fraction. For example a 20 Gy prescription would be delivered in 1 second or less of active irradiation time, but could be divided into 5 pulses of 4 Gy spaced 1

second apart, each pulse delivered in 0.2 seconds, or 20 Gy per second per pulse. Another example is 10 pulses of 2 Gy having a duration of 0.1 seconds spaced 2 seconds apart, or 20 Gy per second per pulse. Pulsed FLASH allows for freedom of duty cycle selection for the delivery so long as the total active delivery time of 1 second or less is enforced for a single treatment session. Pulse separation can vary from an interval between pulses of less than a second to several minutes.

[0106] Fractionated FLASH is the delivery of FLASH or pulsed FLASH ionizing radiation over the course of a longer time interval. This time interval can be hours to days following the established clinical protocols for fractionated radiation therapy, with the actual treatment delivery being either FLASH or pulsed FLASH.

[0107] The term “conventional radiation therapy” or “conventional irradiation” of tissues refers to a dose of 0.5 Gy per second, for example 20 Gy in 40 seconds, 17.5 Gy in 35 seconds, or 15 Gy in 30 seconds (0.5 Gy/sec).

[0108] The term “tumor environment” or “tumor micro-environment” refers to the immediate small-scale environment of an organism or part of an organism, especially as a distinct part of a larger environment, for example, the immediate small-scale environment of the tumor. The term includes not only the tumor cells themselves, but associated blood-vessels (including endothelial cells and smooth muscle cells), immune system cells and secreted cytokines, epithelial cells, fibroblasts, connective tissue, and/or extracellular matrix that is associated with or surrounds the tumor. The term also refers to the cellular and extracellular environment in which the tumor is located.

[0109] The term “standard of care” or “standard radiation treatment protocol” in radiation therapy generally refers to the ionizing radiation dose and administration interval that is generally accepted in the medical field as appropriate treatment for a given tumor, based on the tumor type, size, tissue location, and various other biological parameters. The standard of care or standard treatment protocol varies and is dependent on several factors. For example, for radiation therapy of lung cancer, the standard of care includes multiple fractions (*e.g.*, approximately 30 fractions of low dose radiation, or approximately 60 Gy over 6 weeks) or a smaller number of fractions (*e.g.*, 1-5 fractions) of biologically active doses (*e.g.*, 54 GY in 3

fractions for peripheral tumors, or 48-60 Gy in 4-8 fractions for central tumors) administered to the tumor.

[0110] The term “similar dose of ionizing radiation” refers to a dose of ionizing radiation that is identical to, nearly the same, or substantially the same as the effective dose administered to a tumor in another subject, or administered to a tumor in the same subject undergoing an existing course of treatment. The term encompasses the normal and expected variation in ionizing radiation doses delivered by a medical technician skilled in the art of administering ionizing radiation to a tumor in a subject. For example, the term encompasses variation in the effective dose administered to a tumor of less than 10%, less than 5%, or less than 1%. The subject can be a human or non-human animal, such as a companion animal (*e.g.*, cat, dog) or farm animal (*e.g.*, cow, horse, *etc.*).

[0111] The term “expression level” refers to the amount or level and/or the presence or absence of a biomarker described herein.

[0112] The term “small molecule drug” refers to an organic compound having a molecular weight of less than about 50 kDa, less than about 10 kDa, less than about 1 kDa, less than about 900 daltons, or less than about 500 daltons. The term includes drugs having desired pharmacological properties, and includes compounds that can be administered orally or by injection.

[0113] The term “radiosensitizer” refers to any substance that makes tumor cells easier to kill with radiation therapy. Exemplary radiosensitizers include hypoxia radiosensitizers such as misonidazole, metronidazole, and trans-sodium crocetin, and DNA damage response inhibitors such as Poly (ADP) ribose polymerase (PARP) inhibitors.

[0114] The term “reduced tissue damage” or “reduces damage to normal tissue” refers to a decrease in tissue damage when administering FLASH radiation versus conventional radiation to a subject. The decreased tissue damage can be determined by measuring or quantifying a cellular or tissue response to irradiation, such as but not limited to dermatitis, fibrosis, cell death, or respiratory disorder. In some embodiments, reduced tissue damage refers to a decrease in tissue damage of at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% or more in the

cellular or tissue response to irradiation when administering FLASH versus conventional radiation to a subject.”

[0115] The terms “sample,” “biological sample,” and “tumor sample” refer to bodily fluid, such as but not limited to blood, serum, plasma, or urine, and/or cells or tissues obtained from a subject or patient. In some embodiments, the sample is a formalin-fixed and paraffin embedded tissue or tumor sample. In some embodiments, the sample is a frozen tissue or tumor sample. In some embodiments, the tumor sample can be a biopsy comprising tumor cells from the tumor. In some embodiments, the subject or patient is an animal or mammal. In some embodiments, the subject or patient is a human.

[0116] The term “about” refers to variation in measurements of any value described herein, or administered doses described herein, typically encountered by one of ordinary skill in the art. Thus, the term “about” includes variation of plus or minus 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, or 10.0 percent of any value described herein. Any value described herein is considered modified by the term “about” whether or not the term “about” is used. Any numerical range described herein includes the endpoints and all values in between the endpoints. For example, the range of about 1 to 10 includes about 1.0, about 1.1, about 1.2, . . . about 9.8, about 9.9, or about 10.0.

DETAILED DESCRIPTION OF THE INVENTION

[0117] The methods described herein provide the advantages of anti-tumor efficacy and normal tissue protection when combining a therapeutic agent such as an immune modulator, senolytic agent, radiation sensitizer or nanoparticle with FLASH radiation to treat cancer patients. The methods described herein provide the unexpected result that FLASH radiation in combination with a therapeutic agent (e.g., immune modulator therapy) can increase the anti-tumor response compared to treatment with FLASH radiation therapy or the therapeutic agent therapy alone (monotherapy). The increase in the anti-tumor response can enhance or increase the inhibition of tumor growth that is provided by either monotherapy alone. Methods described herein can be used to treat local and metastatic cancers by administering FLASH radiation therapy to deliver a highly conformal dose to the tumor, and a therapeutic agent. The combination therapy described herein can improve both the efficacy of FLASH radiation therapy

(locally and systemically) and the efficacy of the therapeutic agent therapy. In some embodiments, the therapeutic agent or immune modulator also enhances the anti-cancer response when administered in combination with FLASH radiation, compared to administration of either the therapeutic agent alone or FLASH radiation monotherapy. The methods described herein can also increase the anti-tumor response compared to treatment with conventional radiation therapy or therapeutic agent therapy alone (monotherapy).

[0118] In one aspect, a method for treating a tumor in a subject with cancer comprising administering FLASH RT and an immune modulator to the tumor is provided. The immune modulator can be selected from the group consisting of an inhibitor to an inhibitory checkpoint molecule, an activator of a stimulatory checkpoint molecule, a chemokine inhibitor, an inhibitor of macrophage migration inhibitory factor (MIF), a growth factor, a cytokine, an interleukin, an interferon, an antibody that binds to an immune system cell, such as a bispecific antibody that binds to T-cells and a tumor antigen, a cellular immune modulator such as a CAR-T cell, a vaccine, an oncolytic virus, and any combination thereof. In some embodiments, the inhibitor to the inhibitory checkpoint molecule is a small molecule drug, or an antibody or a fragment thereof that specifically binds to the inhibitory checkpoint molecule and inhibits its activity, wherein the inhibitory checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, BTLA, A2aR, B7-H2, B7-H3, B7-H4, B7-H6, CD47, CD48, CD160, CD244 (2B4), CHK1, CHK2, CGEN-15049, ILT-2, ILT-4, LAG-3, VISTA, gp49B, PIR-B, TIGIT, TIM1, TIM2, TIM3, TIM4, and KIR, and ligands thereof. In other embodiments, the activator of the stimulatory checkpoint molecule is a small molecule drug, polypeptide-based activator, or polynucleotide-based activator that specifically binds to the stimulatory checkpoint molecule and increases its activity, wherein the stimulatory checkpoint molecule is selected from the group consisting of B7-1 (CD80), B7-2 (CD86), 4-1BB (CD137), OX-40 (CD134), HVEM, inducible costimulator (ICOS), glucocorticoid-induced tumor necrosis factor receptor (GITR), CD27, CD28, CD40, and ligands thereof. In certain embodiments, the chemokine inhibitor is a small molecule drug, or antibody or fragment thereof that specifically binds to the chemokine (or its receptor) and inhibits chemokine activity. In some embodiments, the chemokine is selected from the group consisting of CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL5, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7,

CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL5, and CXCL16. In some embodiments, the chemokine inhibitor binds to a chemokine receptor selected from the group consisting of CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7. The inhibitor of MIF can be a small molecule drug, or antibody or fragment thereof that specifically binds to MIF and inhibits MIF activity. Other inhibitors of macrophage migration can also be used. In some embodiments, the immune modulator is an inhibitor of indoleamine 2, 3-dioxygenase (IDO).

[0119] The method can further include: (a) detecting an expression level of one or more biomarkers in a tumor sample from the subject, wherein the one or more biomarkers, e.g., 1, 2, 3, 4, 5 or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), and any combination thereof; (b) comparing the expression level of the one or more biomarkers, e.g., 1, 2, 3, 4, 5 or more biomarkers to the expression level of the one or more biomarkers, e.g., 1, 2, 3, 4, 5 or more biomarkers in a normal tissue sample; and (c) treating the tumor with FLASH RT and an immune modulator if the expression level of the one or more biomarkers, e.g., 1, 2, 3, 4, 5 or more biomarkers is modified compared to the expression level in the normal tissue sample. In some instances, the expression level of the one or more biomarkers, e.g., 1, 2, 3, 4, 5 or more biomarkers is modified if the expression level of at least one of the biomarkers is increased, or the expression level of at least one of the biomarkers is decreased, or the expression level of at least one of the biomarkers is increased and the expression level of at least one of the biomarkers is decreased compared to the expression level in a normal tissue sample. The expression level of the one or more biomarkers, e.g., 1, 2, 3, 4, 5 or more biomarkers can be ranked or weighted.

[0120] In some embodiments, the immune cell biomarker(s) or the tumor cell biomarker(s) or the circulating biomarker(s) is a polynucleotide or a protein. The step of detecting can be performed by using an assay selected from the group consisting of immunohistochemistry, ELISA, Western analysis, HPLC, proteomics, PCR, RT-PCR, Northern analysis, and a microarray.

[0121] The tumor sample can be a biopsy comprising tumor cells. The normal tissue sample can comprise non-tumor cells from the same tissue type as the tumor.

[0122] In another aspect, provided herein is a method of treating a tumor in a subject with cancer comprising: (a) determining an expression level of one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in a tumor sample from the subject, wherein the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), and any combination thereof; (b) comparing the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers to an expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in a normal tissue sample; and (c) administering to the tumor in the subject a treatment comprising FLASH RT and a therapeutic agent if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample.

[0123] In some instances, the step of administering FLASH RT comprises contacting the tumor with a radiosensitizer. The FLASH RT can be administered at a higher dose compared to a standard treatment protocol if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. The FLASH RT can be administered as a hypofractionated radiation treatment if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. In other cases, the FLASH RT is administered as a hyperfractionated radiation treatment if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample.

[0124] In yet another aspect, provided herein is a method of identifying a subject with cancer as a candidate for treatment comprising FLASH RT and a therapeutic agent. The method comprises (a) determining an expression level of one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in a tumor sample from the subject, wherein the one or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), imaging marker(s), and any combination thereof; (b) comparing the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers to an expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in a normal tissue sample; and (c)

classifying the subject as a candidate for treatment comprising FLASH RT and the therapeutic agent if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. In some instances, the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers is modified if the expression level of at least one of the biomarkers is increased, or the expression level of at least one of the biomarkers is decreased, or the expression level of at least one of the biomarkers is increased and the expression level of at least one of the biomarkers is decreased compared to the expression level in a normal tissue sample. In certain cases, the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers is ranked or weighted. In some cases, the method further comprises performing functional imaging of the tumor.

[0125] In another aspect, provided herein is a method of selecting a treatment for a subject with cancer comprising (a) determining an expression level of one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in a tumor sample from the subject, wherein the one or more biomarkers are selected from the group consisting of an immune cell marker(s), tumor cell marker(s), circulating marker(s), and any combination thereof; (b) comparing the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers to an expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in a normal tissue sample; and (c) selecting a treatment comprising FLASH RT and a therapeutic agent if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. In some embodiments, the method comprises performing functional imaging of the tumor; and selecting the treatment comprising FLASH RT and a therapeutic agent based on the functional imaging of the tumor. In some cases, the FLASH RT comprises contacting the tumor with a radiosensitizer.

[0126] In any of the above aspects and embodiments, the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers is modified if the expression level of at least one of the biomarkers is increased, or the expression level of at least one of the biomarkers is decreased, or the expression level of at least one of the biomarkers is increased and the expression level of at least one of the biomarkers is decreased compared to the expression level

in a normal tissue sample. The expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers can be ranked or weighted.

[0127] In any of the above aspects and embodiments, the FLASH RT is administered at a higher dose compared to a standard treatment protocol if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. In certain instances, the FLASH RT is administered as a hypofractionated radiation treatment if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample. In other instances, the FLASH RT is administered as a hyperfractionated radiation treatment if the expression level of the one or more biomarkers, *e.g.*, 1, 2, 3, 4, 5 or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample.

[0128] In any of the above aspects and embodiments, the therapeutic agent is an immune modulator selected from the group consisting of an inhibitor to an inhibitory checkpoint molecule, an activator of a stimulatory checkpoint molecule, a chemokine inhibitor, an inhibitor of macrophage migration inhibitory factor (MIF), a growth factor, a cytokine, an interleukin, an interferon, an antibody that binds to an immune system cell, a cellular immune modulator, a vaccine, an oncolytic virus, and any combination thereof.

[0129] In any of the above aspects and embodiments, the methods described herein can further comprise performing functional imaging of the tumor prior to administering the FLASH RT and/or the therapeutic agent.

[0130] The ionizing radiation (*e.g.*, FLASH RT) and the therapeutic agent can be administered concomitantly. Alternatively, the FLASH RT and the therapeutic agent can be administered sequentially.

[0131] In another aspect, a kit is provided. The kit comprises reagents capable of detecting expression of the biomarkers described herein. In some embodiments, the kit comprises reagents capable of detecting nucleic acid (*e.g.*, RNA) expression of the biomarkers. For example, the kit can comprise oligonucleotide primers that are capable amplifying a nucleic acid expressed by the biomarker genes described herein. In some embodiments, the kit further comprises an

oligonucleotide probe that hybridizes to a biomarker nucleic acid or an amplified biomarker nucleic acid, or a complement thereof. Methods of amplifying and detecting nucleic acids are well known in the art, and can comprise PCR, RT-PCR real-time PCR, and quantitative real-time PCR, Northern analysis, sequencing of expressed nucleic acids, and hybridization of expressed
5 and/or amplified nucleic acids to microarrays. In some embodiments, the kit comprises reagents that are capable of detecting proteins expression by the biomarkers described herein. In some embodiments, the reagents are antibodies that specifically bind to biomarker proteins. Methods of detecting protein expression are well known in the art, and include immunoassays, ELISA, Western analysis, and proteomic techniques.

10 **[0132]** In some embodiments of any of the above aspects and embodiments, the differences in the expression levels of each of the biomarkers in the tumor sample are increased or decreased by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the expression level in normal tissue. In some embodiments, the expression levels of each of the biomarkers in the tumor sample are increased or decreased by at least 1-fold, 2-fold, 3-fold,
15 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10 fold or more relative to the expression level in normal tissue.

[0133] In some embodiments, the average and/or ranked expression level of all the biomarkers in the tumor sample is increased or decreased relative to the expression level in normal tissue. Thus, in some embodiments, the average and/or ranked expression level of all the biomarkers in
20 the tumor sample is increased or decreased by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the expression level in normal tissue. In some embodiments, the expression levels in normal tissue are normalized to a control or baseline level. It will be understood that the expression level can also be compared to the expression level in the tumor sample before, after or during a treatment, course of treatment, or treatment plan. Thus, in some
25 embodiments, the expression levels of each of the biomarkers in the tumor sample are increased or decreased by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the expression level in the tumor sample before, during or after treatment.

[0134] Further, with regard to any of the above aspects and embodiments, the one or more biomarkers can comprise or consist of any combination of the biomarkers, for example, any of
30 the biomarkers described herein, any combination of two or more biomarkers, any combination

of three or more biomarkers, any combination of four or more biomarkers, any combination of five or more biomarkers, any combination of six or more biomarkers, and any combination of seven or more biomarkers.

[0135] In another aspect, the expression level of at least one, two, three, four or more of the biomarkers described herein is determined. The combination of expression levels of two or more biomarkers, *e.g.*, 2, 3, 4, 5, 6 or more biomarkers can indicate that the subject with cancer is more sensitive to radiation compared to a control subject. This subject may be administered a reduced or decreased dose of radiation compared to a standard dose. In other instances, if the combination of expression levels of two or more biomarkers, *e.g.*, 2, 3, 4, 5, 6 or more biomarkers can indicate that the subject with cancer is less sensitive to radiation compared to a control subject. A subject who is less sensitive to radiation may be administered an increased dose, a hypofractionated dose or a hyperfractionated dose of radiation. Optionally, radiation therapy may be administered in combination with an immune modulator, such as but not limited to, an anti-TIM4 antibody, an anti-MFG-E8 antibody, an anti-M199 antibody, and any combination thereof.

[0136] In some embodiments, the biomarker is CD44, MFG-E8, CD68, TGF β , or any combination thereof. In certain embodiments, if a first biomarker has a high level of expression and a second biomarker has a low level of expression in a sample obtained from a subject with cancer relative to a control sample, then it is predicted that radiation treatment monotherapy may result in local tumor control failure. As such, this biomarker profile can indicate that the subject should be administered radiation treatment in combination with an immune modulator. Alternatively, this biomarker profile can indicate that the dose of radiation be increased (*i.e.*, increased over a standard protocol dose). For instance, if the level of CD44 is high and the level of MFG-E8 is low in a subject's tumor sample compared to a control sample, then it is predicted that radiation treatment alone will not lead to a clinical response. In other words, a tumor sample having a high level of CD44 and a low level of MFG-E8 is likely to be insensitive or have a low sensitivity to ionizing radiation or FLASH radiation therapy. In some cases, the biomarker profile described herein indicates that the subject should receive an increased dose of radiation and/or combination therapy comprising FLASH RT and an immune modulator, such as an anti-TIM4 antibody, anti-MFG-E8 antibody, anti-M199 antibody, and any combination thereof.

[0137] In other embodiments, if the level of CD44 is low compared to a normal sample and/or the level of MFG-E8 is high compared to a normal sample, the subject is likely to have a clinical response to ionizing radiation or FLASH monotherapy. In some cases, it is predicted that a subject with low level of CD44 and/or a high level of MFG-E8 is likely to be sensitive to

5 ionizing radiation or FLASH radiation therapy.

[0138] In some embodiments, if a subject's tumor has a high level of CD68 compared to a control sample, the subject is predicted to have decreased survival after radiation monotherapy. As such, this subject can be administered a combination therapy comprising FLASH RT and an immune modulator. In other instances, if a subject's tumor has a low level of CD68 compared to

10 a control sample, the subject is likely to have a clinical response to radiation monotherapy. It is predicted that this subject is sensitive to radiation. In certain cases, it may be indicated that the subject be administered a low dose or reduced dose of radiation compared to a standard protocol dose.

FLASH RADIATION

[0139] FLASH radiation can be administered to tissue (e.g., tumor cells) in a single pulse, or any sequence of pulses, with an interval between pulses of less than a second to several minutes. The dose of FLASH radiation can range from about 40 to greater than 500 Gy/sec. In

15 embodiments, FLASH radiation allows for dose deposition up to 200 centimeters in living tissue at FLASH rates. In some embodiments, the dose of FLASH radiation is sufficient to kill tumor cells located up to 200 centimeters deep in human or animal tissue, for example, from 1 Gy up to more than 500 Gy, with a total beam-on delivery time of not more than 1 second. Each pulse within a FLASH sequence can be continuous or a sequence of shorter micro-pulses. In some

20 embodiments, the dose per pulse is at least 0.1 Gy, and the dose rate per pulse is at least 40 Gy/sec (i.e. 0.1 Gy in 2.5 ms, 4 Gy in 10 ms, 20 Gy in 0.5 sec, etc.) and up to $1e7$ Gy/sec as specified herein. The pulse duty cycle (i.e. temporal spacing) can have any value from 1 to $1e-9$.

[0140] In some embodiments, FLASH radiation is delivered using high-energy particles or waves, such as x-rays, gamma rays, electron beams, or protons. In some embodiments, FLASH radiation is delivered as proton therapy. In some embodiments, the FLASH radiation is not

delivered using electrons. In some embodiments, the proton therapy is delivered according to the following schedule:

FLASH :

1. 20 Gy in 0.5 seconds (40 Gy/sec)
- 5 2. 17.5 Gy in 0.4375 seconds (40 Gy/sec)
3. 15 Gy in 0.375 seconds (40 Gy/sec)

Pulsed-FLASH :

1. 10 pulses of 1.5 Gy in 10 seconds with a duty cycle of 0.1 or 10%
2. 10 pulses of 1.75 Gy in 10 seconds with a duty cycle of 0.1 or 10%
- 10 3. 10 pulses of 2 Gy in 10 seconds with a duty cycle of 0.1 or 10%

In contrast, conventional proton radiation therapy can be delivered according to the following schedule:

1. 20 Gy in 40 seconds (0.5 Gy/sec)
2. 17.5 Gy in 35 seconds (0.5 Gy/sec)
- 15 3. 15 Gy in 30 seconds (0.5 Gy/sec)

[0141] FLASH irradiation using electrons delivered by a linear electron accelerator is described, for example, in V. Favaudon, L. Caplier, V. Monceau, F. Pouzoulet, M. Sayarath, C. Fouillade, M.-F. Poupon, I. Brito, P. Hupé, J. Bourhis, J. Hall, J.-J. Fontaine, M.-C. Vozenin, Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice. *Sci. Transl. Med.* 6, 245ra93 (2014). Systems for producing FLASH radiation are described in U.S. Patent Application No. 15/089,330 (filed April 1, 2016), which is incorporated by reference herein in its entirety for all purposes. Thus, in some embodiments, the radiation therapy or treatment system is a proton pencil beam scanning system that can be used for FLASH RT. More specifically, the system can include an accelerator and beam transport system and a nozzle that can be aimed toward an object. The nozzle includes a beam energy adjuster configured to adjust the beam by, for example, placing different thicknesses of material

in the path of the beam to affect the energies of the particles in the beam. The nozzle is capable of quickly adjusting the particles in the beam to create a scanned beam (as opposed to a scattered beam) that delivers an entire, relatively high therapeutic radiation dose in the target volume. For example, a dose of four grays or more can be delivered along a specified beam direction (e.g., a given ray) in less than one second.

[0142] Each ray is a part of a scan pattern and irradiates tissue along a different line segment through the target volume (a “target line segment”). A high dose that can be delivered in a short period of time along a target line segment may be referred to as a “shot.” In an embodiment, a shot can be adjusted in energy (intensity) or range and delivered to the target volume with a Spread Out Bragg Peak (SOBP) that provides a uniform and otherwise suitably modified dose to an entire target line segment.

[0143] Other types of radiation treatment systems that can be used for FLASH RT are described in the co-pending provisional application Serial No. 62/434,053, filed December 14, 2016, and entitled “Dynamic Three-Dimensional Compensator,” which is hereby incorporated by reference in its entirety.

[0144] In some embodiments the radiation therapy or treatment system comprises an accelerator, a beam transport system, and a beam shaping system. In an embodiment, the accelerator may be radio frequency (RF) (linear accelerator, cyclotron, or synchrotron) or laser-based accelerator. The accelerator may deliver the dose in a pulsed, continuous or quasi-continuous manner. The beam transport may include magnetic elements (dipole, quadrupole, multipole), electrostatic elements, and slits or collimators. The beam shaping can be performed by a combination of magnetic, electrostatic and mechanical elements with the purpose of maximizing the conformality of the dose to the tumor. Embodiments include systems capable of delivering 3D conformal dose to the tumor in a single field or as a combination of multiple beamlets.

[0145] FLASH RT doses can be delivered as a single dose (a single fraction), or the total dose can be divided into multiple fractions that are delivered over time. In an embodiment, continuous monitoring of the tumor using, for example x-ray or magnetic resonance imaging (MRI) may be used by a medical technician to help determine when to turn on the beam when the target is in the defined target field, to further ensure optimal dose conformality.

[0146] FLASH RT can use very high energy charged particles (VHECPs) including, but not limited to, electrons, protons, and heavy ions, high energy photons (x-rays, gamma rays), or any high energy neutral particle, such as neutrons. High energy is defined as any energy which allows for dose deposition up to 200 centimeters in living tissue at FLASH rates. Intensity modulated (IM) FLASH RT and modulated arc (MA) FLASH RT provide means for achieving optimal dose conformity.

RADIATION TREATMENT PLANNING SYSTEMS

[0147] In intensity modulated radiation therapy (IMRT) such as intensity modulated particle therapy (IMPT), beam intensity is varied across each treatment region (target) in a patient.

Instead of being treated with a relatively large and uniform field, the patient is treated with a number of smaller beams (e.g., beam segments or beamlets), each of which can have its own energy and/or intensity value, and each of which can be delivered from a different direction or angle (which may be referred to as beam geometry). Because of the many possible beam geometries, the number of beams, and the range of beam energies or intensities across the beams and within each beam, there is effectively an infinite number (or at least a very large number) of potential treatment plans, and therefore consistently and efficiently generating and evaluating high-quality treatment plans is beyond the capability of a human and relies on the use of a computing system, particularly considering the time constraints associated with the use of radiation therapy to treat ailments like cancer, and particularly considering the large number of patients that are undergoing or need to undergo radiation therapy during any given time period.

[0148] FIG. 1 shows a cross-sectional view of a possible beam geometry to a tumor (target 710) and the surrounding tumor microenvironment 720. In FIG. 1, a prescribed dose to be delivered into and across the target is determined. Each portion of the target can be represented by at least one 3D element known as a voxel; a portion may include more than one voxel. A portion of a target or a voxel may also be referred to herein as a sub-volume; a sub-volume may include one or more portions or one or more voxels. As will be described in detail below, each portion or voxel may receive radiation from one or more beams delivered from different directions. The prescribed dose defines, for example, a dose value, or a minimum dose value and a maximum dose value, for each portion or voxel of the target. In an embodiment, the prescribed

dose is the same for all portions (sub-volumes or voxels) of the target, such that a uniform dose is prescribed for the entire target.

[0149] In FIG. 1, directions (e.g., gantry angles relative to the patient or target, or nozzle directions relative to the patient or target) for delivering beams 705-707 into the target 710 are determined. The beams can be proton beams, electron beams, photon beams, ion beams, or atom nuclei beams. The operation of determining beam directions can include determining the number of beams (the number of directions from which beams are to be delivered). The beams' paths may or may not overlap within the target, and may or may not overlap outside the target. In general, when generating the radiation treatment plan, one goal is to determine beam paths that minimize the irradiation dose of each sub-volume or voxel of the tissue outside the target. In some cases, organs deemed critical by the radiation oncologist may be required to receive less dose than other organs in the vicinity. Therefore, conventional treatment planning is driven by prescription thresholds of the calculated integral dose to the tumor and surrounding organs.

[0150] The current disclosure provides additional criteria for determining the organ dose thresholds outside the target 710. FIG. 2 contains a 3-dimensional surface plot of dose vs time and one other variable. The dose delivered to a tumor will depend on more variables, but two are selected for the purposes of describing the embodiments without added complexity. In general the dose surface can depend on any number of variables, and will in generally be n-dimensional with n being greater than or equal to 1. In the case of the tumor microenvironment 720, the other parameters could be immune-sensitivity, response of the tumor to an immune modulator, or some other biological parameter. While in general the shape of this surface can vary, FIG. 2 shows an increasing dose threshold with decreasing irradiation time, and a slightly increasing dose threshold with increasing "immune-biological indicator".

[0151] In an embodiment, the time constraint can be met through geometrical construction of the beam configuration. Ideally, each sub-volume or voxel outside the target is intersected, at most, by only a single beam. If some overlap between beam paths is permitted, then ideally each sub-volume or voxel outside the target is intersected by not more than two beams, with most intersected by only a single beam. In an embodiment, as one means of achieving the aforementioned goal, the beam directions are determined such that the total amount of overlap between the beams' paths is minimized outside the target. In one such embodiment, the

directions are determined such that the paths of the beams overlap within the target and such that the total amount of overlap of the beams' paths outside the target is less than the total amount of the overlap of the beams' paths within the target. In another such embodiment, the directions are determined so that the paths of the beams do not overlap at all outside the target. The beams' paths can lie within the same plane, or they can be in different planes.

[0152] The beams (705-707) are shown as passing through the patient, however this disclosure is not so limited. The beams may also terminate within the target as would be the case for ion and proton beams. In this case, beam energy determines the termination point within the target, which would need to be considered by the calculation engine and optimizer. Otherwise all other optimization parameters are the same.

[0153] While the operations in FIG. 1 are presented as occurring in series and in a certain order, the present disclosure is not so limited. The operations may be performed in a different order and/or in parallel, and they may also be performed in an iterative manner, as the number of beams (and accordingly, the number of directions), the beam directions, and the beam energies or intensities (and/or beam segment energies or intensities) used to deliver the prescribed dose are interrelated. As noted above, because of the different parameters that need to be considered, the range of values for those parameters, the interrelationship of those parameters, the need for treatment plans to be effective yet minimize risk to the patient, and the need to generate high-quality treatment plans quickly, the use of the optimizer model 150 executing consistently on the computing system 106 (FIG. 3) for radiation treatment planning as disclosed herein is important.

[0154] Although multiple beams are shown in FIG. 1, this does not mean that all beams are necessarily delivered at the same time or in overlapping time periods, although they can be. The number of beams delivered at any one time depends on the number of gantries or nozzles in the radiation treatment system and on the treatment plan.

[0155] In operation, in an embodiment, the beam segments are delivered sequentially. For example, the beam segment 705 is delivered to the target (turned on) and then turned off, then the beam segment 706 is turned on then off, then the beam segment 707 is turned on then off, and so on. Each beam segment may be turned on for only a fraction of a second (on the order of milliseconds). In an embodiment, all beams 705-707 may be turned on simultaneously.

[0156] As shown in FIG. 1, a sub-volume can be traversed by more than two beams, in which case the cumulative dose for the sub-volume is represented by adding the appropriate value for each beam that reaches the sub-volume. Such sub-volumes are known as overlap regions 730. That is, a total value is determined for each sub-volume in the target 710 by adding together the values for each beam region of each beam that reaches the sub-volume. Dose in the overlap regions can also be optimized by the constraints defined by the surface in FIG. 2.

[0157] The optimizer model can adjust the parameters that affect the calculated doses delivered to the target 710 to achieve a satisfactorily uniform cumulative dose across the target 710. A satisfactorily uniform cumulative dose is indicated when all the total values per sub-volume in the target 710 are the same or when the differences between the total values per sub-volume satisfy a threshold value.

[0158] The threshold value can be, for example, a value that specifies the range of differences that is permitted or that specifies a maximum permitted difference.

[0159] Dose limits can include, but are not limited to: a limit on irradiation time for each sub-volume (voxel) in the target (e.g., for each voxel of target tissue, treatment time < 0.5 seconds); a limit on irradiation time for each sub-volume (voxel) outside the target (e.g., for each voxel of normal tissue, treatment time < 0.5 second²); a limit on dose rate for each sub-volume (voxel) in the target (e.g., for each voxel of target tissue, dose rate > 40 Gy/sec); and a limit on dose rate for each sub-volume (voxel) outside the target (e.g., for each voxel of normal tissue, dose rate > 40 Gy/sec), a limit on dose and dose rate in the tumor micro-environment. In general, the limits are intended to minimize the amount of time that normal tissue is irradiated and maximize the immunological response.

[0160] In summary, embodiments described herein improve radiation treatment planning and the treatment itself by expanding FLASH RT to a wider variety of treatment platforms and target sites as well as optimizing delivery of FLASH RT to enhance the immune response. Treatment plans generated as described herein are superior for sparing normal tissue from radiation in comparison to conventional techniques even for non-FLASH dose rates by reducing, if not minimizing, the magnitude (and the integral in some cases) of the dose to normal tissue (outside the target) by design. When used with FLASH dose rates, management of patient motion is simplified. Treatment planning, while still a complex task of finding a balance between

competing and related parameters, is simplified relative to conventional planning. The techniques described herein may be useful for stereotactic radiosurgery as well as stereotactic body radiotherapy with single or multiple metastases.

[0161] In some embodiments, the methods described herein can be used with other types of external beam radiotherapy such as, but not limited to, IMPT, intensity modulated radiation therapy (IMRT), image-guided radiotherapy (IGRT), RapidArc™ radiotherapy, stereotactic body radiotherapy (SBRT), and stereotactic ablative radiotherapy (SABR).

[0162] FIG. 3 shows a block diagram of an example of a computing system 100 that can be used for radiation treatment planning. In its most basic configuration, the system 100 includes at least one processing unit 102 and memory 104. This most basic configuration is illustrated in FIG. 3 by dashed line 106. The system 100 may also have additional features and/or functionality. For example, the system 100 may also include additional storage (removable and/or non-removable) including, but not limited to, magnetic or optical disks or tape. Such additional storage is illustrated in FIG. 3 by removable storage 108 and non-removable storage 120. The system 100 may also contain communications connection(s) 122 that allow the device to communicate with other devices, e.g., in a networked environment using logical connections to one or more remote computers.

[0163] The system 100 also includes input device(s) 124 such as keyboard, mouse, pen, voice input device, touch input device, etc. Output device(s) 126 such as a display device, speakers, printer, etc., are also included.

[0164] In the example of FIG. 3, the memory 104 includes computer-readable instructions, data structures, program modules, and the like associated with a treatment planning system 150. However, the treatment planning system 150 may instead reside in any one of the computer storage media used by the system 100, or may be distributed over some combination of the computer storage media, or may be distributed over some combination of networked computers.

[0165] The treatment planning system 150 can be used to develop a radiation treatment plan for a particular patient by receiving patient-specific information (e.g., geometry information) that is input to and processed by the treatment planning system. The input patient-specific information may contain any combination of parameters that can practically affect the radiation

treatment plan. For example, the patient-specific information may be organized as a vector or a data structure including feature elements for: size and shape of the target volume; location of the target volume; size and shape of an organ at risk; type of an organ at risk; a part of the target volume that overlaps an organ; and a part of an organ that overlaps the target volume.

5 **[0166]** The treatment planning system 150 may be used to predict dose parameters for a treatment plan corresponding to a particular patient. The dose treatment planning system 150 may include a dose-volume histogram (DVH) estimation model, where the predicted quantity is a dose volume histogram. In other embodiments, the treatment planning system 150 also generates a prediction based on a distance to a target (DTH) histogram, which expresses the
10 distance from a region of interest (ROI) to a radiation target. In general, the treatment planning system 150 is implemented as any model suitable for predicting dosage (as a dose histogram or spatial 3D dose distribution) for a radiation treatment plan.

15 **[0167]** As mentioned above, the radiation treatment planning system 150 can account for both the spatial domain and the time domain. A time-dependent component can be included in treatment planning for IM or MA FLASH RT with VHECPs, for example.

20 **[0168]** In some embodiments, the treatment planning system 150 considers the combination of FLASH RT and immunotherapy. The treatment planning system 150 incorporates immunotherapy into treatment planning, including how immunotherapy affects the dose regimen and the impact of immunotherapy on radiation delivery and dose rate. In general, treatment
planning system 150 determines a final radiation treatment plan that integrates FLASH RT and immunotherapy. For example, the treatment planning system 150 can iteratively evaluate the FLASH aspects of radiation treatment and the immunotherapy/biological aspects of radiation treatment to generate a final radiation treatment plan that optimizes the combination of both aspects.

25 **[0169]** For example, the planner defines a set of quality metrics. For planning, the metrics are defined such that a smaller value is preferred over a larger value. The planner also defines a relative priority or weight (w_i) for each of the quality metrics. The task of developing an optimal plan is then formulated as a quadratic cost function C : $C = \sum(w_i(Q_i - q_i)^2)$, where q_i is the value of the quality metric that can be achieved for a particular treatment plan. The optimal plan
30 is determined by minimizing the cost function C .

[0170] Another way to assist the planner is to use a multi-criteria optimization (MCO) approach for treatment planning. Pareto surface navigation is an MCO technique that facilitates exploration of the tradeoffs between clinical goals. For a given set of clinical goals, a treatment plan is considered to be Pareto optimal if it satisfies the goals and none of the metrics can be improved without worsening at least one of the other metrics. The set of Pareto optimal plans define a Pareto surface related to the set of clinical goals. Movement along the Pareto surface results in tradeoffs between the clinical goals; some metrics will improve at the cost of worsening one or more other metrics. The planner can navigate along the Pareto surface and choose a final (optimized) radiation treatment plan that seems to be the best according to the criteria applied by the planner, or a treatment plan can be selected automatically based on its proximity to the Pareto surface.

[0171] Metrics associated with the combination of FLASH RT and immunotherapy include metrics associated with, for example, target homogeneity, critical organ sparing, prescribed doses, dose delivery rates, normal and tumor tissue toxicity, etc.

[0172] FIG. 4 is a block diagram illustrating an embodiment of an automated radiation therapy treatment planning system 150. The system 150 includes an input interface 210 to receive patient-specific information (data) 201, and an output interface 230. The system 150 in whole or in part may be implemented as a software program, hardware logic, or a combination thereof on/using the computing system 100 (FIG. 3).

[0173] The radiation therapy treatment planning system 150 also receives or accesses FLASH RT parameters and metrics and immunotherapy parameters and metrics. Examples of the metrics are described herein. FLASH RT parameters can include, for example, beam type, beam energy, the angle of the beam relative to the patient/target volume, beam shape, dose delivery schedule, total dose and irradiation time for any given normal tissue volume outside the tumor, as well as dose and irradiation time within the tumor microenvironment which optimizes the immunological response. Immunotherapy parameters can include, for example, the type of drug or immune modulator administered to the subject, tumor antigen, neoantigen, or antibody administered, dosage, and delivery schedule (e.g., before, after, and/or during FLASH RT).

[0174] The treatment planning system 150 yields a prediction result, e.g., an achievable dose distribution prediction. A treatment plan based on the prediction result can then be generated. In

an embodiment, the prediction result is accompanied by parameters indicative of the quality of the prediction, such as reliability of the result, complexity of the predicted plan, and probability of the result.

[0175] In some embodiments, the radiation treatment planning systems described herein will result in a prediction that a lower effective dose of radiation, e.g., FLASH radiation, can be administered to a subject based on a response of the subject to immunotherapy. For example, in some embodiments, the radiation treatment planning system(s) will predict that a lower effective dose of radiation, e.g., FLASH radiation, can be administered to a subject if the tumor in the subject responds favorably to immunotherapy, e.g., the tumor is reduced in size following immunotherapy.

[0176] In some embodiments, the radiation treatment planning systems described herein will result in a prediction to change the effective dose of radiation, e.g., FLASH radiation, based on parameters that enhance the immune response, e.g., increase the delivery or activity of an immune modulator to more effectively treat a tumor in a subject.

BIOMARKERS FOR RADIOTHERAPY SELECTION

[0177] The biomarkers described herein can be used to stratify patients to receive individualized, tailored radiotherapy (e.g., FLASH RT) in combination with an immune modulator agent. The biomarkers can also be used to monitor the efficacy of immune modulator therapy on patients with cancer. The biomarkers include, but are not limited to, one or more immune cell biomarkers, one or more tumor cell biomarkers, one or more circulating biomarkers, one or more imaging biomarkers, and any combination thereof. For instances, an immune cell biomarker can provide information about the location and/or activity of a specific cell population, such as a T cell population. An immune cell biomarker or tumor cell biomarker can be a genetic biomarker, polynucleotide biomarker, or a protein biomarker. In some embodiments, an immune cell biomarker is a specific polynucleotide (e.g., RNA and microRNA) or protein that is expressed at a higher level by a particular immune cell compared to a non-immune cell or a different type of immune cell. Similarly, a tumor cell biomarker can be a specific polynucleotide (e.g., RNA and microRNA) or protein that is expressed at a higher level by a tumor cell compared to a non-tumor cell. For example, the tumor cell biomarker can be a protein

or a polynucleotide encoding said protein that is associated with proliferation and/or metastasis of a tumor cell. In some cases, the protein can be involved in angiogenesis or other processes that are activated by a tumor cell. The tumor biomarker can be an oncogene or a tumor suppressor. In some instances, a tumor cell biomarker is a gene variation, gene mutation, copy number variant (CNV), single nucleotide polymorphism (SNP), and the like that is present in a tumor cell, but not in a non-tumor cell. In some embodiments, a circulating biomarker is an exosome (*i.e.*, a cell-derived vesicle that can be found in a body fluid). Examples of useful biomarkers includes those described in U.S. Patent Appl. Publ. No. 20160024594, the disclosure of which is hereby incorporated by reference for all purposes.

[0178] The biomarker set can include, but is not limited to, CD44, milk fat globule-EGF factor 8 (MFG-E8), CD68 and TGF β . CD44 is a cell-surface glycoprotein that plays a role in cell proliferation, cell-cell interactions, cell adhesion, and cell migration of various cell types including lymphocytes and cancer cells. The human CD44 polypeptide sequence is set forth in, *e.g.*, GenBank Accession No. NP_000601. The human CD44 mRNA (coding) sequence is set forth in, *e.g.*, GenBank Accession No. NM_000610. Milk fat globule-EGF factor 8 protein (MFG-E8) is a macrophage-produced protein that promotes engulfment and clearance of apoptotic cells in tumors. The human MFG-E8 polypeptide sequence is set forth in, *e.g.*, GenBank Accession No. NP_005919. The human MFG-E8 mRNA (coding) sequence is set forth in, *e.g.*, GenBank Accession No. NM_005928. CD68 is a 110-kD transmembrane glycoprotein that is highly expressed by human monocytes and tissue macrophages. The protein primarily localizes to lysosomes and endosomes with a smaller fraction circulating to the cell surface. It is a type I integral membrane protein with a heavily glycosylated extracellular domain and binds to tissue- and organ-specific lectins or selectins. CD68 is also a member of the scavenger receptor family. The human CD68 polypeptide sequence is set forth in, *e.g.*, GenBank Accession No. NP_001242. The human CD68 mRNA (coding) sequence is set forth in, *e.g.*, GenBank Accession No. NM_001251. TGF β is a cytokine that is involved in cell growth, cell proliferation, cell differentiation, apoptosis, homeostasis and many other cellular processes. The human TGF β polypeptide sequence is set forth in, *e.g.*, GenBank Accession No. NP_000651. The human TGF β mRNA (coding) sequence is set forth in, *e.g.*, GenBank Accession No. NM_000660.

[0179] It will be understood that the expression levels of each of the biomarkers described herein in the patient sample can increase or decrease relative to the expression level of the tumor biomarker in a normal or control tissue sample. For example, the expression level of one tumor biomarker can increase in the tumor sample compared to the expression level in a normal tissue, whereas the expression level of a second biomarker can decrease in the tumor sample compared to the expression level in a normal tissue. The expression level can also be based on the average, combination or sum of the all the tumor biomarker expression levels in the patient sample. For example, the expression level of each biomarker in the patient sample can be ranked or weighted to produce a ranked value that is higher or lower than the normal tissue value (which can be a normalized value, for example, set to 1).

[0180] In some embodiments, biomarker expression is determined in a biological sample from the subject having a tumor. In some embodiments, the biological sample is a tumor sample. The tumor sample can be a biopsy comprising tumor cells from the tumor. In some embodiments, the biological sample comprises a bodily fluid, such as but not limited to blood, serum, plasma, or urine, and/or cells or tissues from the subject. In some embodiments, the biological sample is a formalin-fixed and paraffin embedded tissue or tumor sample. In some embodiments, the biological sample is a frozen tissue or tumor sample. Thus, in some embodiments, one or more steps of the methods described herein are carried out *in vitro*. For example, in some embodiments, biomarker expression is determined *in vitro*.

[0181] In some embodiments, the normal tissue sample comprises non-tumor cells from the same tissue type as the tumor. In some embodiments, the normal tissue sample is obtained from the same subject diagnosed with the tumor. A normal tissue sample can also be a control sample of the same tissue-type from a different subject. The expression level of the normal tissue sample can also be an average or mean value obtained from a population of normal tissue samples.

[0182] The level of expression of the biomarkers described herein can be determined using any method known in the art. For example, the level of expression can be determined by detecting the expression of a nucleic acid (*e.g.*, RNA, mRNA or microRNA) or the protein encoded by the nucleic acid.

[0183] Exemplary methods for detecting expression levels of nucleic acids include, without limitation, Northern analysis, polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), real-time PCR, quantitative real-time PCR, and DNA microarrays.

[0184] Exemplary methods for detecting expression levels of proteins (*e.g.*, polypeptides)

include, without limitation, immunohistochemistry, ELISA, Western analysis, HPLC, and proteomics assays. In some embodiments, the protein expression level is determined by immunohistochemistry using the Allred method to assign a score (*see, e.g.*, Allred, D. C., Connection 9:4-5, 2005, which is incorporated by reference herein). For example, formalin-fixed, paraffin embedded tissues are contacted with an antibody that specifically binds a biomarker described herein. The bound antibody is detected with a detectable label or secondary antibody coupled with a detectable label, such as a colorimetric label (*e.g.*, an enzymatic substrate produce by HRP or AP). The antibody positive signal is scored by estimating the proportion of positive tumor cells and their average staining intensity. Both the proportion and intensity scores are combined into a total score that weighs both factors.

[0185] In some embodiments, the protein expression level is determined by digital pathology. Digital pathology methods include scanning images of tissues on a solid support, such as a glass slide. The glass slides are scanned into whole slide images using a scanning device. The scanned images are typically stored in an information management system for archival and retrieval. Image analysis tools can be used to obtain objective quantitative measurements from the digital slides. For example, the area and intensity of immunohistochemical staining can be analyzed using the appropriate image analysis tools. Digital pathology systems can include scanners, analytics (visualization software, information management systems and image analysis platforms), storage and communication (sharing services, software). Digital pathology systems are available from numerous commercial suppliers, for example, Aperio Technologies, Inc. (a subsidiary of Leica Microsystems GmbH), and Ventana Medical Systems, Inc. (now part of Roche). Expression levels can be quantified by commercial service providers, including Flagship Biosciences (CO), Pathology, Inc. (CA), Quest Diagnostics (NJ), and Premier Laboratory LLC (CO).

[0186] In some embodiments, imaging of the tumor, such as functional imaging is also used to identify or select a cancer patient who should receive the combination therapy described herein.

Non-limiting examples of functional imaging include single-photon emission computed tomography, optical imaging, ultrasonography, positron emission tomography (PET), computed tomography (CT), perfusion computed tomography, magnetic resonance imaging (MRI), functional magnetic resonance imaging, magnetic resonance spectroscopic imaging, dynamic contrast-enhanced imaging, diffusion-weighted imaging, blood-oxygenation level dependent imaging, magnetic resonance spectroscopy, magnetic resonance lymphography, and any combination thereof. Any type of functional imaging such as multimodality imaging can be performed to characterize the tumor, to determine the delineation of the tumor, the extent of the tumor, the tumor volume, and/or to assess the tumor microenvironment (*e.g.*, the environment surrounding the tumor). Functional imaging can aid in selecting the best treatment option and/or in monitoring response to the treatment.

METHODS FOR SELECTING A COURSE OF TREATMENT

[0187] The expression levels of the biomarkers can be used to determine or select a course of treatment in a subject diagnosed with a tumor. For example, in some embodiments, the treatment comprises administering ionizing radiation, such as FLASH RT, to the tumor in the subject. The ionizing radiation can also be administered to the entire subject or a portion thereof, especially if the tumor is dispersed or mobile. In some embodiments, the treatment further comprises contacting the tumor with a radiosensitizer. In some embodiments, the treatment further comprises administering a compound or biologic drug, such as an antibody, that inhibits an immune checkpoint pathway, to the subject. In some embodiments, the treatment comprises administering a FLASH radiation treatment protocol in combination with an immune modulator.

[0188] The course of treatment can be selected based on the expression levels of the biomarkers. For example, the expression levels can be used to determine if radiation therapy is appropriate for the subject (*i.e.*, for making a go/no go decision on radiotherapy). Further, if the expression levels of the biomarkers are increased relative to a normal or control value, then the effective radiation dose to the tumor can be increased, and/or the fractionation schedule modified accordingly. In some embodiments, the effective dose of FLASH RT to the tumor is increased. The radiation dose to the blood vessels feeding the tumor can also be increased. In some cases, a hypofractionated radiation treatment is administered. Alternatively, a hyperfractionated

radiation treatment is administered. In some embodiments, FLASH radiation treatment is provided in combination with immune modulator treatment.

[0189] In some embodiments, if the expression levels of the biomarkers are increased relative to a normal or control value, then the treatment can comprise administering ionizing radiation, such as FLASH RT, to the tumor. In some embodiments, if the expression levels of the biomarkers are decreased relative to a normal or control value, then the treatment can comprise decreasing the amount of ionizing radiation or FLASH RT administered to the tumor.

[0190] The treatment can also comprise modifying an existing course of treatment. For example, in some embodiments, the existing course of treatment is modified to increase the effective dose of the ionizing radiation, e.g., FLASH radiation, administered to the tumor. In some embodiments, the effective dose of ionizing radiation, such as FLASH radiation, is increased by increasing the amount of ionizing radiation administered to the tumor and/or contacting the tumor with a radiosensitizer. In some embodiments, the existing course of treatment is modified to decrease the effective dose of the ionizing radiation administered to the tumor. In some embodiments, the treatment comprises modifying a standard radiation treatment protocol in combination with administering an immune modulator.

[0191] In some embodiments, the effective dose of ionizing radiation, e.g., FLASH radiation, administered to the tumor is increased if the level of one or more biomarkers described herein is elevated in the tumor environment. For example, the effective dose of ionizing radiation is increased as compared to the standard of care for a subject that does not have elevated levels of the biomarker(s) in the tumor environment. This applies to subjects who are currently not undergoing radiation therapy as well as modifying an existing course of treatment for subjects undergoing radiation therapy. Thus, the effective dose of ionizing radiation can be increased from the current effective dose if the subject is already undergoing radiation therapy for a tumor. The radiation therapy can be modified to reduce the constraints on neighboring healthy tissue. For example, if the biomarker level in the tumor environment indicates more aggressive radiation therapy is required, the treatment plan can be modified so that the constraints on the border between healthy tissue and tumor tissue are decreased. This would result in a trade-off between damaging some healthy tissue in order to kill more of the tumor tissue.

[0192] In some embodiments, the treatment comprises a combination of radiation therapy and an immune modulator agent (including a radiosensitizer). In some embodiments, the treatment comprises a combination of FLASH radiation therapy and an immune modulator agent (including a radiosensitizer). In some embodiments, the effective dose of ionizing radiation administered to the tumor is not changed (*e.g.*, relative to the standard of care or relative to an existing course of treatment) when an immune modulator agent is administered to the subject. For example, in some embodiments, the subject is administered an effective dose of ionizing radiation that is the same or similar to that administered to a subject that does not have elevated levels of one or more biomarkers described herein in the tumor environment, and the subject is further administered an immune modulator agent. In some embodiments, the effective dose of ionizing radiation administered to the tumor is based on the standard of care for a subject that does not have elevated levels of the biomarker(s) in the tumor environment, and the subject is further administered an immune modulator agent. In some embodiments involving an existing course of treatment, the effective dose of ionizing radiation is maintained at the current effective dose, and an anti-cancer agent is administered to the subject in combination with the ionizing radiation if the level of one or more biomarkers described herein is elevated in the tumor environment.

[0193] In some embodiments, the treatment plan is developed and/or modified based on the expression levels of the biomarkers described herein.

[0194] The course of treatment can also be selected by using an algorithm, for example a computer-implemented algorithm, that analyses or determines the expression level of the biomarkers in the tumor sample relative to the level in the normal sample. The algorithm can be a linear regression algorithm that includes the biomarker expression levels and coefficients (*i.e.*, weights) for combining the expression levels. In some embodiments, the algorithm comprises a least squares fit to calculate the coefficients. If the algorithm determines that the expression level of the biomarkers in the tumor sample is increased or decreased relative to the normal sample, then the appropriate course of treatment can be assigned. In some embodiments, the algorithm is a nonparametric regression tree. In some embodiments, treatment comprising FLASH radiation and an immune modulator is selected in dependence on the algorithm determining that the expression level of the biomarkers in the tumor sample is increased or

decreased relative to the normal sample. In some embodiments, standard statistical methods were used to analyze the data to determine which biomarkers were most predictive of clinical survival or local tumor control failure.

[0195] In some embodiments, the method described herein is a computer implemented method. In some embodiments, the computer implemented method comprises a linear regression model that assigns a ranked or weighted value to the expression levels of the biomarkers described herein. In some embodiments, the disclosure provides a computer-readable medium, the medium providing instructions to cause a computer to perform a method described herein. For example, the medium can provide instructions to cause a computer to assign a ranked or weighted value to the expression levels of the biomarkers described herein.

RADIATION THERAPY

[0196] The expression levels of the tumor biomarkers described herein can be used to optimize treatment of patients with radiotherapy, such as FLASH RT. For example, the therapeutic dose of the radiation administered to the tumor or subject can be adjusted based on the expression levels of the biomarkers. The effective dose of ionizing radiation varies with the type of tumor and stage of cancer that needs to be treated. The effective dose can also vary based on other treatment modalities being administered to the patient, for example chemotherapeutic treatments and surgical treatments, and whether the radiation is administered pre- or post-surgery. In general, a conventional curative therapeutic dose for a solid epithelial tumor ranges from about 60 to 80 gray (Gy), whereas a curative dose for a lymphoma is about 20 to 40 Gy. In general, preventative doses can be 45-60 Gy. For FLASH irradiation, the curative therapeutic dose for a solid epithelial tumor can range from about 20 to 200 gray (Gy), whereas a curative dose for a lymphoma is about 10 to 200 Gy, with preventative doses from 5-500 Gy.

[0197] The therapeutic dose can be delivered in fractions. Fractionation refers to spreading out the total dose of radiation over time, for example, over days, weeks or months. The dose delivered in each fraction can be about 1.5-2 Gy per day. The treatment plan can include a fraction treatment one or more times per day, every other day, weekly, *etc.* depending on the treatment needs of each patient. For example, a hypofractionation schedule comprises dividing the total dose into several relatively large doses, and administering the doses at least one day

apart. Exemplary hypofraction doses are 3 Gy to 20 Gy per fraction. An exemplary fractionation schedule that can be used to treat lung cancer is Continuous Hyperfractionated Accelerated Radiation therapy (CHART), which consists of three small fractions per day.

[0198] In some embodiments, FLASH RT includes contacting the tumor in the subject with a radiosensitizer. Exemplary radiosensitizers include hypoxia radiosensitizers such as misonidazole, metronidazole, and trans-sodium crocetin, a compound that helps to increase the diffusion of oxygen into hypoxic tumor tissue. The radiosensitizer can also be a DNA damage response inhibitor interfering with base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), recombinational repair comprising homologous recombination (HR) and non-homologous end-joining (NHEJ), and direct repair mechanisms. SSB repair mechanisms include BER, NER, or MMR pathways whilst DSB repair mechanisms consist of HR and NHEJ pathways. Radiation causes DNA breaks that if not repaired are lethal. Single strand breaks are repaired through a combination of BER, NER and MMR mechanisms using the intact DNA strand as a template. The predominant pathway of SSB repair is the BER utilizing a family of related enzymes termed poly-(ADP-ribose) polymerases (PARP). Thus, the radiosensitizer can include DNA damage response inhibitors such as Poly (ADP) ribose polymerase (PARP) inhibitors.

[0199] The biomarkers described herein are useful in developing and modifying treatment plans for patients diagnosed with a tumor or cancer. The treatment plan can include visualizing or measuring the tumor volume that needs to be irradiated, the optimal or effective dose of radiation administered to the tumor, and the maximum dose to prevent damage to nearby healthy tissue or organs at risk. Algorithms can be used in treatment planning, and include dose calculation algorithms based on the particular radiotherapy technique parameters employed, *e.g.*, gantry angle, MLC leaf positions, *etc.*, and search algorithms which use various techniques to adjust system parameters between dose calculations to optimize the effectiveness of the treatment. Exemplary dose calculation algorithms include various Monte Carlo ("MC") techniques and pencil beam convolution ("PBC"). Exemplary search algorithms include various simulated annealing ("SA") techniques, algebraic inverse treatment planning ("AITP"), and simultaneous iterative inverse treatment planning ("SIITP"). Such techniques, and others, are included within the scope of this disclosure.

[0200] Treatment planning algorithms may be implemented as part of an integrated treatment planning software package which provides additional features and capabilities. For example, a dose calculation algorithm and search algorithm may be used to optimize a set of fluence maps at each gantry angle, with a separate leaf sequencer used to calculate the leaf movements needed to deliver them. Alternatively, a dose calculation algorithm and search algorithm may be used to directly optimize leaf movements and other machine parameters. The Eclipse™ Treatment Planning System offered by the assignee of the present application includes such an integrated software program. Methods for optimizing treatment plans are described in U.S. Patent No. 7,801,270, which is incorporated by reference herein.

[0201] In some embodiments, the biomarkers described herein can be used to monitor the progress of tumor control after FLASH radiation therapy. For example, the expression levels of the biomarkers before and after ionizing FLASH radiation therapy can be compared. In some embodiments, if the expression levels of biomarkers increase after radiotherapy, this suggests that the tumor is continuing to grow in size. Thus, the radiation treatment can be modified based on monitoring tumor growth using the biomarkers described herein.

[0202] The biomarkers described herein can be used with any radiation therapy technique known in the art. Radiation therapy techniques include external-beam radiotherapy (“EBRT”) and Intensity Modulated Radiotherapy (“IMRT”), which can be administered by a radiotherapy system, such as a linear accelerator, equipped with a multileaf collimator (“MLC”). The use of multileaf collimators and IMRT allows the patient to be treated from multiple angles while varying the shape and dose of the radiation beam, thereby avoiding excess irradiation of nearby healthy tissue. Other exemplary radiation therapy techniques include stereotactic body radiotherapy (SBRT), volumetric modulated arc therapy, three-dimensional conformal radiotherapy (“3D conformal” or “3DCRT”), image-guided radiotherapy (IGRT). The radiation therapy techniques can also include Adaptive radiotherapy (ART), a form of IGRT that can revise the treatment during the course of radiotherapy in order to optimize the dose distribution depending on patient anatomy changes, and organ and tumor shape. Another radiation therapy technique is brachytherapy. In brachytherapy, a radioactive source is implanted within the body of the subject, such that the radioactive source is near the tumor. As used herein, the term radiotherapy should be broadly construed and is intended to include various techniques used to

irradiate a patient, including use of photons (such as high energy x-rays and gamma rays), particles (such as electron and proton beams), FLASH RT, and radiosurgical techniques. Further, any method of providing conformal radiation to a target volume is intended to be within the scope of the present disclosure.

5 THERAPEUTIC AGENTS

[0203] FLASH radiation therapy described herein can be administered in combination with one or more therapeutic agents. Example of therapeutic agents include immune modulators, senolytic agents, radiosensitizers, and nanoparticles.

10 [0204] In some embodiments, the therapeutic agent is a mitotic spindle inhibitor. In some embodiments, flash radiation therapy is combined with a mitotic spindle inhibitor such as a cyclin inhibitor, for example, a CDK4/6 inhibitor (e.g., Pablociclib), AURKA inhibitors such as the small molecules Alisertib and Tozasertib, compounds that block TPX2-AURKA complexes such as a complex disruptor (GSK1070916) (Asteriti et al., 2015; Janeček et al., 2016), and Taxanes (such as Docetaxel, Paclitaxel).

15 [0205] In some embodiments, the therapeutic agent is a DNA repair and response pathway inhibitor. In some embodiments, flash radiation therapy is combined with a PARP inhibitor (e.g., Talazoparib, Rucaparib, Olaparib) (Lord and Ashworth, 2016; Murai et al., 2012), a RAD51 inhibitor (RI-1), or an inhibitor of DNA damage response kinases such as CHCK1 (AZD7762), ATM (KU-55933, KU-60019, NU7026, VE-821), and ATR (NU7026).

20 [0206] In some embodiments, the therapeutic agent is an inhibitor of the KRAS and/or MAPK pathway. In some embodiments, flash radiation therapy is combined with inhibitors upstream and/or downstream of KRAS. Upstream inhibitors can target EGFR (Erlotinib, Gefitinib, Lapatinib, Afatinib) and/or SHP2 (RMC-4550). Downstream inhibitors include inhibitors of MEK (Trametinib, Selumetinib, PD0325901), BRAF (Dabrafenib, Vermurafenib, PLX-4720), or
25 ERK (SCH772984, LY3214996, GDC-0994).

[0207] In some embodiments, the therapeutic agent is an inhibitor of the Epithelial to Mesenchymal (EMT) Transition. TGF-beta is a known driver of EMT. Thus, in some embodiments, flash radiation therapy is combined with an inhibitor of the EMT Transition, such

as a small molecule inhibitor (e.g., SD-208, LY2109761, LY21157299) or an antibody or fragments thereof that binds TGF-beta.

[0208] In some embodiments, the therapeutic agent induces or is an activator of the TH1 Pathway. T helper type 1 (Th1) cells are a subset of CD4⁺ effector T cells and play a key role in immune response as well as cancer immunotherapy. Th1 subtype cells are involved in activating antigen presenting cells (APCs) and also of recruitment of macrophages or mast cells to the tumor site [1]. They are also involved in direct activation of cytokines in the tumor microenvironment that can lead to enhanced tumor cell killing [2]. The pathway is inhibited by Flash radiation compared to untreated mice. Therefore, in some embodiments, flash radiation therapy is combined with an activator of the TH1 pathway, such as an adjuvant that induces Th1 activity. The adjuvants that can be used in combination with FLASH radiation include but are not limited to 1) cytokines associated with Th1 activation such as IL-12, IFN- alpha, beta or gamma, IL-2, IL-18, IL-27, CD80 (drug target abatacept, beletcept, galaximab), ICAM1 and TNF-alpha [3]; 2) Toll-like receptor agonists for TLR4 and TLR9, such as monophosphoryl lipid A and Bacillus Calmette-Guerin, Agatolimod, ISS-1018, HYB2055, and MGN1703; 3) STAT3 modulators like danvatirsen and OPB-31121; and 4) Inactivated bacteria/parasites or their derivatives that trigger interferon gamma or IL-12 production, included but not limited to *Listeria monocytogenes*, *Leishmania major* and *Toxoplasma gondii*, *Mycobacterium tuberculosis*, staphylococcus enterotoxin B and unmethylated CpG nucleotides that activate Th1 response in the body[4]. In addition gene therapy systems including bacterial or virus based gene expression systems that lead to production of IL2, IL-12 and IFN-gamma when injected at the tumor site can be used to activate the Th1 response.

[0209] In some embodiments, the therapeutic agent is an activator of the Phosphatase and Tensin Homolog (PTEN) pathway, which is protective for carcinogenesis. The PTEN pathway was activated in Flash irradiated tissues, and therefore Flash irradiation is predicted to have protective effects on tissue compared to conventional radiation. The inventors also observed reduced lung fibrosis in Flash vs conventional irradiated mice, and therefore Flash irradiation could reduce lung fibrosis through activation of the PTEN pathway. Therefore, in some embodiments, flash radiation therapy is combined with an activator of the PTEN pathway. Activators/agonists of the PTEN pathway are described in US 2011/0189169 A1, and include

mTOR inhibitors such as rapamycin (Rapamune®, sirolimus, ATC code L04AA10 commercially available from Wyeth) and its chemical analogues such as CCI-779 (temsirolimus, Anatomical Therapeutic Chemical (ATC) code L01XE09, commercially available from Wyeth), RAD-001 (everolimus, ATC code L04AA18, commercially available from Novartis) and AP-2357 (Granville et al., Clin. Cancer Res. 12:679, 2006), which are herein incorporated by reference. Additional activators of PTEN activity include Src inhibitors, p38 MAPK modulators, NF-kB inhibitors, PPAR-gamma modulators, and IGF-1R modulators; Ublituximab, Rituximab, Sunitinib, (Induces PTEN), Trastuzumab and Pertuzumab (Increases PTEN through Src inhibition), Resistin (p38 MAPK modulator, increases PTEN), Simvastatin (NF-kB inhibitor), Lovastatin and Rosiglitazone (PPAR-gamma modulators), NVP-AEW541 (IGF-1R modulator that increases PTEN), and PP1 Herbimycin (Src inhibitors) (see Boosani et al Expert Opin Ther Pat. 2013 May; 23(5): 569–580.)

[0210] In some embodiments, the therapeutic agent is an inhibitor of the TGF-beta pathway. TGF-beta is a protein known to be involved in several normal tissue toxicity effects including lung fibrosis. Bone morphogenic proteins (BMPs) are members of the TGF-beta superfamily, and the BMP pathway is downregulated following Flash treatment. Flash treated mice also exhibited reduced lung fibrosis compared to mice treated with conventional radiation. Thus, in some embodiments, flash radiation therapy is combined with an inhibitor of the TGF-beta pathway, such as a small molecule inhibitor (e.g., SD-208, LY2109761, LY21157299) or an antibody or fragment thereof that binds TGF-beta.

[0211] In some embodiments, the therapeutic agent is an activator or inducer of Type 1 interferons (IFNs), which influence the development of innate and adaptive immune responses. The IFN signaling pathway was downregulated in FLASH treated animals when compared to conventional radiation treatment. Type 1 interferons modulate innate immune responses in a balanced manner that promotes antigen presentation and natural killer cell functions while restraining pro-inflammatory pathways and cytokine production. Type 1 interferons also activate the adaptive immune system, promoting the development of high-affinity antigen-specific T and B cell responses and immunological memory. Type 1 interferons in the microenvironment promote the maturation and antigen presentation of DCs and boost endogenous NK or CD8+ T-cell mediated antitumor immune responses. Thus, in some embodiments, flash radiation therapy

is combined with an activator or inducer of Type 1 interferons, which is expected to create an environment in which immune cells, including T-cells, can eradicate tumor cells.

[0212] In some embodiments, the activator or inducer of Type 1 interferons is an activator of the STING/cGAS pathway. Activators of the STING/cGAS pathway include, synthetic CDN
5 STING agonists, small molecule STING agonists, small molecule STING pathway agonists, viruses encoding STING pathway agonists, bacteria encoding STING pathway agonists, or STING agonist encapsulated nanoparticles and liposomes. In some embodiments, the interferon activator includes compounds that bind and activate Toll-like receptors (TLR) such as TLR4 and TLR9, and compounds that active the MAVS pathway.

10 [0213] In some embodiments, the therapeutic agent is an activator of Dendritic Cell (DC) maturation. As described herein, DC maturation is down-regulated following FLASH treatment. Regulation of phagocytic functions in dendritic cells, and thereby antigen processing and presentation by innate signaling, represents a critical level of integration of the adaptive and innate immune systems. Thus, in some embodiments, flash radiation therapy is combined with an
15 activator of DC maturation, such as a synthetic peptide vaccine.

[0214] Furthermore, inhibitors of CD47/SIRP-alpha can be used to enhance antigen-cross presentation by dendritic cells and increased T-cell priming. Thus, in some embodiments, flash radiation therapy is combined with an inhibitors of CD47/SIRP-alpha, such as, among others, antibodies, antibody derivatives or fragments thereof, and compounds or small molecules that
20 inhibit the CD47/SIRP-alpha interaction.

[0215] In some embodiments, the therapeutic agent is an Aryl Hydrocarbon Receptor (ahR) inhibitor. In some embodiments, flash therapy is combined with an ahR inhibitor, such as SR1, CH-223191, UM729, or Galangin.

IMMUNE MODULATORS

25 [0216] The FLASH radiation therapy described herein can be administered in combination with one or more immune modulators. The combination therapy can provide an increased anti-tumor response (a positive clinical response) compared to administration of either treatment as monotherapy. In some cases, the immune modulator can be selected from the group consisting of an inhibitor to an inhibitory checkpoint molecule, an activator of a stimulatory checkpoint

molecule, a chemokine inhibitor, an inhibitor of macrophage migration inhibitory factor (MIF), a growth factor, a cytokine, an interleukin, an interferon, an antibody that binds to an immune system cell, such as a bispecific antibody that binds to T-cells and a tumor antigen, a cellular immune modulator such as a CAR-T cell, a vaccine, an oncolytic virus, and any combination thereof.

[0217] Immune modulators can include small molecules and biologic therapies (*e.g.*, antibodies, fragments thereof, and derivatives thereof) that bind molecules expressed on the surface of immune system cells, such as antigen presenting cells and T-cells. Biologic therapies can also include bispecific antibodies, fragments thereof, and derivatives thereof that bind to both antigen presenting tumor cells and T-cells. Immune modulators also can include small molecules that inhibit or stimulate the immune system. In some instances, the immune modulator stimulates CD27⁺ immune cells, or inhibits one or more inhibitory checkpoint molecule(s) including PD-1, PD-L1, PD-L2, CTLA-4, BTLA, A2aR, B7-H2, B7-H3, B7-H4, B7-H6, CD47, CD48, CD160, CD244 (2B4), CHK1, CHK2, CGEN-15049, ILT-2, ILT-4, LAG-3, VISTA, gp49B, PIR-B, TIGIT, TIM1, TIM2, TIM3, TIM4, KIR, and ligands thereof, and others. Immune checkpoint pathways and signaling molecules are described in, *e.g.*, Pardoll, *Nature Rev Cancer*, 2012, 12:252-264; and Mellman *et al.*, *Nature*, 2011, 480:480- 489.

[0218] An inhibitor of an inhibitory checkpoint molecule can be an antibody or fragment thereof that specifically binds or recognizes PD-1, PD-L1, PD-L2, CTLA-4, BTLA, A2aR, B7-H2, B7-H3, B7-H4, B7-H6, CD47, CD48, CD160, CD244 (2B4), CHK1, CHK2, CGEN-15049, ILT-2, ILT-4, LAG-3, VISTA, gp49B, PIR-B, TIGIT, TIM1, TIM2, TIM3, TIM4, KIR, and ligands thereof. In some embodiments, the CTLA-4 inhibitor is selected from the group consisting of ipilimumab, tremelimumab, and the like. One non-limiting example of a small molecule immune modulator is an inhibitor of the enzyme indolamine 2,3-dioxygenase (IDO). In some embodiments, the immune modulator is an inhibitor of PD-1, PD-L1, PD-L2, or CTLA-4.

[0219] In some embodiments, the PD-1 inhibitor is selected from the group consisting of pembrolizumab, nivolumab, lambrolizumab, pidilizumab, AMP-244, MEDI-4736, MPDL328 OA, MIH1, IBI-308, mDX-400, BGB-108, MEDI-0680, SHR-1210, PF-06801591, PDR-001, GB-226, STI-1110, biosimilars thereof, biobetters thereof, and bioequivalents thereof. In some

embodiments, the PD-L1 inhibitor is selected from the group consisting of durvalumab, atezolizumab, avelumab, BMS-936559, ALN-PDL, TSR-042, KD-033, CA-170, STI-1014, KY-1003, biosimilars thereof, biobetters thereof, and bioequivalents thereof.

[0220] In some embodiments, the activator of the stimulatory checkpoint molecule is a small molecule, antibody or a fragment thereof, a polypeptide-based activator, a polynucleotide-based activator (*i.e.*, an aptamer), agonist, agonist antibody or fragment thereof, and the like. The stimulatory checkpoint molecule can be B7-1 (CD80), B7-2 (CD86), 4-1BB (CD137), OX40 (CD134), HVEM, inducible costimulator (ICOS), glucocorticoid-induced tumor necrosis factor receptor (GITR), CD27, CD28, CD40, or a ligand thereof.

[0221] In some embodiments, a chemokine inhibitor is administered as an immune modulator. The chemokine inhibitor can be a small molecule, or antibody or fragment thereof that specifically binds to the chemokine (or its receptor) and inhibits its activity. In some embodiments, the chemokine is selected from the group consisting of CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, and CXCL16, or any other chemokine that is associated with cancer such as trafficking leukocytes into the tumor microenvironment (*e.g.*, control leukocyte infiltration to the tumor). In some embodiments, the chemokine inhibitor binds to a chemokine receptor selected from the group consisting of CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7.

[0222] Additional examples of an immune modulator include but are not limited to an anti-TIM4 antibody, an anti-MFG-E8 antibody, an anti-M199 antibody, any combination thereof, and the like. In some embodiments, the immune modulator includes agents (antibodies or small molecules) involved in priming and activation of the immune systems, and includes agents targeting CTLA4, B7 (B7-1 or B7-2), PD-L1/PD-L2, or PD-1, or agents targeting the binding interactions between CTLA4 and B7-1/B7-2, or PD-1 and PD-L1/PD-L2. Agents targeting CTLA4, B7 (B7-1 or B7-2), PD-L1/PD-L2, and PD-1 include antibodies that specifically bind

these molecules, such as monoclonal antibodies. In some embodiments, the agent is an antibody that specifically binds to LAG 3, TIM1, TIM3, MFG-E8, IL-10, or Phosphatidylserine.

[0223] The immune modulators described herein can be administered at therapeutically effective doses. Therapeutically effective doses can be determined by one of ordinary skill in the art based on the type of immune modulator administered. Dosage, routes of administration, and administration schedules described in the art can be used. Representative doses are available in the Merck Manual Professional Edition (see the internet at merckmanuals.com/professional).

[0224] Further, doses of immune modulators administered to animals can be converted to equivalent doses for humans based on the body surface area (BSA) (represented in mg/m^2) normalization method (see, e.g., Reagan-Shaw, S. et al., “Dose translation from animal to human studies revisited,” *FASEB J.* 22, 659-661 (2007); and “Guidance for Industry – Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers,” U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), July 2005, Pharmacology and Toxicology; which are incorporated by reference herein). For example, the human equivalent dose (HED) based on BSA is can be calculated by the following formula I:

[0225] I. $\text{HED} = \text{animal dose in mg/kg} \times (\text{animal weight in kg} / \text{human weight in kg})^{0.33}$

[0226] Alternatively, the HED can be determined by the following formula II:

[0227] II. $\text{HED (mg/kg)} = \text{animal dose (mg/kg)} \times (\text{animal Km} / \text{human Km})$

[0228] The Km factor is determined based on the following Table (see Guidance for Industry, Id.):

[0229] Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area

Species	To Convert Animal Dose in mg/kg to Dose in mg/m ² , Multiply by k _m	To Convert Animal Dose in mg/kg to HED ^a in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg) ^b	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:	12	3.1	0.32
Monkeys ^c	6	6.2	0.16
Marmoset	7	5.3	0.19
Squirrel monkey	20	1.8	0.54
Baboon	27	1.4	0.73
Micro-pig	35	1.1	0.95
Mini-pig			

Assumes 60 kg human.

[0230] Thus, a 5 mg/kg dose in mice is equivalent to a 0.4 mg/kg dose in a 60 kg human. A
 5 0.4 mg/ml dose in a 60 kg human is equivalent to a dose of 14.8 mg/m².

[0231] In some embodiments, the immune modulators described herein are administered in therapeutically effective amounts for periods of time effective to treat a cancer or tumor. The effective amount of the immune modulators described herein can be determined by one of ordinary skill in the art and includes dosage amounts for a mammal of from about 0.5 to about
 10 200 mg/kg, about 0.5 to about 150 mg/kg, about 0.5 to 100 mg/kg, about 0.5 to about 75mg/kg, about 0.5 to about 50mg/kg, about 0.01 to about 50mg/kg, about 0.05 to about 25 mg/kg, about 0.1 to about 25 mg/kg, about 0.5 to about 25 mg/kg, about 1 to about 20 mg/kg, about 1 to about 10 mg/kg, about 20mg/kg of body weight, about 10 mg/kg, about 5 mg/kg, about 2.5 mg/kg, about 1.0 mg/kg, or about 0.5 mg/kg of body weight of the immune modulator, or any range

derivable therein. In some embodiments, the dosage amounts of the immune modulators are from about 0.01 mg/kg to about 10 mg/kg of body weight. In some embodiments, the dosage amount of the immune modulator is from about 0.01 mg/kg to about 5 mg/kg, or from about 0.01 mg/kg to about 2.5 mg/kg of body weight. The compositions described herein can be administered in a single dose or in the form of individual divided doses, such as from 1 to 4 times per day, or once every 2 days, 3 days, 4 days, 5 days, 6 days, weekly, or monthly. The compositions described herein can also be administered for various treatment cycles, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 treatment cycles. The treatment cycles can be different lengths of time depending on the cancer to be treated, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 week treatment cycles. In addition, the effective amount of an immune modulator described herein can be determined during pre-clinical trials and clinical trials by methods known to physicians and clinicians.

EXAMPLES

[0232] The following examples are offered to illustrate, but not to limit, the claimed embodiments.

Example 1

[0233] This example provides results from a study comparing gene expression profiles and tissue damage in mice treated with Conventional and FLASH irradiation.

Microarray Methods:

[0234] **Samples:** C57BL/6 mice were either irradiated with Conventional proton dose rate (1Gy/s), Flash Dose rate (40Gy/s), Split Flash (total dose split into 10 equal parts, each fraction separated by a time period of 1 sec, instantaneous dose at the time of delivery is 40Gy/s overall dose rate is 4Gy/s). 392 age and sex matched mice were treated in 6 Cohorts and sacrificed 1hr-36 wks after lung irradiation. 4 Groups in each cohort: Sham: no radiation; Conventional: 1Gy/s; FLASH: 40Gy/s beam on; Split-FLASH: 4Gy/0.1sec, pulsed delivery (see Fig. 5). Animals were euthanized either 24 hours, 8 weeks, 16 weeks or 24 weeks after radiation and lungs collected. For gene expression analysis a total of 96 lung samples (24 samples / timepoint) were taken, three male and three female each from four different treatment groups, i.e. Sham, Conventional 15 Gy, Flash 15 Gy and Split Flash 15 Gy.

[0235] RNA isolation and quality control: Total RNA was isolated using Qiagen RNasy Tissue microarray kit (Qiagen cat 74104). Briefly, frozen middle left lung from mice (20-30 mg) was homogenized in liquid nitrogen and RNA isolated using the manufacturers protocol. Integrity of the isolated RNA was assessed using the 2100 Bioanalyzer (Agilent technologies) and only samples with RNA integrity number of at least 7 or 28/18S ratio of 1.3 or higher were considered for further processing.

[0236] Sample amplification and labelling: Fluorescent Cy3 Labelling was done as recommended by Agilent Technologies using the One-Color Microarray-Based Gene Expression Analysis Low input Quick Amp Labeling. 100 ng RNA of each sample was amplified and labelled with the One-Color Microarray-Based Gene Expression Analysis Low input Quick Amp Labelling kit and 600 ng of Cy3 labelled cRNA was used for hybridization on each patch. Amplification and integration of Cy3 was measured using the Nanodrop and only samples having specific activity of above 8 were used further.

[0237] Gene-expression profiles were generated using the SurePrint G3 Mouse Gene Expression v2 microarray 8x60K produced by Agilent Technologies (Palo Alto, CA, USA). Labeling and hybridization was performed following the manufacturer's protocol. Each sample was loaded in triplicate onto the Agilent array for a total of 72 slots. Then, 600 ng of Cy3-labeled cRNA was hybridized on the 8 × 60K arrays using Agilent's High-RPM Gene Expression Hyb Kit. Hybridization was performed for 20 hrs at 65 °C in a rotating hyb oven at 10 rpm. After washing, arrays were scanned using Innopsys 710 Innoscan. Resulting TIFF-images were processed using Innopsys Feature Extraction software Mapix.

[0238] Expression Profile pre-processing & normalization: After image extraction using Mapix software correlation analysis between technical replicates was conducted to identify any technical replicates which did not correlate with their sibling replicates. Any arrays that passed QC were consolidated into one file by calculating the median of each technical replicate's mean intensity and median background. Data files were analyzed using an R package Limma (Ritchie et al., 2015). After uploading data, mean fluorescent intensities arrays were quantile normalized to allow comparisons across different groups(Bolstad et al., 2003). In order to handle flagged spots a weight of 0 for all flags with values less than -50 was assigned. This ensured that during the linear fitting of the data that these points are not be considered for linear fitting.

[0239] Gene Set Enrichment Analysis (GSEA): After normalization data was used for GSEA analysis to identify enriched pathways in each treatment group (Subramanian et al., 2005). A classic GSEA analysis was run using the HALLMARKS gene sets using probe level data between treatments groups and sham. For downstream analysis, pathways with FDR-q values less than 25% and corrected p-values less than or equal to 0.05 were considered for reporting and downstream purposes.

[0240] Differentially expressed genes analysis: To identify differentially expressed genes between treatment groups, Limma's package was used which implements a linear model fitting of the data and then uses a moderated T-Test to compare probes between comparison groups. To correct for multiple hypothesis testing p-values using Bonferroni-Hochberg method were adjusted and genes with adjusted p-value less than or equal to 0.05 for downstream analysis (Benjamini and Hochberg, 1995) were considered.

[0241] Ingenuity Pathway Analysis (IPA): Traditional analysis of the microarray data by generating a list of most regulated genes is not enough to understand the functions of the regulated genes and their roles in biological processes. Hence, molecular pathway analysis was also performed using IPA to predict what canonical pathways, upstream regulators and biofunctions were altered to identify the molecular events and further unveil the molecular mechanisms being regulated by altering the dose rate of radiation from 1Gy/s to 40Gy/s IPA searches through the gene list and determines genes that are involved in well documented canonical signal transduction or metabolic pathways.

[0242] IPA queries the Ingenuity Pathways Knowledge Base for interactions between the focused genes first and then all other gene objects stored in the knowledge base. For our data, genes that met the parametric P value of 0.05 were queried for IPA Core analysis followed by identification of major canonical pathways modulated. The significance of the association between the data set and the canonical pathway was determined based on two parameters: (1) A ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway and (2) a P value calculated using Fischer's exact test, followed by Benjamini Hotchberg correction (FDR cutoff value of ≤ 0.1) determining the probability that the association between the genes in the data set and the canonical pathway is due to chance alone 3) An absolute value cutoff of Z score 1.5 or above to predict the enrichment

and activation status of a particular pathway. The pathways that met all these criterion were predicted to be significantly regulated.

TUNEL Methods:

[0243] TUNEL Fluorescence Staining: Apoptosis was determined by Terminal

5 deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) fluorescence staining using the In Situ Cell Death Detection Kit, Fluorescein (Sigma-Aldrich Corporation, Saint Louis, MO). Snap frozen lung tissues were sectioned 5 μ m thick, then fixed, permeabilized, and stained according to the manufacturer's directions. Tissue sections were also treated with DAPI (NucBlue™ Fixed Cell ReadyProbes™ Reagent (Thermo Fisher Scientific, Waltham, MA), 2
10 drops per mL of PBS) as a counterstain. Images were acquired at 100X magnification with Immersion Oil, type LDF (Cargille-Sacher Laboratories Inc., Cedar Grove, NJ) using the DAPI and FITC channels on a Keyence BZ-X700 microscope (Keyence Corporation, Osaka, Japan).

[0244] Analysis: Several fields of view were imaged for each sample on a Nikon ECLIPSE Ni-E microscope (Nikon Corporation, Chiyoda, Japan) at 100X magnification with oil

15 immersion in the DAPI and FITC fluorescent channels (nuclei and TUNEL respectively). These images were imported into QuPath (Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. Sci. Rep. 7, 16878 (2017)) and the watershed cell detection plugin was used to segment and count the nuclei in the DAPI channel (Fig. 22). Finally, the Fiji distribution of ImageJ (Rueden, C. T.; Schindelin, J. & Hiner, M. C. et al. (2017), "ImageJ2: ImageJ for the
20 next generation of scientific image data", BMC Bioinformatics 18:529, doi:10.1186/s12859-017-1934-z); and Schindelin, J.; Arganda-Carreras, I. & Frise, E. et al. (2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676-682, PMID 22743772, doi:10.1038/nmeth.2019) was used to quantify the number of TUNEL positive cells in each field of view. A gaussian blur was first applied to the image, and then thresholding was used to
25 segment the cells (Fig. 23A and 23B).

[0245] Lung Function: Lung and penh was evaluated using Whole Body Plethysmograph (Jackson et Health Physics 2014). A baseline measure of penh was taken and thereafter animals were monitored every 2 weeks for lung function. The results are shown in FIG. 6.

[0246] Lung Fibrosis: Mice were either untreated or treated either conventional, Flash or split flash radiation. At various time points 16, 24 and 36 weeks the mice were euthanized, lung tissue was fixed in formalin and embedded in parafilm. The lung sections were stained with masson trichome and H&E stains and microscopic evaluation was performed by a trained pathologist who scored for fibrosis using the MFSS scoring scheme from 0-8 (Ashcroft Journal of Clinical Pathology 1988 and Hubner et al Biotechniques 2008), with 0 being no fibrosis and 8 corresponding to complete obliteration of the pulmonary architecture by severe fibrosis with complete loss of airspace (obliterative fibrosis). The results are shown in FIG. 10.

[0247] Dermatitis in the mice was scored by using the Douglas and Fowler scale. The scoring was done between 0 and 3.5, with 0 being normal and 3.5 being moist desquamation in most of the irradiated area along with necrosis (Ryan et al Journal of investigative Dermatology 2012). The results are shown in FIG. 9.

[0248] Survival: Mice were treated with 15, 17.5 and 20 Gy of conventional or Flash radiation dose. Each radiation dose group was composed of 20 age-matched animals (50% male and 50% female). Animals were monitored thrice weekly and euthanized if there was more than 20% body weight loss with no recovery within 2 days or severe dermatitis as scored by a veterinarian. The results are shown in FIG. 7 and FIG. 8.

Results:

Lung function is improved and fibrosis is decreased using FLASH radiation

[0249] FIG. 6 shows improved lung function for FLASH when compared to Conventional radiation treatment. This difference confirms the presence of dose rate dependence of radiation toxicity. FIG. 10 shows a 23% reduction in average lung fibrosis severity for FLASH over Conventional group at 17.5 Gy. This difference confirms the presence of dose rate dependence of radiation toxicity. FIG. 11 shows an increased average lung weight for Conventional when compared to FLASH treatment – 0.46 g vs. 0.40 g (M); 0.37 g vs. 0.32 g (F)). This study indicates normal tissue sparing for FLASH.

Survival of mice is increased after irradiation with FLASH compared with conventional radiation

[0250] FIG. 7 shows an improved median survival for FLASH over Conventional radiation treatment groups: 20.0 Gy: 14% increase; 17.5 Gy: 18% increase. FIG. 8 shows an improved median survival for FLASH over Conventional radiation treatment groups and the potential for a therapeutic window that is dose rate dependent: 17.5 Gy FLASH > 17.5 Gy CON; 17.5 Gy

5 FLASH = 20.0 Gy CON.

FLASH decreases dermatitis compared with conventional radiation

[0251] FIG. 9 shows a reduction in average dermatitis for FLASH when compared to Conventional radiation: FLASH: 34% reduction; Split-FLASH: 52% reduction. This difference confirms the presence of dose rate dependence of radiation toxicity related to dermatitis.

10 **Microarray:**

Flash radiotherapy (FLASH RT) modalities synergize with mitotic spindle inhibitors

[0252] FIG. 12 shows Venn Diagrams of Differentially Expressed genes at each time point. GSEA at 24 hours revealed G2M checkpoint and E2F Targets were repressed by all radiotherapy modalities (Fig. 13A). These signatures suggest that cells are arresting at G1 and G2M post treatment. E2F targets include a broad range of genes; however, cyclins are major targets for this pathway {cite}. Therefore, the use of Flash RT in combination with CDK4/6 inhibitor (Pablociclib) may sustain cell cycle arrest caused by radiotherapy.

[0253] The mitotic spindle was specifically downregulated in both flash and split flash groups (Fig. 14A, 14B). An overlap analysis of the core enrichment genes for each group shows that key regulators of mitotic spindle genes were downregulated such as AURKA, Kinesin-Like protein family genes (KIF11, KIF23, KIF23, KIF4A) and TPX2 (Fig. 14C, 14D) Emerging evidence that AURKA and TPX2 play major roles in driving acquired resistance in the context of several targeted therapies (Donnella et al., 2018; Panicker et al., 2017). In these models AURKA expression increases to drive drug resistance, thus blocking AURKA expression may be important for the efficacy of Flash and Split Flash therapies. Specifically targeting AURKA with small molecule inhibitors (Alisertib, Tozasertib). TPX2 is a driver of AURKA and blocking TPX2-AURKA complexes using a complex disruptor (GSK1070916) (Asteriti et al., 2015; Janeček et al., 2016) is indicated. Finally, mitotic spindle integrity can be targeted by using Taxanes (Docetaxel, Paclitaxel). At the end of 24 weeks, FLASH RT upregulated Mitotic

Spindle genes (Fig. 13D), in support that adaptive gene expression changes may play a critical role to the efficacy of radiotherapy.

Flash RT modalities combinations with DNA Damage Repair & Response inhibitors

[0254] Flash RT specifically repressed DNA repair pathways genes. At the pathway level

- 5 Flash RT downregulated the Hallmark DNA Repair pathway (Fig. 13A & 15A). Core enrichment analysis reveals the downregulation of key Homology Directed Repair (HR) genes such as LIG1, RAD51, and BRCA2 (Fig. 15B for full list). The immediate implication of these downregulated genes suggest that flash therapy induces a *BRCAness* phenotype, therefore targeting tumors with PARP inhibitors (Talazoparib, Rucaparib, Olaparib) in combination with
- 10 FLASH RT (Lord and Ashworth, 2016; Murai et al., 2012) may be indicated.

[0255] Additionally, in the context of inhibited DNA repair (i.e. BRCA1 mutations, or

BRCAness) the DNA damage response is blocked by blocking key regulators of this pathway.

For example, RAD51 is required for the repair of double stranded breaks thus to the combination of FLASH RT and RAD51 inhibitors (RI-1) may synergize. Additionally, targeting other DNA

- 15 damage response kinases such as CHCK1 (AZD7762), ATM (KU-55933, KU-60019, NU7026, VE-821), ATR (NU7026), can also be effective in the context of flash RT induced *BRCAness* (Ashworth and Lord, 2018; Lord and Ashworth, 2016).

Flash RT in combination with MAPK pathway inhibitors

[0256] Post 24hrs, many pathways increase in expression (Fig. 13B-D), specifically KRAS Signaling upregulated across time in all treatment groups, specifically 'KRAS Signaling Up' was enriched in conventional and FLASH groups between 8-24 weeks (Figure 16A-C). KRAS signaling is a major driver of cancer and drives many biological programs through the MAPK pathway including proliferation, cell cycle progressing and pro-survival signaling (Sun et al., 2015). There is evidence that conventional radiation therapy depends on blocking EGF/EGFR signaling (Wang et al., 2011). While direct inhibitor of RAS are still in early stages, the aim is to block up and downstream RAS signaling (Downward, 2003) using the combination of FLASH RT with inhibitors upstream and downstream of KRAS. As an example, upstream inhibitors such as, EGFR (Erlotinib, Gefitinib, Lapatinib, Afatinib) and SHP2 (RMC-4550) can be targeted in combination with FLASH therapy. As another example, downstream inhibitors such as MEK (Trametinib, Selumetinib, PD0325901), BRAF (Dabrafenib, Vermurafenib, PLX-4720), or ERK (SCH772984, LY3214996, GDC-0994) can be combined with FLASH RT.

Radiotherapy induced EMT

[0257] During the time course of 8-10 weeks Epithelial to Mesenchymal (EMT) Transition was an upregulated pathway in Flash between 8 to 16 weeks (Fig. 13B-C); however, at the end of 24 weeks EMT was upregulated in both Conventional and Flash Treatments (Figure 17A-B). EMT is known to drive resistance to both targeted and non-targeted therapies and thus may pose a challenge to the efficacy of radiotherapy modalities (Kitai and Ebi, 2016; Liang et al., 2015). EMT is a consequence of gene expression changes that drive cells from one state to another, thus targeting EMT may pose a challenge. Overlap analysis of the EMT signature for Conventional and Flash at 24 weeks reveals that there are a total of 56 genes overlapping (Fig. 17C, Table 5). Within the overlap TGFB1 and TGFBI, both were upregulated in conventional and flash treated mice. TGFB is a known driver of EMT and thus TGFB inhibitors (SD-208, LY2109761, LY21157299) may prevent EMT from occurring and give rise to radioresistant tumors (Foroutan et al., 2017)

Differential gene expression analysis:

[0258] To get an assessment of early response to flash vs conventional treatment, microarray assessment was performed for irradiated mice euthanized 24 hrs post-irradiation. Initial cluster analysis reveals that for early time point Flash and sham samples cluster together while conventionally treated mice clustered with Split flash group. This follows the pattern initially seen with survival and dermatitis where Flash fared much better than conventional initially but later cluster closer to conventional treatment. Based on the gene expression analysis, at 24 hrs conventional irradiation (1Gy/s) most significantly regulated 2131 genes (p value of 0.05) while Flash regulated only 257 genes at 24Hr time point, out of these 175 common genes were regulated by both these radiation types (Fig. 5).

IPA analysis:

[0259] IPA analysis revealed that many canonical pathways were altered by these different radiation treatment regimens. Major changes were observed at the initial time point of 24 hrs where most of the regulation at RNA level happens that is captured by the genome wide microarray. Most of the pathways significantly upregulated (p value ≤ 0.05 , Z score of ≥ 1.5 and FDR 0.1) in the conventional radiation were involved in inflammatory response like Interferon signaling, IL-8 signaling, STAT3 signaling, GP6 signaling pathway and phospholipase C signaling. Some cancer associated pathways were upregulated as well, like the pancreatic adenocarcinoma signaling and colorectal cancer metastasis signaling. Cyclin and cell cycle regulation pathway was predicted to be inhibited after conventional irradiation. (Figure 18 and Table 6A-C). At 24 hrs for Flash radiation most of the pathways were downregulated and that included Mitotic roles of polo-like kinases, estrogen-mediated S phase entry, Aryl hydrocarbon receptor signaling, cyclin and cell cycle regulation, TH1 for helper T cells pathway, dendritic cell maturation and Calcium induced T lymphocyte apoptosis. In the split flash pathways regulated mostly involved cell cycle with distinct phases of cell cycle been modulated differentially along with the p53 pathway. The common pathway modulated by all the treatment types was Cyclin and cell cycle regulation, that is known to be disrupted after radiation treatment.

[0260] When comparison between conventional and Flash radiation was performed (p value of overlap >0.05 and Z score of 1.5), few pathways were shown to be differentially regulated

between these treatments (Fig. 18). Dendritic cell maturation, PKC signaling in lymphocytes, TH1 pathway and calcium-induced T lymphocyte apoptosis was shown to go up after conventional radiation and down after Flash treatment. While many pathways were significantly upregulated after conventional radiation and unaffected after Flash radiation like IL-8, production of ROS and MO in macrophages, interferon signaling, Rho family GTPases, GP6 signaling, colorectal cancer metastasis signaling, Sphingosine-1-phosphate signaling etc.

[0261] TH1 Pathway: T helper type 1 (Th1) cells are a subset of CD4⁺ effector T cells and play a key role in immune response as well as cancer immunotherapy. Th1 subtype are involved in activating antigen presenting cells (APCs) and also of recruitment of macrophages or mast cells to the tumor site[1]. They are also involved in direct activation of cytokines in the tumor microenvironment that can lead to enhanced tumor cell kill[2]. This pathway was found to be inhibited by Flash compared to untreated mice, therefore flash radiation and immunotherapy combinations will work more effectively when combined with adjuvants that induce Th1 activity. The adjuvants to be used in combination with FLASH delivery include but are not limited to 1) cytokines associated with Th1 activation such as IL-12, IFN- alpha, beta or gamma, IL-2, IL-18, IL-27, CD80 (drug target abatacept, beletcept, galaximab), ICAM1 and TNF-alpha[3]; 2) Toll-like receptor agonist for TLR4 and TLR9 such as monophosphoryl lipid A and Bacillus Calmette-Guerin, Agatolimod, ISS-1018, HYB2055, and MGN1703; 3) STAT3 modulators like danvatirsen and OPB-31121; 4) Live bacteria (such as *Listeria monocytogenes*) or intracellular parasites (such as *Leishmania major* and *Toxoplasma gondii*), or compounds derived therefrom that trigger interferon gamma or IL-12 production, Bacterial lipopolysaccharide, killed *Mycobacterium tuberculosis*, bacterial superantigens such as staphylococcus enterotoxin B and unmethylated CpG nucleotides that activate Th1 response in the body[4].

[0262] Calcium-mediated T cell apoptosis: One important pathway through which T cells undergo apoptosis is through calcium channel involving binding of MHC-class II complex on Antigen Presenting Cells to the TCR-CD3 complex on T cells, that leads to activation of PLC-gamma1 which in turns activate PKC and accumulation of calcium in the cytosol. The free calcium through interaction with calcineurin, CABIN and NFAT triggers an apoptotic pathway. This pathway was downregulated after Flash and was activated by conventional irradiation.

Therefore, Flash radiation may spare T lymphocytes both by nature of its being quicker treatment modality (higher dose rate) that avoids irradiating more blood and also through downregulation of this pathway thus leading to molecular sparing of T lymphocytes.

Lymphocytes are considered an organ-at-risk during radiation therapy and many organs with a high blood flow like lungs and brain have to be limited in dose to reduce lymphocyte killing[5]. Thus, Flash radiation in combination with treatment planning algorithms can be used to treat organ sites with a high lymphocyte count. This lymphocyte sparing will also have implications in providing effective immunotherapy combinations with radiation.

[0263] For 16-week analysis, two canonical pathways were found to be significantly modulated in the flash grp. The cyclin and cell cycle regulation pathway was activated while the NFAT pathway in cardiac hypertrophy was downregulated (p value ≤ 0.05 , Z score of ≥ 1.5 and FDR 0.1). In comparative analysis between Conventional 15 Gy and Flash 15 Gy radiation using Fischer exact statistical analysis (p value ≤ 0.05 and Z score of ≥ 1.5) many pathways were found to be regulated by both the treatments. Interferon signaling was found to be upregulated in both through was found to be more activated in the conventional grp (Figure 18). BMP signaling pathway was found to be downregulated in Flash vs Conventional.

[0264] PTEN: Phosphatase and tensin homolog (PTEN) pathway that is protective for carcinogenesis was activated in Flash, therefore predicting that maybe perhaps Flash can have protective effects on tissue compared to conventional. Loss of PTEN expression in mouse fibroblast is shown to result in lung fibrosis (Parapuram et al, Matrix Biol 2015). PTEN knockout mice treated with bleomycin (radio-mimetic drug) showed increased lung fibrosis. Because reduced lung fibrosis was observed in Flash vs conventional radiation treatment(Fig. 10), one of the mechanisms through which Flash results in reduced lung fibrosis could be through the activation of PTEN pathway. Activators/agonists of the PTEN pathway are described in US 2011/0189169 A1, and include mTOR inhibitors such as rapamycin (Rapamune®, sirolimus, ATC code L04AA10 commercially available from Wyeth) and its chemical analogues such as CCI-779 (temsirolimus, Anatomical Therapeutic Chemical (ATC) code L01XE09, commercially available from Wyeth), RAD-001 (everolimus, ATC code L04AA18, commercially available from Novartis) and AP-2357 (Granville et al., Clin. Cancer Res. 12:679, 2006, which is herein incorporated by reference). Additional Activators of PTEN

activity include Ublituximab, Rituximab, Sunitinib, (Induces PTEN), Trastuzumab and Pertuzumab (Increases PTEN through Src inhibition), Resistin (p38 MAPK modulator, increases PTEN), Simvastatin (NF0-kB inhibitor), Lovastatin and Rosiglitazone (PPAR-gamma modulators), NVP-AEW541 (IGF-1R modulator that increases PTEN), and PP1 Herbimycin (Src inhibitors) (see Boosani et al Expert Opin Ther Pat. 2013 May; 23(5): 569–580.)

[0265] BMP pathway: Bone morphogenic proteins (BMPs) are members of the TGF-beta superfamily. TGF-beta is a protein known to be involved in several normal tissue toxicity effects including lung fibrosis. The BMP pathway was downregulated at 16 week time point in the Flash treatment group. Flash treated samples also exhibited reduced lung fibrosis at 16 and 24 week time points. Therefore, inhibiting the TGF-beta pathway may reduce normal tissue toxicity.

Interferon signaling:

[0266] Type 1 interferons (IFNs) influence the development of innate and adaptive immune responses. One of the most dysregulated canonical pathways, the IFN signaling pathway, was downregulated in FLASH when compared to CONV. Type 1 interferons modulate innate immune responses in a balanced manner that promotes antigen presentation and natural killer cell functions while restraining pro-inflammatory pathways and cytokine production. Type 1 interferons also activate the adaptive immune system, promoting the development of high-affinity antigen-specific T and B cell responses and immunological memory. Type 1 interferons in the microenvironment promote the maturation and antigen presentation of DCs and boost endogenous NK or CD8+ T-cell mediated antitumor immune responses. Thus, type 1 interferon activators can be used in combination with FLASH delivery to create an environment in which immune cells, including T-cells, can thrive to eradicate tumor cells.

[0267] One key pathway involved in the production of type 1 interferons is the STING/cGAS pathway. Stimulator of interferon genes (STING) is an intracellular signaling molecule that senses cyclic dinucleotides (CDNs). CDNs are derived from infectious agents exogenously, or are produced by the mammalian dsDNA sensor cGAS (cyclic guanosine monophosphate – adenosine monophosphate; cyclic GMP-AMP synthase). The STING/cGAS sensing mechanism

induces innate immune responses including the production and release of type 1 interferons. Thus, activators of the STING/cGAS pathway can be used in combination with FLASH delivery to create an environment in which immune cell can thrive to eradicate tumor cells. These activators include, among others, synthetic CDN STING agonists, small molecule STING agonists, small molecule STING pathway agonists, viruses encoding STING pathway agonists, bacteria encoding STING pathway agonists, or STING agonist encapsulated nanoparticles and liposomes. Other type 1 interferon activators include compounds that bind and activate Toll-like receptors (TLR) such as TLR4 and TLR9, and compounds that active the MAVS pathway.

Dendritic cell maturation:

- 10 [0268] Dendritic cell maturation is down-regulated in FLASH. Regulation of phagocytic functions in dendritic cells, and thereby antigen processing and presentation by innate signaling, represents a critical level of integration of the adaptive and innate immune systems. Combination of FLASH and synthetic peptide vaccines could be beneficial to induce long-lasting immune responses.
- 15 [0269] Furthermore, inhibitors of CD47/SIRP-alpha can be used enhance antigen-cross presentation by dendritic cells and increased T-cell priming. These include, among others, antibodies, antibody derivatives and small molecules inhibiting the CD47/SIRP-alpha interaction.

Nanoparticles:

- 20 [0270] Nanoparticles are small particles between 1 and 100 nm in size. They can have different shapes such as spheres, rods, or stars. They can be passively targeted to tumors through the enhanced permeability and retention effect or actively targeted, such as by conjugating antibodies or peptides to the nanoparticles. Furthermore, they can be encapsulated in vesicles or inserted into gel matrices to increase tumor targeting.
- 25 [0271] It has been previously shown that nanoparticles with a high effective atomic number, such as gold or gadolinium, can have a synergistic effect when administered prior to radiotherapy by providing a dose enhancement (Hainfeld et al., 2004). This is because when hit by photons, the photoelectric effect occurs in the nanoparticles, whereby electrons and additional x-rays are emitted. Furthermore, radiation of nanoparticles can also cause hyperthermia in the

tumor, increasing the treatment efficacy. It has also been shown that a dose enhancement can be achieved when using nanoparticles in conjunction with proton therapy (Lin, et al. 2014). Finally, radiation can also be used to modulate and enhance the delivery of nanoparticles to the tumor by altering the tumor microenvironment, such as by reducing the tumor interstitial pressure

(Stapleton et al., 2017).

Flash RT can be used in conjunction with nanoparticles

[0272] One of the main hurdles of using nanoparticles in conjunction with radiotherapy is that nanoparticles can have a short half-life in the body. It is also challenging and impractical to deliver nanoparticles before each of several treatments in a traditional fractionated schedule. In addition, some nanoparticles can be toxic in high enough concentrations, especially when not quickly cleared from the body. These challenges can be overcome by using nanoparticles in conjunction with Flash radiotherapy. By treating the tumor or tumors in only a single fraction, the nanoparticle concentration in the tumor or tumors could remain high enough throughout the entire treatment, even if the nanoparticles had a relatively short half-life. Furthermore, because nanoparticles with a short half-life would still be effective, possible toxicity would be reduced as the nanoparticles would be cleared from the body quickly. One example of a workflow for Flash radiotherapy in conjunction with nanoparticles, is the administration of nanoparticles followed by imaging, such as by CT or MRI. These imaging data are used to create a Flash radiotherapy treatment plan, considering the distribution of the nanoparticles. Then, prior to treatment, nanoparticles are reinjected and the planned Flash treatment delivered.

Aryl Hydrocarbon Receptor Pathway:

[0273] The Aryl Hydrocarbon Receptor (AhR) Signaling pathway was downregulated in Flash treated lungs. The general function of this pathway is to detect aromatic (aryl) hydrocarbons and activate a suite of xenobiotic-metabolizing genes, specifically cytochrome P450 enzymes. This has major implication for drug combination strategies as the downregulation of this pathway may alter the clearance of other drugs in a patient. Additionally, the activation of this pathway may also promote pan drug resistance to small molecules or the cell's ability to process toxic agents due to radiotherapy. AhR pathway activation has also been associated with cancer immunotherapy, AhR regulates both innate and adaptive immunity, it activates anti-inflammatory Treg cells and M2 macrophages. Therefore the combination of radiotherapy with

ahR inhibitors (such as SR1, CH-223191, UM729, Galangin) may suppress the tumors ability to clear toxic agents and activate immune cells.

Apoptosis in Lung Tissue

[0274] *MERGEFORMAT*MERGEFORMATFIG. 24 is a graph showing the TUNEL

5 results. The graph shows the average number of counts per animal for the roughly 20 animals per group (males and females pooled). Quantifying TUNEL cells constitutes a counting problem in which the number of TUNEL positive cells is counted per group of animal samples and therefor should follow Poisson statistics. The error bars on the chart indicate the 95% confidence intervals calculated using the exact estimation method articulated by Ulm et al. (Ulm, 10 K., "A simple method to calculate the confidence interval of a standardized mortality ratio (SMR)", Am J Epidemiol, 1990, 131(2): 373-5). The overlap of 95% confidence intervals indicates that SplitFlash (pulsed Flash) and FLASH are more similar to Sham than Conventional, thereby indicating a lower occurrence of apoptosis for these novel treatment techniques.

Table 2 : 24 Hours Top Enriched Pathways

Table 2A Conventional Versus Sham:

Hallmark Pathway	NES	NOM p-val	FDR q-val
HALLMARK_PANCREAS_BETA_CELLS	-1.53295	0.017857	0.109607
HALLMARK_G2M_CHECKPOINT	-1.52134	0.020704	0.083659
HALLMARK_E2F_TARGETS	-1.50024	0.008529	0.074088
HALLMARK_SPERMATOGENESIS	-1.4566	0.029536	0.10317

Table 2B Flash Versus Sham:

Hallmark Pathway	NES	NOM p-val	FDR q-val
HALLMARK_MITOTIC_SPINDLE	-1.75448	0.003968	0.04197
HALLMARK_G2M_CHECKPOINT	-1.73351	0.001	0.024433
HALLMARK_E2F_TARGETS	-1.64836	0.005988	0.063113

HALLMARK_ALLOGRAFT_REJECTION	-1.57263	0.026465	0.132354
HALLMARK_DNA_REPAIR	-1.50316	0.041016	0.208294

Table 2C Split Flash Versus Sham

Hallmark Pathway	NES	NOM p-val	FDR q-val
HALLMARK_INTERFERON_ALPHA_RESPONSE	1.638147	0.009653	0.218787
HALLMARK_G2M_CHECKPOINT	-1.6643	0.001	0.042827
HALLMARK_E2F_TARGETS	-1.61984	0.001	0.040858
HALLMARK_MITOTIC_SPINDLE	-1.61318	0.018145	0.031744

NES= Normalized Enrichment Score, Nom p-val = Normalized -values, FDR-q-val = False

5 Discovery Rate value

Table 3: 8 Weeks Top Enriched Pathways**Table 3A Conventional Versus Sham:**

Hallmark Pathways	NES	NOM p-val	FDR q-val
HALLMARK_CHOLESTEROL_HOMEOSTASIS	1.61763 2	0.008602	0.24828 9
HALLMARK_P53_PATHWAY	1.58845 3	0.018595	0.18615 6
HALLMARK_ANGIOGENESIS	1.56595 6	0.001	0.14802 7
HALLMARK_UV_RESPONSE_UP	1.54882 3	0.002079	0.13248
HALLMARK_MITOTIC_SPINDLE	1.54585 9	0.012579	0.10919 6
HALLMARK_GLYCOLYSIS	1.52934 5	0.024948	0.10219 2
HALLMARK_MTORC1_SIGNALING	1.52647	0.033058	0.08984

	3		6
HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.5244	0.002088	0.08127 1
HALLMARK_HYPOXIA	1.51058 2	0.012552	0.08401 1
HALLMARK_APOPTOSIS	1.50634 9	0.008493	0.07181 7
HALLMARK_ANDROGEN_RESPONSE	1.46668 1	0.021277	0.09527 6
HALLMARK_PI3K_AKT_MTOR_SIGNALING	1.46244 3	0.04277	0.09178 7
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	1.44437 3	0.027542	0.10201 3
HALLMARK_COAGULATION	1.43281 5	0.033543	0.10604 9
HALLMARK_COMPLEMENT	1.42985 6	0.004301	0.09521 7
Hallmark Pathways	NES	NOM p-val	FDR q-val

HALLMARK_KRAS_SIGNALING_UP	1.42324 9	0.018828	0.09570 5
HALLMARK_INFLAMMATORY_RESPONSE	1.39063 1	0.035124	0.11403 4
HALLMARK_IL2_STAT5_SIGNALING	1.31162 9	0.038136	0.18504

Table 3B Flash Versus Sham:

HALLMARK_COAGULATION	1.406174	0.045714	0.225907
HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.382228	0.036329	0.225861
HALLMARK_APICAL_JUNCTION	1.3685	0.016	0.231629
HALLMARK_WNT_BETA_CATENIN_SIGNALING	1.361896	0.02924	0.227167
HALLMARK_KRAS_SIGNALING_UP	1.351275	0.043561	0.228315

NES= Normalized Enrichment Score, Nom p-val = Normalized -values, FDR-q-val = False Discovery Rate value

Table 4: 16 Weeks Top Enriched Pathways

5

Table 4A Conventional Versus Sham:

Hallmark Pathways	NES	NOM p-val	FDR q-val
HALLMARK_COAGULATION	1.5755655	0.001	0.17153585
HALLMARK_HEDGEHOG_SIGNALING	-1.6175224	0.0056926	0.032724753

Table 4B Flash Versus Sham:

Hallmark Pathway	NES	NOM p-val	FDR q-val
EPITHELIAL_MESENCHYMAL_TRANSITION	1.5123537	0.0116505	0.2488241
HALLMARK_COAGULATION	1.4792827	0.0223577	0.2174025
HALLMARK_APOPTOSIS	1.446631	0.0371094	0.24221855
HALLMARK_KRAS_SIGNALING_UP	1.438819	0.0233463	0.22549081
HALLMARK_HYPOXIA	1.4367952	0.0039526	0.20197491
HALLMARK_MYOGENESIS	1.406268	0.0380228	0.2080547

10 **Table 4C Flash Versus Sham:**

Hallmark Pathway	NES	NOM p-val	FDR q-val
HALLMARK_P53_PATHWAY	1.5119545	0.0087912	0.24653515

NES= Normalized Enrichment Score, Nom p-val = Normalized -values, FDR-q-val = False Discovery Rate value

Table 5: 24 Weeks Top Enriched Pathways

Table 5A Conventional Versus Sham:

Hallmark Pathways	NES	NOM p-val	FDR q-val
EPITHELIAL_MESENCHYMAL_TRANSITION	1.715518	0.001	0.066558
HALLMARK_P53_PATHWAY	1.657078	0.002045	0.065537
HALLMARK_COAGULATION	1.629851	0.001	0.062578
HALLMARK_IL2_STAT5_SIGNALING	1.566532	0.004016	0.099271
HALLMARK_INFLAMMATORY_RESPONSE	1.559741	0.014257	0.090297
HALLMARK_HYPOXIA	1.546812	0.004124	0.087968
HALLMARK_ALLOGRAFT_REJECTION	1.517242	0.028169	0.107425
HALLMARK_INTERFERON_GAMMA_RESPONSE	1.513903	0.010101	0.098017
HALLMARK_MITOTIC_SPINDLE	1.505295	0.021359	0.094306
HALLMARK_E2F_TARGETS	1.502026	0.032882	0.088742
HALLMARK_MYC_TARGETS_V2	1.496078	0.026477	0.085209
HALLMARK_GLYCOLYSIS	1.465007	0.035928	0.10566
HALLMARK_KRAS_SIGNALING_UP	1.45314	0.044715	0.084997
HALLMARK_APICAL_JUNCTION	1.447435	0.02008	0.078949
HALLMARK_ESTROGEN_RESPONSE_LATE	1.287919	0.041667	0.181257

5

Table 5B Flash Versus Sham:

Hallmark Pathways	NES	NOM p-val	FDR q-val
HALLMARK_COAGULATION	1.754068	0.001	0.030982
EPITHELIAL_MESENCHYMAL_TRANSITION	1.743082	0.002066	0.017006
HALLMARK_MYC_TARGETS_V2	1.66075	0.014056	0.036597
HALLMARK_GLYCOLYSIS	1.598933	0.004193	0.076556

HALLMARK_IL6_JAK_STAT3_SIGNALING	1.580787	0.006098	0.078151
HALLMARK_IL2_STAT5_SIGNALING	1.571376	0.011858	0.07368
HALLMARK_MITOTIC_SPINDLE	1.553222	0.001927	0.075656
HALLMARK_INFLAMMATORY_RESPONSE	1.522947	0.029528	0.093476
HALLMARK_HYPOXIA	1.468816	0.001972	0.130024
HALLMARK_APOPTOSIS	1.416187	0.03937	0.141715
HALLMARK_XENOBIOTIC_METABOLISM	1.390655	0.030181	0.151785
HALLMARK_ESTROGEN_RESPONSE_LATE	1.348313	0.023715	0.158576
HALLMARK_ESTROGEN_RESPONSE_EARLY	1.327884	0.02449	0.175084

NES= Normalized Enrichment Score, Nom p-val = Normalized -values, FDR-q-val = False Discovery Rate value.

Table 6 : 24 Hours Top Canonical Pathways by IPA**Table 6A Conventional Versus Sham:**

Ingenuity Canonical Pathways	Neg LogP value	Ratio	Z score
Cyclins_and_Cell_Cycle_Regulation	1.09	2.05E-01	1.941
GP6_Signaling_Pathway	1.03	1.76E-01	3.545
STAT3_Pathway	1.09	1.96E-01	1.606
Phospholipase_C_Signaling	1.09	1.60E-01	2.785
Colorectal_Cancer_Metastasis_Signaling	1.19	1.60E-01	3.781
Pancreatic_Adenocarcinoma_Signaling	1.60	2.03E-01	2.183
IL-8_Signaling	1.60	1.79E-01	4.352
Interferon_Signaling	1.75	3.45E-01	2.333
Salvage_Pathways_of_Pyrimidine_Ribonucleotides	1.88	2.31E-01	1.528

5 Table 6B Flash Versus Sham:

Ingenuity Canonical pathways	Neg LogP value	Ratio	Z score
Mitotic_Roles_of_Polo-Like_Kinase	8.52	1.97E-01	-1.667
Estrogen-mediated_S-phase_Entry	2.30	1.54E-01	-2
Aryl_Hydrocarbon_Receptor_Signaling	2.30	6.11E-02	-2.121
Cyclins_and_Cell_Cycle_Regulation	2.13	7.69E-02	-2.449
Th1_Pathway	1.99	6.03E-02	-2.236
Calcium-induced_T_Lymphocyte_Apoptosis	1.30	7.27E-02	-2
Dendritic_Cell_Maturation	1.30	4.29E-02	-1.633

Table 6C Split Flash Versus Sham

Ingenuity Canonical pathways	Neg LogP value	Ratio	Z score
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	1.15E+01	3.00E-01	1.604
Mitotic Roles of Polo-Like Kinase	7.00E+00	1.97E-01	-1.897
p53 Signaling	7.00E+00	1.40E-01	1.508
Aryl Hydrocarbon Receptor Signaling	3.58E+00	9.16E-02	-1.897
Cyclins and Cell Cycle Regulation	1.94E+00	8.97E-02	-1.89
Estrogen-mediated S-phase Entry	1.67E+00	1.54E-01	-2

Example 2

[0275] This example demonstrates that Flash radiation leads to altered activation of hedgehog (Hh) signaling.

[0276] The Hh signaling pathway has recently been recognized as an important signaling pathway and a therapeutic target in cancer. In adults, mutation or deregulation of this pathway plays a key role in both proliferation and differentiation leading to tumorigenesis or tumor growth acceleration in a wide variety of tissues. Inappropriate activation of the Hh signaling pathway has been implicated in the development of several other types of cancer including lung, prostate, breast, and pancreas. In addition, some recent findings suggest that Hh might also promote tumorigenesis by signaling in a paracrine manner from the tumor to the surrounding stroma or in cancer stem cells (CSCs). As shown in Figure 25 and Table 7, flash radiation leads to reduced activation of the Hedgehog signaling pathway when compared to conventional proton irradiation. The cumulative enrichment score is indicative of downregulation of genes. The components of the Hh/GLI signaling pathway involved in the signaling transfer to GLI transcription factors include Hedgehog ligands (Sonic Hh (SHh), Indian Hh (IHh), and Desert Hh (DHh)), Patched receptor (Ptch1, Ptch2), Smoothened receptor (Smo), Suppressor of fused homolog (Sufu), kinesin protein Kif7, protein kinase A (PKA), and cyclic adenosine monophosphate (cAMP). The activator form of GLI travels to the nucleus and stimulates the

transcription of the target genes by binding to their promoters. The main target genes of the Hh signaling pathway are PTCH1, PTCH2, and GLI family zinc finger 1 (GLI1).

- [0277] Several hedgehog antagonists including but not limited to hedgehog antagonist Smo antagonist, PTCH1 inhibitors like RU-SKI 43, Cyclopamine, Vismodegib, LDE 225, saridegib, BMS 833923, LEQ 506, PF- 04449913 , PF-5274857, GANT61, SANT-1, Glasdegib (PF- 04449913), Taladegib (LY2940680) and TAK-441 may work in combination with Flash therapy.

Table 7. Hedgehog pathway genes downregulated by Flash radiation compared to conventional radiation.

Gene Name	UniprotID	Cumulative Enrichment Score (Flash Vs Conv.)	
CELSR1	Q9NYQ6	-0.002235843	
TLE3	Q04726	0.010807141	
OPHN1	O60890	-0.06859329	
GPR56	Q9Y653	-0.15772812	
PTCH1	Q13635	-0.14803493	
TLE1	Q04724	-0.39281052	
MYH9	P35579	-0.52821416	
RASA1	P20936	-0.5298179	
HEY1	Q9Y5J3	-0.5706669	
ETS2	P15036	-0.5470168	
HEY2	Q9UBP5	-0.51258683	
LDB1	Q86U70	-0.47611424	
UNC5C	O95185	-0.42774034	

NF1	P21359	-0.38596168	
CDK6	Q00534	-0.34588408	
VLDLR	P98155	-0.26430693	
NRP2	O60462	-0.17468892	
DPYSL2	Q16555	-0.08375509	
NRP1	O14786	0.025754608	

References:

Ashworth, A., and Lord, C.J. (2018). Synthetic lethal therapies for cancer: what's next after PARP inhibitors? *Nat. Rev. Clin. Oncol.* 1.

- 5 Asteriti, I.A., De Mattia, F., and Guarguaglini, G. (2015). Cross-Talk between AURKA and Plk1 in Mitotic Entry and Spindle Assembly. *Front. Oncol.* 5.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300.

- 10 Bolstad, B.M., Irizarry, R.A., Åstrand, M., and Speed, T.P. (2003). A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 19, 185–193.

Donnella, H.J., Webber, J.T., Levin, R.S., Camarda, R., Momcilovic, O., Bayani, N., Shah, K.N., Korkola, J.E., Shokat, K.M., Goga, A., et al. (2018). Kinome rewiring reveals AURKA limits PI3K-pathway inhibitor efficacy in breast cancer. *Nat. Chem. Biol.*

Downward, J. (2003). Targeting RAS signalling pathways in cancer therapy. *Nat. Rev. Cancer* 3, 11–22.

- 15 Foroutan, M., Cursons, J., Hediye-Zadeh, S., Thompson, E.W., and Davis, M.J. (2017). A Transcriptional Program for Detecting TGFβ-Induced EMT in Cancer. *Mol. Cancer Res. MCR* 15, 619–631.

Hainfeld, JF et al. (2004). The use of gold nanoparticles to enhance radiotherapy in mice. *Phys Med Biol.*

- 20 Janeček, M., Rossmann, M., Sharma, P., Emery, A., Huggins, D.J., Stockwell, S.R., Stokes, J.E., Tan, Y.S., Almeida, E.G., Hardwick, B., et al. (2016). Allosteric modulation of AURKA kinase activity by a small-molecule inhibitor of its protein-protein interaction with TPX2. *Sci. Rep.* 6, 28528.

Kitai, H., and Ebi, H. (2016). Key roles of EMT for adaptive resistance to MEK inhibitor in KRAS mutant lung cancer. *Small GTPases* 0, 00–00.

- 25 Liang, S.-Q., Marti, T.M., Dorn, P., Froment, L., Hall, S.R.R., Berezowska, S., Kocher, G., Schmid, R.A., and Peng, R.-W. (2015). Blocking the epithelial-to-mesenchymal transition pathway abrogates resistance to anti-folate chemotherapy in lung cancer. *Cell Death Dis.* 6, e1824.

Lin, Y et al. (2014). Comparing gold nano-particle enhanced radiotherapy with protons, megavoltage photons and kilovoltage photons: a Monte Carlo simulation. *Phys Med Biol*.

Lord, C.J., and Ashworth, A. (2016). BRCAness revisited. *Nat. Rev. Cancer* 16, 110–120.

Murai, J., Huang, S.N., Das, B.B., Renaud, A., Zhang, Y., Doroshow, J.H., Ji, J., Takeda, S., and Pommier, Y. (2012). Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. *Cancer Res.* 72, 5588–5599.

Panicker, R.C., Coyne, A.G., and Srinivasan, R. (2017). Allosteric Targeting of Aurora A Kinase Using Small Molecules: A Step Forward Towards Next Generation Medicines? *Curr. Med. Chem.*

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G.K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 43, e47.

Stapleton, S et al. (2017). Radiation effects on the tumor microenvironment: Implications for nanomedicine delivery. *Adv Drug Deliv Rev.*

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., et al. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U. S. A.* 102, 15545–15550.

Sun, Y., Liu, W.-Z., Liu, T., Feng, X., Yang, N., and Zhou, H.-F. (2015). Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. *J. Recept. Signal Transduct. Res.* 35, 600–604.

Wang, M., Morsbach, F., Sander, D., Gheorghiu, L., Nanda, A., Benes, C., Kriegs, M., Krause, M., Dikomey, E., Baumann, M., et al. (2011). EGF Receptor Inhibition Radiosensitizes NSCLC Cells By Inducing Senescence In Cells Sustaining DNA Double-Strand Breaks. *Cancer Res.* 71, 6261–6269.

[1] T. Nishimura, K. Iwakabe, M. Sekimoto, Y. Ohmi, T. Yahata, M. Nakui, T. Sato, S. Habu, H. Tashiro, M. Sato, A. Ohta, Distinct role of antigen-specific T helper type 1 (Th1) and Th2 cells in tumor eradication in vivo, *J Exp Med*, 190 (1999) 617-627.

[2] H.M. Xu, Th1 cytokine-based immunotherapy for cancer, *Hepatobiliary Pancreat Dis Int*, 13 (2014) 482-494.

[3] G.E. Kaiko, J.C. Horvat, K.W. Beagley, P.M. Hansbro, Immunological decision-making: how does the immune system decide to mount a helper T-cell response?, *Immunology*, 123 (2008) 326-338.

[4] G. Hasko, C. Szabo, IL-12 as a therapeutic target for pharmacological modulation in immune-mediated and inflammatory diseases: regulation of T helper 1/T helper 2 responses, *Br J Pharmacol*, 127 (1999) 1295-1304.

[5] C. Tang, Z. Liao, D. Gomez, L. Levy, Y. Zhuang, R.A. Gebremichael, D.S. Hong, R. Komaki, J.W. Welsh, Lymphopenia association with gross tumor volume and lung V5 and its effects on non-small cell lung cancer patient outcomes, *Int J Radiat Oncol Biol Phys*, 89 (2014) 1084-1091.

[0278] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, patent applications, sequence
5 accession numbers and UniProt numbers cited herein are hereby incorporated by reference in their entirety for all purposes.

Informal Sequence Listing:

SEQ ID NO:1: Sonic hedgehog protein (SHH) (HHG-1) (Shh unprocessed N-terminal signaling and C-terminal autoprocessing domains) (ShhNC) [Cleaved into: Sonic hedgehog protein N-product (ShhN) (Shh N-terminal processed signaling domains) (ShhNp)]. (UniProt ID Q15465)

5 >sp|Q15465|SHH_HUMAN Sonic hedgehog protein OS=Homo sapiens OX=9606 GN=SHH
PE=1 SV=1
MLLLARCLLLVLVSSLLVCSGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKT LGASG
RYEGKISRNSERFKELTPNYNPDIIKFDEENTGADRLMTQRCKDKLNALISVMNQWPGV
KLRVTEGWDEDGHHSEESLHYEGRAVDITTSRDRSKYGMRLARLAVEAGFDWVYYESKAH
10 IHCSVKAENSVAAKSGGCFPGSATVHLEQGGTKLVKDLSPGDRVLAADDQGRLLYSDFLT
FLDRDDGAKKVFIYVIETREPRERLLLTAHLLFVAPHNDSATGEPEASSGSGPPSGGALG
PRALFASRVPGQRVYVVAERDGDRLPAAVHSVTLSEEAAGAYAPLTAQGTLINRVL
ASCYAVIEEHSWAHRAFPRLAHALLAALAPARTDRGGDSGGGDRGGGGGRVALTAPGA
ADAPGAGATAGIHWYSQLLYQIGTWLLDSEALHPLGMAVKSS

15 SEQ ID NO:2: Indian hedgehog protein (IHH) (HHG-2) [Cleaved into: Indian hedgehog protein N-product; Indian hedgehog protein C-product] (UniProt ID Q14623)

>sp|Q14623|IHH_HUMAN Indian hedgehog protein OS=Homo sapiens OX=9606 GN=IHH
PE=1 SV=4
20 MSPARLRPRHLFCLVLLLLLVPAAWGCGPGRVVGSRRRPPRKLVPPLAYKQFSPNVPEKT
LGASGRYEGKIARSSERFKELTPNYNPDIIKFDEENTGADRLMTQRCKDRLNSLAISVMN
QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSRDRNKYGLLARLAVEAGFDWVYY
ESKAHVHCSVKSEHSAAAKTGGCFPAGAQRVLES GARVALSAVRPGDRVLAMGEDGSPTF
SDVLIFLDREPHRLRAFQVIETQDPPRRLLALTPAHLFTADNHTPEAARFRATFASHVQP
25 GQYVLVAGVPGLQPARVA AVSTHVALGAYAPLTKHGTLVVEDVVASCF AAVADHHLAQLA
FWPLRLFHSLAWGSWTPGEGVHWYPQLLYRLGRLLLEEGSFHPLGMSGAGS

SEQ ID NO:3: Desert hedgehog protein (DHH) (HHG-3) [Cleaved into: Desert hedgehog protein N-product; Desert hedgehog protein C-product] (UniProt ID O43323)

30 >sp|O43323|DHH_HUMAN Desert hedgehog protein OS=Homo sapiens OX=9606 GN=DHH
PE=1 SV=1
MALLTNLLPLCCLALLALPAQSCGPGRGPVGRRRYARKQLVPLLYKQFVPGVPERTLGAS
GPAEGRVARGSERFRDLVPNYNPDIIKFDEENSGADRLMTERCKERVNALAIAMNMWPG
VRLRVTEGWDEDGHHQAQDSLHYEGRALDITTSRDRNKYGLLARLAVEAGFDWVYYESRN
35 HVHVSVKADNSLAVRAGGCFPGNATVRLWSGERKGLRELHRGDWVLAADASGRVVPTPVL
LFLDRDLQRRASFVAVETEWPPRKLLLTWPWHLVFAARGPAPAPGDFAPVFARRLRAGDSV
LAPGGDALRPARVARVAREEAVGVFAPLTAHGTLVNDVLASCYAVLESHQWAHRAFPRL
RLLHALGALLPGGAVQPTGMHWYSRLLYRLAEELLG

40 SEQ ID NO:4: Protein patched homolog 1 (PTC) (PTC1). UniProt ID Q13635. Isoform L (UniProt Q13635-1)

>sp|Q13635|PTC1_HUMAN Protein patched homolog 1 OS=Homo sapiens OX=9606
GN=PTCH1 PE=1 SV=2
MASAGNAAEPQDRGGGSGCIGAPGRPAGGGRRRRRTGGLRRAAAPDRDYLHRPSYCDAAF
45 ALEQISKGKATGRKAPLWLRAKFQRLFLKGCYIQKNCCKFLVVGLLIFGAFVGLKAAN
LETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQHLD
SALQASRVHVYMYNRQWKLEHLCYKSGELITETGYMDQIEYLYPCLITPLDCFWEGAK

LQSGTAYLLGKPPLRWTNFDPLEFLEELKKINYQVDSWEEMLNKAIEVGHGYMDRPNLPA
 DPDCPATAPNKNSTKPLDMALVLNGGCHGLSRKYMHWQEELIVGGTVKNSTGKLVSAHAL
 QTMFQLMTPKQMYEHFKGYEYVSHINWNEDKAAAILEAWQRTYVEVVHQSV AQNSTQKVL
 SFTTTTLDLILKSFSDVSVIRVASGYLLMLAYACL TMLRWDCSKSQGAVGLAGVLLVALS
 5 VAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAFSETGQNKRIPFEDRTGEC
 LKRTGASVALTSISNVTAFMAALIPIPALRAFSLQA AVVVVFNFAMVLLIFPAILSMDL
 YRREDRRLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYSPPPYSSHSFAHETQITMQS
 TVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDTLSCQSPESTSSTRDLLSQFSDSSL
 10 HCLEPPCTKWTLSFAEKHYAPFLKPKAKVVVIFLFLGLLGVSLYGTTRVRDGLDLTDI
 VPRETREYDFIAAQFKYFSFYNNYIVTQKADYPNIQHLLYDLHRSFSNVKYVMLEENKQL
 PKMWLHYFRDWLQGLQDAFDSWETGKIMPNNYKNGSDDGVLAYKLLVQTGSRDKPIDIS
 QLTQRLVDADGIINPSAFYIYLTAWVSNDPVAYAA SQANIRPHRPEWVHDKADYMPETR
 LRIPAAEPIEYAQFPFYLNGLRDTSDFVEAIEKVRTICSNYTSLGLSSYPNGYPFLFWEQ
 YIGLRHWLLLFISSVLACTFLVCAVFLNPNWTAGIIVMVLALMTVELFGMMGLIGIKLSA
 15 VPVILIASVGIGVEFTVHVALAFLTAIGDKNRRRAVLAEHMFAPVLDGAVSTLLGVLM
 AGSEFDFIVRYFFAVLAILTILGVNLGLVLLPVLLSFFGPYPEVSPANGLNRLPTPSPEP
 PPSVVRFAMPPGHTHSGSDSSDSEYSSQTTVSGLSEELRHYEAQQGAGGPAHQVIVEATE
 NPVFAHSTVVHPESRHHPPSNPRQQPHLDSGSLPPGRQGGQPPRRDPPREGLWPPPYRPRR
 DAFEISTEGHSGPSNRARWGPRGARSHNPRNPASTAMGSSVPGYCQPITTVTASASVTVA
 20 VHPPVPGPGRNPRGGLCPGYPETDHGLFEDPHVPFHVRCERRDSKVEVIELQDVECEER
 PRGSSSN

SEQ ID NO:5. Isoform L'. UniProt ID Q13635-2.

>sp|Q13635-2|PTC1_HUMAN Isoform L' of Protein patched homolog 1 OS=Homo sapiens
 OX=9606 GN=PTCH1

25 MELLNRNRLVIVSPRCTPPKASGGPARRGFYFRSFCKDGGGGEEEEENGGEKDDRDK
 ETRSDKGKATGRKAPLWLRAKFQRLFLKLGCIYQKNCCKFLVVGLLIFGAFVGLKAANL
 ETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQHLD
 ALQASRVHVYMYNRQWKLEHLCYKSGELITETGYMDQIIEYLYPCLITPLDCFWEAKL
 30 QSGTAYLLGKPPLRWTNFDPLEFLEELKKINYQVDSWEEMLNKAIEVGHGYMDRPNLPA
 DPDCPATAPNKNSTKPLDMALVLNGGCHGLSRKYMHWQEELIVGGTVKNSTGKLVSAHALQ
 TMFQLMTPKQMYEHFKGYEYVSHINWNEDKAAAILEAWQRTYVEVVHQSV AQNSTQKVL
 FTTTTLDDILKSFSDVSVIRVASGYLLMLAYACL TMLRWDCSKSQGAVGLAGVLLVALSV
 AAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAFSETGQNKRIPFEDRTGECL
 35 KRTGASVALTSISNVTAFMAALIPIPALRAFSLQA AVVVVFNFAMVLLIFPAILSMDLY
 RREDRRLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYSPPPYSSHSFAHETQITMQST
 VQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDTLSCQSPESTSSTRDLLSQFSDSSLH
 CLEPPCTKWTLSFAEKHYAPFLKPKAKVVVIFLFLGLLGVSLYGTTRVRDGLDLTDIV
 PRETREYDFIAAQFKYFSFYNNYIVTQKADYPNIQHLLYDLHRSFSNVKYVMLEENKQLP
 40 KMWLHYFRDWLQGLQDAFDSWETGKIMPNNYKNGSDDGVLAYKLLVQTGSRDKPIDISQ
 LTKQRLVDADGIINPSAFYIYLTAWVSNDPVAYAA SQANIRPHRPEWVHDKADYMPETRL
 RIPAAEPIEYAQFPFYLNGLRDTSDFVEAIEKVRTICSNYTSLGLSSYPNGYPFLFWEQY
 IGLRHWLLLFISSVLACTFLVCAVFLNPNWTAGIIVMVLALMTVELFGMMGLIGIKLSA
 45 PVVILIASVGIGVEFTVHVALAFLTAIGDKNRRRAVLAEHMFAPVLDGAVSTLLGVLM
 GSEFDFIVRYFFAVLAILTILGVNLGLVLLPVLLSFFGPYPEVSPANGLNRLPTPSPEP
 PSVVRFAMPPGHTHSGSDSSDSEYSSQTTVSGLSEELRHYEAQQGAGGPAHQVIVEATEN
 PVFAHSTVVHPESRHHPPSNPRQQPHLDSGSLPPGRQGGQPPRRDPPREGLWPPPYRPRR
 AFEISTEGHSGPSNRARWGPRGARSHNPRNPASTAMGSSVPGYCQPITTVTASASVTVA
 50 HPPVPGPGRNPRGGLCPGYPETDHGLFEDPHVPFHVRCERRDSKVEVIELQDVECEER
 RGSSSN

SEQ ID NO:6. Isoform M. UniProt ID Q13635-3.

>sp|Q13635-3|PTC1_HUMAN Isoform M of Protein patched homolog 1 OS=Homo sapiens
OX=9606 GN=PTCH1

5 MGKATGRKAPLWLRAKFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFVGLKAAANLETNVE
ELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQHLDLSDALQAS
RVHVYMYNRQWKLEHLCYKSGELITETGYMDQIIELYPCLIITPLDCFWEAGAKLQSGTA
YLLGKPPLRWTNFDPLEFLEELKKINYQVDSWEEMLNKAEVGHGYMDRPNLNPADPDCPA
10 TAPNKNSTKPLDMALVLNNGGCHGLSRKYMHWQEELIVGGTVKNSTGKLVSAHALQTMFQL
LDDILKSFSDDVSVIRVASGYLLMLAYACLTMLRWDCSKSQGAVGLAGVLLVALSVAAGLG
LCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAFSETGQNKRIFFEDRTGECLKRTGA
SVALTSISNVTAFFMAALIPALRAFSLQAAVVVVFNFAMVLLIFPAILSMIDLRRDR
RLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYSPPPPYSSHSFAHETQITMQSTVQLRT
15 EYDPHTHVYYTTAEPRSEISVQPVTVTQDTLSCQSPESTSSSTRDLSQFSDSSLHCLEPP
CTKWTLSFAEKHYAPFLLKPKAKVVVIFLFLGLLGVSLYGTTRVRDGLDLTDIVPRETR
EYDFIAAQFKYFSFYNNYIVTQKADYPNIQHLLYDLHRSFSNVKYVMLEENKQLPKMWLH
YFRDWLQGLQDAFDSWETGKIMPNNYKNGSDDGVLAYKLLVQTGSRDKPIDISQLTKQR
LVDADGIINPSAFYIYLTAWVSNPVAAYASQANIRPHRPEWVHDKADYMPETRLRIPAA
20 EPIEYAQFPFYLNGLRDTSDFVEAIEKVRTICSNYTSLGLSSYPNGYPFLFWEQYIGLRH
WLLLFISVVLACTFLVCAVFLNPNWTAGIIVMVLALMTVELFGMMGLIGIKLSAVPVVIL
IASVGIGVEFTVHVALAFLTAIGDKNRRAVLALEHMFAPVLDGAVSTLLGVLMLAGSEFD
FIVRYFFAVLAILTILGVNLGLVLLPVLLSFFGYPYEVSPANGLNRLPTSPPEPPSVVR
FAMPPGHTHSGSDSSDSEYSSQTTVSGLSEELRHVEAQAGGPAHQVIVEATENPVFAH
25 STVVHPESRHHPPSNPRQQPHLDSGSLPPGRQGGQPRRDPPREGLWPPPYRPRRDAFEIS
TEGHSGPSNRARWGPRGARSHNPRNPASTAMGSSVPGYCQPITTVTASASVTVAVHPPPV
PGPGRNPRGGLCPGYPETDHGLFEDPHVPFHVRCERRDSKVEVIELQDVECEERPRGSSS
N

30 SEQ ID NO:7. Isoform S. UniProt ID Q13635-4.

>sp|Q13635-4|PTC1_HUMAN Isoform S of Protein patched homolog 1 OS=Homo sapiens
OX=9606 GN=PTCH1

MFNPQLMIQTPKEEGANVLTTEALLQHLDLSDALQASRVHVYMYNRQWKLEHLCYKSGELIT
ETGYMDQIIELYPCLIITPLDCFWEAGAKLQSGTAYLLGKPPLRWTNFDPLEFLEELKKI
35 NYQVDSWEEMLNKAEVGHGYMDRPNLNPADPDCPATAPNKNSTKPLDMALVLNNGGCHGLS
RKYMHWQEELIVGGTVKNSTGKLVSAHALQTMFQLMTPKQMYEHFKGYEYVSHINWNEDK
AAAILEAWQRTYVEVVHQSVAQNSTQKVLSTTTTTLDDILKSFSDDVSVIRVASGYLLMLA
YACLTMLRWDCSKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVG
VDDVFLLAHAFSETGQNKRIFFEDRTGECLKRTGASVALTSISNVTAFFMAALIPALR
40 AFSLQAAVVVVFNFAMVLLIFPAILSMIDLRRRLDIFCCFTSPCVSRVIQVEPQAYT
DTHDNTRYSPPPPYSSHSFAHETQITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPV
VTQDTLSCQSPESTSSSTRDLSQFSDSSLHCLEPPCTKWTLSFAEKHYAPFLLKPKAKV
VVIFLFLGLLGVSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSFYNNYIVTQKAD
YPNIQHLLYDLHRSFSNVKYVMLEENKQLPKMWLHYFRDWLQGLQDAFDSWETGKIMP
45 NYKNGSDDGVLAYKLLVQTGSRDKPIDISQLTKQRLVDADGIINPSAFYIYLTAWVSNP
VAYASQANIRPHRPEWVHDKADYMPETRLRIPAAEPIEYAQFPFYLNGLRDTSDFVEAI
EKVRTICSNYTSLGLSSYPNGYPFLFWEQYIGLRHWLLLFISVVLACTFLVCAVFLNPNW
TAGIIVMVLALMTVELFGMMGLIGIKLSAVPVVILIASVGIGVEFTVHVALAFLTAIGDK
NRRAVLALEHMFAPVLDGAVSTLLGVLMLAGSEFDIVRYFFAVLAILTILGVNLGLVLL
50 PVLLSFFGYPYEVSPANGLNRLPTSPPEPPSVVRFAMPPGHTHSGSDSSDSEYSSQTTV

SGLSEELRHYEAQQGAGGPAHQVIVEATENPVFAHSTVVHPESRHHPPSNPRQQPHLDSG
SLPPGRQGGQPRRDPPREGLWPPPYRPRRDAFEISTEGHSGPSNRARWGPRGARSHNPRN
PASTAMGSSVPGYCQPITTVTASASVTVAVHPPPVPGPGRNPRGGGLCPGYPETDHGLFED
PHVPFHVRCERRDSKVEVIELQDVECEERPRGSSSN

5

SEQ ID NO:8. Protein patched homolog 2 (PTC2). UniProt ID Q9Y6C5. Isoform 1 (Q9Y6C5-1).

>sp|Q9Y6C5|PTC2_HUMAN Protein patched homolog 2 OS=Homo sapiens OX=9606
GN=PTCH2 PE=1 SV=2

10 MTRSPPLRELPPSYTPPARTAAPQILAGSLKAPLWLRAYFQGLLFSLGCGIQRHCGKVLFL
LGLLAFGALALGLRMAIETNLEQLWVEVGSRVSQELHYTKEKLGEEAAYTSQMLIQATAR
QEGENILTPEALGLHLQAALTASKVQVSLYGKSWDLNKICYKSGVPLIENGMIERMIEKL
FPCVILTPLDCFWEAKLQGGSAAYLPGRPDIQWTNLDPEQLLEELGPFASLEGFRELDDK
15 AQVGQAYVGRPCLHPDDLHCPPSAPNHHSRQAPNVAHELSSGGCHGFSHKFMHWQEELLLG
GMARDPQGELLRAEALQSTFLLMSPRQLYEHFRGDYQTHDIGWSEEQASTVLQAWQRRFV
QLAQEALPENASQQIHAFSSTTLDLILHAFSEVSAARVVGGYLLMLAYACVTMLRWDCAQ
SQGSVGLAGVLLVALAVASGLGLCALLGITFNAATTQVLPFLALGIGVDDVFLLAHAFTE
ALPGTPLQERMGECLQRTGTSVVLTSINNMAAFLMAALVPIPALRAFSLQAAIVVGCTFV
AVMLVFPAISLDLRRRHCRQLDVLCCFSSPCSAQVIQILPQELGDGTVPVGIAHLTATV
20 QAFTHCEASSQHVVITLPPQAHLVPPPSDPLGSELFSPGGSTRDLLGQEEETRQKAACKS
LPCARWNLAHFARYQFAPLLLQSHAKAIVLVLFALLGLSLYGATLVQDGLALTDVVRG
TKEHAFLSAQLRYFSLYEVALVTQGGFDYAHSQRALFDLHQRFSLLKAVLPPPATQAPRT
WLHYRNWLQGIQAAFDQDQWASGRITRHSYRNGSEDGALAYKLLIQTGDAQEPLDFSQLT
TRKLVDREGLIPPELFYMGLTVWVSSDPLGLAASQANFYPPPPEWLHDKYDITGENLRIP
25 PAQPLEFAQFPFLRLGLQKTADFVEAIEGARAACAEGAGQAGVHAYPSGSPFLFWEQYGLL
RRCFLLAVCILLVCTFLVCALLLNPNWTAGLIVLVLAMMTVELFGIMGFLGIKLSAIPVV
ILVASVGIGVEFTVHVALGFLTQGSRNLRAAHALEHTFAPVTDGAISTLLGLMLLAGSH
FDIVRYFFAALTVLTLGLLHGLVLLPVLLSILGPPPEVIQMYKESPEILSPPAPQGGG
LRWGASSSLPQSFAVTTSMVAIHPPPLPGAYIHPAPDEPPWSPAATSSGNLSSRGPGP
30 ATG

SEQ ID NO:9. Protein patched homolog 2 (PTC2). UniProt ID Q9Y6C5. Isoform 2 (Q9Y6C5-2).

>sp|Q9Y6C5-2|PTC2_HUMAN Isoform 2 of Protein patched homolog 2 OS=Homo sapiens
OX=9606 GN=PTCH2

35 MTRSPPLRELPPSYTPPARTAAPQILAGSLKAPLWLRAYFQGLLFSLGCGIQRHCGKVLFL
LGLLAFGALALGLRMAIETNLEQLWVEVGSRVSQELHYTKEKLGEEAAYTSQMLIQATAR
QEGENILTPEALGLHLQAALTASKVQVSLYGKSWDLNKICYKSGVPLIENGMIERMIEKL
FPCVILTPLDCFWEAKLQGGSAAYLPGRPDIQWTNLDPEQLLEELGPFASLEGFRELDDK
40 AQVGQAYVGRPCLHPDDLHCPPSAPNHHSRQAPNVAHELSSGGCHGFSHKFMHWQEELLLG
GMARDPQGELLRAEALQSTFLLMSPRQLYEHFRGDYQTHDIGWSEEQASTVLQAWQRRFV
QLAQEALPENASQQIHAFSSTTLDLILHAFSEVSAARVVGGYLLMLAYACVTMLRWDCAQ
SQGSVGLAGVLLVALAVASGLGLCALLGITFNAATTQVLPFLALGIGVDDVFLLAHAFTE
ALPGTPLQERMGECLQRTGTSVVLTSINNMAAFLMAALVPIPALRAFSLQAAIVVGCTFV
45 AVMLVFPAISLDLRRRHCRQLDVLCCFSSPCSAQVIQILPQELGDGTVPVGIAHLTATV
QAFTHCEASSQHVVITLPPQAHLVPPPSDPLGSELFSPGGSTRDLLGQEEETRQKAACKS
LPCARWNLAHFARYQFAPLLLQSHAKAIVLVLFALLGLSLYGATLVQDGLALTDVVRG
TKEHAFLSAQLRYFSLYEVALVTQGGFDYAHSQRALFDLHQRFSLLKAVLPPPATQAPRT
WLHYRNWLQGIQAAFDQDQWASGRITRHSYRNGSEDGALAYKLLIQTGDAQEPLDFSQLT
50 TRKLVDREGLIPPELFYMGLTVWVSSDPLGLAASQANFYPPPPEWLHDKYDITGENLRIP

PAQPLEFAQFPFLRLGLQKTADFVEAIEGARAACAEAGQAGVHAYPSGSPFLFWEQYLGL
 RRCFLAVCILLVCTFLVCALLLLNPWTAGLIVLVLAMMTVELFGIMGFLGIKLSAIPVV
 ILVASVGIGVEFTVHVALGFLTTQGSRLRAAHAEHTFAPVTDGAISTLLGLLMLAGSH
 FDFIVRYFFAALTVLTLGLLHGLVLLPVLLSILGPPPEVIQMYKESPEILSPPAPQGGG
 5 LRPEEI

SEQ ID NO:10. Smoothened homolog (SMO) (Protein Gx). UniProt ID Q99835.

>sp|Q99835|SMO_HUMAN Smoothened homolog OS=Homo sapiens OX=9606 GN=SMO
 PE=1 SV=1

10 MAAARPARGPPELLGLLLLLLLGDPGRGAASSGNATGPGPRSAGGSARRSAAVTGPPPP
 LSHCGRAAPCEPLRYNVCLGSVLPYGATSTLLAGSDSQEEAHGKLVLWSGLRNAPRCWA
 VIQPLLCAVYMPKCENDRVELPSRTLCAATRGPCAIVERERGWPDFLRCTPDRFPEGCTN
 EVQNIKFNSGQCEVPLVRTDNPKSWYEDVEGCGIQCQNPLFTEAEHQDMHSYIAAFGAV
 TGLCTLFTLATFVADWRNSNRYPAVILFYVNACFFVGSIGWLAQFMDGARREIVCRADGT
 15 MRLGEPTSNETLSCVIIIFVIVYYALMAGVVWFVLTLYAWHTSFKALGTTYQPLSGKTSYF
 HLLTWSLPPFVLTVAILAVAQVDGDSVSGICFVGYKNYRYRAGFVLAPIGLVLIVGGYFLI
 RGVMTLFSIKSNHPGLLSEKAASKINETMLRLGIFGFLAFGFVLITFSCHFYDFFNQAEW
 ERSFRDYVLCQANVTIGLPTKQPIPDCEIKNRPSLLVEKINLFAMFGTGIAMSTWVWTKA
 TLLIWRRTWCRLTGQSDDEPKRIKSKMIAKAFSKRHELLQNPGQELSFSMHTVSHDGPV
 20 AGLAFDLNEPSADVSSAWAQHVTKMVARRGAILPQDISVTPVATPVPPEEQANLWLVEAE
 ISPELQKRLGRKKKRRKRKKEVCPLAPPELHPPAPAPSTIPRLPQLPRQKCLVAAGAWG
 AGDSCRQGAWTLVSNPFCPEPSPPQDPFLPSAPAPVAWAHGRRQGLGPIHSRTNLMDEL
 MDADSDF

25 SEQ ID NO:11. Suppressor of fused homolog (SUFUH). UniProt ID Q9UMX1. Isoform 1.

>sp|Q9UMX1|SUFU_HUMAN Suppressor of fused homolog OS=Homo sapiens OX=9606
 GN=SUFU PE=1 SV=2

MAELRPSGAPGPTAPPAPGPTAPPAFASLFPPGLHAIYGECRRLYPDQPNPLQVTAIVKY
 WLGGPDPLDYVSMYRNVGSPSANIPEHWHYISFGLSDLYGDNRVHEFTGTDGSPGFGFEL
 30 TFRCLKRETGESAPPTWPAELMQGLARYVFQSENTFCSGDHVSWHSPLDNSESRIQHMLLT
 EDPQMOPVQTPFGVVTFLQIVGVCTEELHSAQQWNGQGILELLRTVPIAGGPWLITDMRR
 GETIFEIDPHLQERVDKGIETDGSNLSGVSAKCAWDDLSRPPEDDEDSRSICIGTQPRRL
 SGKDTEQIRETLRRGLEINSKPVLPPINPQRQNGLAHDRAPS RKDSLES DSSTAIIPHEL
 IRTQLESVHLKFNQESGALIPLCLRGRL LHGRHFTYKSITGDMAITFVSTGVEGAFATE
 35 EHPYAAHGPPWLQILLTEEFVEKMLEDLEDLTSPEEFKLPKEYSWPEKKLVKSILPDVVFD
 SPLH

SEQ ID NO:12. Suppressor of fused homolog (SUFUH). UniProt ID Q9UMX1-2. Isoform 2.

>sp|Q9UMX1-2|SUFU_HUMAN Isoform 2 of Suppressor of fused homolog OS=Homo sapiens
 OX=9606 GN=SUFU

40 MAELRPSGAPGPTAPPAPGPTAPPAFASLFPPGLHAIYGECRRLYPDQPNPLQVTAIVKY
 WLGGPDPLDYVSMYRNVGSPSANIPEHWHYISFGLSDLYGDNRVHEFTGTDGSPGFGFEL
 TFRCLKRETGESAPPTWPAELMQGLARYVFQSENTFCSGDHVSWHSPLDNSESRIQHMLLT
 EDPQMOPVQTPFGVVTFLQIVGVCTEELHSAQQWNGQGILELLRTVPIAGGPWLITDMRR
 45 GETIFEIDPHLQERVDKGIETDGSNLSGVSAKCAWDDLSRPPEDDEDSRSICIGTQPRRL
 SGKDTEQIRETLRRGLEINSKPVLPPINPQRQNGLAHDRAPS RKDSLES DSSTAIIPHEL
 IRTQLESVHLKFNQESGALIPLCLRGRL LHGRHFTYKSITGDMAITFVSTGVEGAFATE
 EHPYAAHGPPWLQL

SEQ ID NO:13. Suppressor of fused homolog (SUFUH). UniProt ID Q9UMX1-3. Isoform 3.
 >sp|Q9UMX1-3|SUFU_HUMAN Isoform 3 of Suppressor of fused homolog OS=Homo sapiens
 OX=9606 GN=SUFU

MAELRPSGAPGPTAPPAPGPTAPPASFLLFPPGLHAIYGECRRLYPDQPNPLQVTAIVKY
 5 WLGGPDPLDYVSMYRNVGSPSANIPEHWHYISFGLSDLYGDNRVHEFTGTDGPGSGFGFEL
 TFRLLKRETGESAPPTWPAELMQGLARYVFQSENTFCSGDHVSWHSPLDNSESRIQHMLLT
 EDPQMGPVQTPFGVVTFLQIVGVCTEELHSAQQWNGQGILELLRTVPIAGGPWLITDMRR
 GETIFEIDPHLQERVDKGIETDGSNLSGVSAKCAWDDLSPPEDEDSRSICIGTQPRRL
 10 SGKDTEQIRETLRRGLEINSKPVLPINPQRQNGLAHDRAPSRKDSLESSTAIIPHEL
 IRTQLESVHLKFNQESGALIPLCLRGRLHGRHFTYKSITGDMAITFVSTGVEGAFATE
 EHPYAAHGPWLQVRRPFFSLLPFIDFLAHPSSPLAALDGTSPSWGAGHECLMDSGPGAC
 V

SEQ ID NO:14. Kinesin-like protein KIF7. UniProt ID Q2M1P5.

>sp|Q2M1P5|KIF7_HUMAN Kinesin-like protein KIF7 OS=Homo sapiens OX=9606 GN=KIF7
 PE=1 SV=2

MGLEAQRLPGAEEAPVRVALRVRPLLPKELLHGHQSCLQVEPGLGRVTLGRDRHFGFHV
 LAEDAGQEAVYQACVQPLLEAFFEGFNATVFAYGQTGSGKTYTMGEASVASLLEDEQGIV
 PRAMAEAFKLIDENDLLDCLVHVSYLEVYKEEFRDLLEVGTASRDQLREDERGNVVLG
 20 VKEVDVEGLDEVLSLLEMGNAAHRTGATHNLSSRSHTVFTVTLQQRGRAPSRLPRPAP
 GQLLVSKFHFVDLAGSERVLKTGSTGERLKESIQINSSLLALGNVISALGDPQRRGSHIP
 YRDSKITRILKDSLGGNAKTVMIACVSPSSSDFDETLNTLNYASRAQNIRNRATVNWPE
 AERPPEETASGARGPPRHRSETRIIHRGRRAPGPATASAAAAMRLGAECARYRACTDAAY
 SLLRELQAEPGLPGAAARKVRDWLCAVEGERSALSSASGPDSGIESASVEDQAAQAGGR
 25 KEDEGAQQLLTLQNQVARLEENRDFLALEDAMEQYKLQSDRLREQQEEMVELRLRLLEL
 VRPGWGGPRLNLPGSFVPRPHTAPLGGAHAHVLMVPPACLPGDEVGSEQRGEQVTN
 GREAGAELLTEVNRLGSGSSAASEEEEEEEPPRRTLHLRRNRISNCSQRAGARPGSLPE
 RKGPELCEELDAAIPGSRAVGGSKARVQARQVPPATASEWRLAQAQKIRELAINIRMK
 EELIGELVRTGKAAQALNRQHSQRIRELEQAEQVRAELSEGQRQLRELEGKELQDAGER
 30 SRLQEFRRRVAAAQSQVQVLKEKKQATERLVLSAQSEKRLQELERNVQLMRQQQGLQQR
 RLREETEQKRRLEAEMSKRQHRVKELELKHEQQQKILKIKTEEIAAFQRKRSSGNGSVV
 SLEQQQKIEEQKKWLDQEMEKVLQQRRALEELGEELHKREAILAKKEALMQEKTGLESKR
 LRSSQALNEDIVRVSSRLEHLEKELSEKSGQLRQGSAAQSQQQIRGEIDSLRQEKDSSLKQ
 RLEIDGKLQGSLLSPEERTLFLQDEAIEALDAAIEYKNEAITCRQVRVLRASASLLSQ
 35 EMNLMAKLSYLSSETRALLCKYFDKVVTLREEHQHQQIAFSELEMQLEEQQRLVYWLEV
 ALERQRLEMDRQLTLQKEHEQNMQLLQLQSRDHLGEGGLADSRQYEAIRIQALEKELGRY
 MWINQELKQKLGGVNAVGHSGGKRLCSEGRQAPGNEDELHLAPELLWLSPLTEGAPR
 TREETRDLVHAPLPLTWKRSSLCGEEQGSPEELRQREAAEPLVGRVLPVGEAGLPWNFGP
 LSKPRRELRRASPGMIDVRKNPL

SEQ ID NO:15. Cadherin EGF LAG seven-pass G-type receptor 1 CELR1. UniProt ID Q9NYQ6.
 Isoform 1.

>sp|Q9NYQ6|CELR1_HUMAN Cadherin EGF LAG seven-pass G-type receptor 1 OS=Homo sapiens
 OX=9606 GN=CELSR1 PE=1 SV=1

MAPPPPPVLPVLLLLAAAAALPAMGLRAAAWEPRVPGGTRAFALRPACTYAVGAACTPRA
 45 PRELLDVGRDGRLAGRRRVSGAGRPLPLQVRLVARSAPTALSRLRLRARTHLPGCGARARL
 CGTGARLCGALCFVPVGGCAAQAHSALAAPTTLACRCPPRPRPCPRPICLPVGGSVR
 LRLLCALRRAAGAVRVGLALEAATAGTPSASPSPPPLPPNLPEARAGPARRARRGTSGR
 GSLKFPMPNYQVALFENEPAGTLILQLHAHYTIEGEEERVSYYMEGLFDESRGYFRIDS
 50 ATGAVSTDSVLDRETKETHVLRVKAVDYSTPPRSATTYITVLVKDTNDHSPVFEQSEYRE

RVRENLEVGYEVL TIRASDRDSPINANLRYRVLGGAWDVFQLNESSGVVSTRAVL DREEA
 AEYQLLVEANDQGRNPGPLSATATVYIEVEDENDNYPQFSEQNYVVQVPEDVGLNTAVLR
 VQATDRDQGGNAAIHYSILSGNVAGQFYLSLSGILDVINPLDFEDVQKYSLSIKAQDGG
 RPPLINSSGVVSVQVL DVNDNEPIFVSSPFQATVLENVPLGYPVVHIQAVDADSGENARL
 5 HYRLVDTASTFLGGGSAGPKNPAPTDPFPFIHNSSGWITVCAELDREEVEHYSFGVEAV
 DHGSPPMSSSTSVSITVL DVNDNDPVFTQPTYELRLNEDAAVGSSVLTQLQARDRDANSVI
 TYQLTGGNTRNRFALSSQRGGGLITLALPLDYKQEQQYVLAVTASDGTRSHTAHVLINVT
 DANTHRPVFQSSHYTVSVSEDRPVGTSIATLSANDED TGENARITYVIQDPVPQFRIDPD
 10 SGTMYTMMELDYENQVAYTLTIMAQDNGIPQKSDTTTLEILILDANDNAPQFLWDFYQGS
 IFEDAPPSTSILQVSATDRDSGPNGRLLYTFQGGDDGDGDFYIEPTSGVIRTQRRLDREN
 VAVYNLWALAVDRGSPTPLSASVEIQVTILDINDNAPMFEKDELELFEENNPVGSVVAK
 IRANDPDEGPNAQIMYQIVEGDMRHFFQLDLLNGDLRAMVELDFEVRREYVLVVQATSAP
 LVS RATVHILLVDQNDNPPVLPDFQILFN NYVTNKSNSFPTGVIGCIPAHDPDVS DSLNY
 TFVQGNELRLLLLD PATGELQLSRDL DNNRPLEALMEVSVSDGIHSVTAFC TLRVTIITD
 15 DMLTNSITVRLNMSQEKFLSPLLALFVEGVAAVLSTTKDDVFVFN VQNDTDVSSNINLV
 TFSALLPGGVRGQFFPSEDLQE QIYLNRTLLTTISTQRVLPFDDNICLREPCENYMKCVS
 VLRFDSSAPFLSSTTVLFRPIHPINGLRRCRPPGFTGDY CETEIDLCYSDPCGANGRCRS
 REGGYTCECFEDFTGEHCEVDARSGR CANGVCKNGGTCVNLLIGGFHCVCPPGEYERPYC
 EVTTRSFPFQSFVTFRGLRQRHFHTISLTFATQERNGLLLYNGRFNEKHDFIALEIVDEQ
 20 VQLTFSAGETTTT VAPKVP SGVSDGRWHSVQVQYYNKPNIGHLGLPHGPSGEKMAVVTV D
 DCDTTMAVRFGKDIGNYSCAAQGTQTGSKKSLDLTG PLLLGVPNL PEDFPVHNRQFVGC
 MRNLSVDGKNVDMAGFIANNGTREGCAARRNFC DGRRCQNGGTCVNRWNMYLCECPLRFG
 GKNCEQAMPHPQLFSGESVVSWSDLNIIISVPWYLG LMFRTRKEDSVLMEATSGGPTSFR
 LQILNNYLQFEVSHGPSDVESVMLSGLRVTDGEWHLLIELKNVKEDSEM KHLVTMTLDY
 25 GMDQNKADIGGMPLGLTVRSVVVG GASEDKVSRRGFRGCMQGV RMGGTPTNVATLNMNN
 ALKVRVKDGCDDDPCTSSPCPPNSRCHDAWEDYSCVCDKGYLG INCVDACHLNPENMG
 ACVRSPGSPQGYVCECGPSHYGPYCENKLDLPCPRGW WGNPVC GPCHCAVSKGFDPCNK
 TNGQCQCKENYYKLLAQDTC LPCDCFPHGSHSRTCDMATGQCACKPGVIGRQCNRCDNPF
 AEVTTLGCEVIYNGCPKA FEAGIWWPQTKFGQPA AVPCPKGSVGN AVRHCSGEKGWLPPE
 30 LFNCTTISFVDLRAMNEKLSRNETQVDGARALQLVRALRSATQHTGT LFGNDVRTAYQLL
 GHVLQHESWQQGFDLAATQDADFHE DVIHSGSALLAPATRAAWEQIRSEG GT AQLLRRL
 EGYFSNVARNVRRTYLRPFVIVTANMILA VDIFDKFNFTGARVPRFD TIHEEFPRELESS
 VSFPA DFFRPPEEKEG PLLRPAGRRTPQTTRPGPGTEREAPISRRRRHPDDAGQFAVAL
 VIIYRTL GQLLPERYDPDRRSLRLPHRPIINTPMVSTLVYSEGAPLPRPLERPVLVEFAL
 35 LEVEERTKPV CVFWNHSLAVGGTGGWSARGCELLSRNRTHVACQCSHTASFAVLMDISRR
 ENGEVLPLKIVTYAAVSLSLAALLVAFVLLSLVRMLRSNLHSHKHLAVALFLSQLVFVI
 GINQ TENPFLCTVVA ILLHYIYMSTFAWTLVESLHVYRMLTEVRNIDTGP MRFYVVGWG
 IPAIVTGLAVGLDPQGYGNPDFCWLSLQDTLIWSFAGPIGAVIINTVTSVLSAKVSCQR
 KHHYYGKKGIVSLLRTAFL LLLLISATWLLG LLAVNRDALSFHYLFAIFSGLQGPVLLF
 40 HCVLNQEV RKHLKGV LGGRKLHLED SATTRATLLTRSLNCNTTFGDGPDMLRTDLGESTA
 SLDSIVRDEGIQKLGVSSGLVRGSHGEPDASLM PRSCKDPPGHDS DSDSELSLDEQSSSY
 ASSHSSDSEDDGVGAEEKWDPARGAVHSTPKGDAVANHVPAGWPDQSLAESDSEDP SGKP
 RLKVETKVSVELHREEQGSHRGEYPPDQESGGAARLASSQPPEQRKGILKNKVITYPPPLT
 45 LTEQTLKGRLREKLADCEQSPTSSRTSSLGSGGPDCAITVKSPGREPGRDHLNGVAMNVR
 TGSAQADGSDSEKP

SEQ ID NO:16. Cadherin EGF LAG seven-pass G-type receptor 1 CELR1. UniProt ID Q9NYQ6-2. Isoform 2.

50 >sp|Q9NYQ6-2|CELR1_HUMAN Isoform 2 of Cadherin EGF LAG seven-pass G-type receptor 1
 OS=Homo sapiens OX=9606 GN=CELSR1

MAPPPPPVLPVLLLLAAAAALPAMGLRAAAWEPRVPGGTRAFALRPGCTYAVGAACTPRA
 PRELLDVGRDGRLAGRRRVSGAGRPLPLQVRLVARSAPTALSRRLRARTHLPGCGARARL
 CGTGARLCGALCFVPVGGCAAQAHSALAAPTTLPACRCPPRPRPCPRPICLPPGGSVR
 LRLLCALRRAAGAVRVGLALEAATAGTPSASPSPPPLPPNLPEARAGPARRARRGTSGR
 5 GSKFMPMPNYQVALFENEPAGTLILQLHAHYTIEGEEERVSYMEGLFDERSRGYFRIDS
 ATGAVSTDSVLDRETKETHVLRVKAVDYSTPPRSATTYITVLVKDTNDHSPVFEQSEYRE
 RVRENLEVGYEVLTIASDRDSPINANLRYRVLGGAWDVFQLNESSGVVSTRAVLDRREA
 AEYQLLVEANDQGRNPGPLSATATVYIEVEDENDNYPQFSEQNYVVQVPEDVGLNTAVLR
 10 VQATDRDQGGQNAAIHYSILSGNVAGQFYLSLSGILDVINPLDFEDVQKYSLSIKAQDGG
 RPPLINSSGVVSVQVLDVNDNEPIFVSSPFQATVLENVPLGYPVVHIQAVDADSGENARL
 HYRLVDTASTFLGGGSAGPKNPATPDFPFQIHNSSGWITVCAELDREEVEHYSFGVEAV
 DHGSPPMSSSTSISITVLDVNDNDPVFTQPTYELRLNEDAAVGSSVLTQLQARDRDANSVI
 TYQLTGGNTRNRFALSSQRGGGLITLALPLDYKQEQYVLAVTASDGTRSHTAHVLINVT
 15 DANTRHPVFQSSHYTVSVSEDRPVGTSIATLSANDEDGTENARITYVIQDPVPQFRIDPD
 SGTMYTMMELDYENQVAYTLTIMAQDNGIPQKSDTTTLEILILDANDNAPQFLWDFYQGS
 IFEDAPPSTSILQVSATDRDSGPNGRLLYTFQGGDDGDGDFYIEPTSGVIRTQRRLDREN
 VAVYNLWALAVDRGSPTPLSASVEIQVTILDINDNAPMFEKDELELFVEENNPVGSVVAK
 IRANDPDEGPNAQIMYQIVEGDMRHFFQLDLLNGDLRAMVELDFEVRREYVLVVQATSAP
 LVSRAVHILLVDQNDNPPVLPDFQILFNYYVTNKSNSFPTGVIGCIPAHDPDVSLSNY
 20 TFFVQGNELRLLLLDPATGELQLSRDLNDRPLEALMEVSVSDGIHSVTAFTCLRVTHITD
 DMLTNSITVRLNMSQEKFLSPLLALFVEGVAAVLSTTKDDVFVFNVDNDTDVSSNINLV
 TFSALLPGGVRGQFFPSEDLQEQIYLNRTLLTTISTQRVLPFDDNICLREPCENYMKCVS
 VLRFDSSAPFLSSTTVLFRPIHPINGLRCRCPPGFTGDYCETEIDLCYSDPCGANGRCRS
 REGGYTCECFEDFTEIS

25 SEQ ID NO:17. Transducin-like enhancer protein 3 TLE3. UniProt ID Q04726. Isoform 1.

>sp|Q04726|TLE3_HUMAN Transducin-like enhancer protein 3 OS=Homo sapiens OX=9606
 GN=TLE3 PE=1 SV=2

MYPPQGRHPAPHQPGQPGFKFTVAESCDRIKDEFQFLQAQYHSLKVEYDKLANEKTQMQRH
 30 YVMYYEYMSYGLNIEMHKQTEIAKRLNTILAQIMPFLSQEHQQQVAQAVERAQVMTMELN
 AIIGQQQLQAQHLSHATHGPPVQLPPHPSGLQPPGIPPTGSSSGLLALGALGSQAHLTV
 KDEKNHHELDHRERESSANNSVSPESLRASEKHRSADYSMEAKKRKAEEKDSLRYDS
 DGDKSDDLVDVSNEDPATPRVSPAHSPPENGLDKARSLKKDAPTSPASVASSSSTPSSK
 TKDLGHNDKSSTPGLKSNTPTPRNDAPTPTGTSTTPGLRSMGKPPGMDPIGIMASALRTP
 35 ISITSSYAAPFAMMSHHEMNGSLTSPGAYAGLHNIPPQMSAAAAAAAAAAYGRSPMVSFGA
 VGFDPHPPMRATGLPSSLASIPGGKPAYSFHVSADGQMMPVFPHDALAGPGIPRHARQI
 NTLSHGEVVCVAVTISNPTRHVYTGKGKCVKIWDISQPGSKSPISQLDCLNRDNYIRSKL
 LPDGRTLIVGGEASTLTIWDLASPTPRIKAELTSSAPACYALAISPDAKVCFSCCSDGNI
 AVWDLHNQTLVRQFQGHTDGASCIDISHDGTKLWTGGLDNTVRSWDLREGRQLQHQHFTS
 40 QIFSLGYCPTGEWLAVGMESSENVEVLHHTKPKDYQLHLHESCVLSLKFAFCGKWFVSTGK
 DNLLNAWRTPYGASIFQSKESSSVLSCDISADDKYIVTGSGDKKATVYEVY

45 SEQ ID NO:18. Transducin-like enhancer protein 3 TLE3. UniProt ID Q04726-2. Isoform 2.

>sp|Q04726-2|TLE3_HUMAN Isoform 2 of Transducin-like enhancer protein 3 OS=Homo sapiens
 OX=9606 GN=TLE3

MYPPQGRHPAPHQPGQPGFKFTVAESCDRIKDEFQFLQAQYHSLKVEYDKLANEKTQMQRH
 50 YVMYYEYMSYGLNIEMHKQTEIAKRLNTILAQIMPFLSQEHQQQVAQAVERAQVMTMELN
 AIIGQQQLQAQHLSHATHGPPVQLPPHPSGLQPPGIPPTGSSSGLLALGALGSQAHLTV
 KDEKNHHELDHRERESSANNSVSPESLRASEKHRSADYSMEAKKRKAEEKDSLRYDS
 DGDKSDDLVDVSNEDPATPRVSPAHSPPENGLDKARSLKKDAPTSPASVASSSSTPSSK

TKDLGHNDKSSTPGLKSNTPTPRNDAPTPGTSTTPGLRSMPPASALRTPISITSSYAAPFA
 MMSHHEMNGSLTSPGAYAGLHNIPPQMSAAAAAAAAAAYGRSPMVSFAGVDFPHPPMRAT
 GLPSSLASIPGGKPAYSFHVSADGQMOPVFPFDALAGPGIPRHARQINTLSHGGEVCAV
 TISNPTRHVYTGGKGCVKIWDISQPGSKSPISQLDCLNRDNYIRSKLLPDGRTLIVGGE
 5 ASTLTIWDLASPTPRIKAELTSSAPACYALAI SPDAKVCFSCSDGNIAVWDLHNQTLVR
 QFQGHTDGASCIDISHDGTKLWTGGLDNTVRSWDLREGRQLQQHDFTSQIFSLGYCPTGE
 WLA VGMESNNVEVLHHTKPKDKYQLHLHESCVLSLKFA YCGKWFVSTGKDNLLNAWRTPYG
 ASIFQSKESSSVLSCDISADDKYIVTGSGDKKATVYEVY

- 10 SEQ ID NO:19. Transducin-like enhancer protein 3 TLE3. UniProt ID Q04726-3. Isoform 3.
 >sp|Q04726-3|TLE3_HUMAN Isoform 3 of Transducin-like enhancer protein 3 OS=Homo sapiens
 OX=9606 GN=TLE3
 MYPQGRHPAPHQPGQPGFKFTVAESCDRIKDEFQFLQAQYHSLKVEYDKLANEKTMRH
 YVMYYEMSYGLNIEMHKQTEIAKRLNTILAQIMPFLSQEHQQQVAQAVERAQVMTMELN
 15 AIIGQQQLQAQHL SHA THGPPVQLPPHPSGLQPPGIPPVTGSSSGLLALGALGSQAHLTV
 KDEKNHHELDHRERESSANNSVSPSESLRASEKHRGSADYSMEAKKRKAEEKDSLRYDS
 DGDKSDDLVVDVSNEDPATPRVSPAHSPPENGLDKARSLKKDAPTSPASVASSSSTPSSK
 TKDLGHNDKSSTPGLKSNTPTPRNDAPTPGTSTTPGLRSMPPGKPPGMDPIASALRTPISI
 TSSYAAPFAMMSHHEMNGSLTSPGAYAGLHNIPPQMSAAAAAAAAAAYGRSPMVGFDPHPP
 20 MRATGLPSSLASIPGGKPAYSFHVSADGQMOPVFPFDALAGPGIPRHARQINTLSHGGEV
 VCAVTISNPTRHVYTGGKGCVKIWDISQPGSKSPISQLDCLNRDNYIRSKLLPDGRTLIV
 VGGEASTLTIWDLASPTPRIKAELTSSAPACYALAI SPDAKVCFSCSDGNIAVWDLHNQ
 TLVRQFQGHTDGASCIDISHDGTKLWTGGLDNTVRSWDLREGRQLQQHDFTSQIFSLGYC
 PTGEWLA VGMESNNVEVLHHTKPKDKYQLHLHESCVLSLKFA YCGKWFVSTGKDNLLNAWR
 25 TPYGASIFQSKESSSVLSCDISADDKYIVTGSGDKKATVYEVY

- SEQ ID NO:20. Transducin-like enhancer protein 3 TLE3. UniProt ID Q04726-4. Isoform 4.
 >sp|Q04726-4|TLE3_HUMAN Isoform 4 of Transducin-like enhancer protein 3 OS=Homo sapiens
 OX=9606 GN=TLE3
 MPPPPPLSCLRGLQAPHQPGQPGFKFTVAESCDRIKDEFQFLQAQYHSLKVEYDKLANEKT
 MRHYVMYYEMSYGLNIEMHKQTEIAKRLNTILAQIMPFLSQEHQQQVAQAVERAQVMT
 TMELNAIIGQQQLQAQHL SHA THGPPVQLPPHPSGLQPPGIPPVTGSSSGLLALGALGSQ
 AHLTVKDEKNHHELDHRERESSANNSVSPSESLRASEKHRGSADYSMEAKKRKAEEKDSL
 35 SRYDSGDGKSDDLVVDVSNEDPATPRVSPAHSPPENGLDKARSLKKDAPTSPASVASSSS
 TPSSKTKDLGHNDKSSTPGLKSNTPTPRNDAPTPGTSTTPGLRSMPPGKPPGMDPIGIMAS
 ALRTPISITSSYAAPFAMMSHHEMNGSLTSPGAYAGLHNIPPQMSAAAAAAAAAAYGRSPM
 VGFDPHPPMRATGLPSSLASIPGGKPAYSFHVSADGQMOPVFPFDALAGPGIPRHARQI
 NTLSHGEVVC A V TISNPTRHVYTGGKGCVKIWDISQPGSKSPISQLDCLNRDNYIRSKL
 40 LPDGRTLIVGGEASTLTIWDLASPTPRIKAELTSSAPACYALAI SPDAKVCFSCSDGNI
 AVWDLHNQTLVRQFQGHTDGASCIDISHDGTKLWTGGLDNTVRSWDLREGRQLQQHDFTS
 QIFSLGYCPTGEWLA VGMESNNVEVLHHTKPKDKYQLHLHESCVLSLKFA YCGKWFVSTGK
 DNLLNAWRTPYGASIFQSKESSSVLSCDISADDKYIVTGSGDKKATVYEVY

- 45 SEQ ID NO:21. Transducin-like enhancer protein 3 TLE3. UniProt ID Q04726-5. Isoform 5.
 >sp|Q04726-5|TLE3_HUMAN Isoform 5 of Transducin-like enhancer protein 3 OS=Homo sapiens
 OX=9606 GN=TLE3
 MYPQGRHPAPHQPGQPGFKFTVAESCDRIKDEFQFLQAQYHSLKVEYDKLANEKTMRH
 YVMYYEMSYGLNIEMHKQTEIAKRLNTILAQIMPFLSQEHQQQVAQAVERAQVMTMELN
 50 AIIGQQQLQAQHL SHA THGPPVQLPPHPSGLQPPGIPPVTGSSSGLLALGALGSQAHLTV

KDEKNHHELDHRERESSANNSVSPESLRASEKHRGSADYSMEAKKRKAEEKDSLRYDS
 DGDKSDDLVDVSNEDPATPRVSPAHSPPENGLDKARSLKKDAPTSPASVASSSSTPSSK
 TKDLGHNDKSSTPGLKSNTPTPRNDAPTPGTSTTPGLRSMGKPPGMDPIASALRTPISI
 TSSYAAPFAMMSHHEMNGSLTSPGAYAGLHNIPPQMSAAAAAAAAAAYGRSPMVSGAVGF
 5 DPHPPMRATGLPSSLASIPGGKPAYSFHVSADGQMOPVFPDHALAGPGIPRHARQINTL
 SHGEVVCAVTISNPTRHVYTGKGKCVKIWDISQPGSKSPISQLDCLNRDNYIRSKLLPD
 GRTLIVGGEASTLTIWDLASPTPRIKAELTSSAPACYALAI SPDAKVCFSCCSDGNIAVW
 DLHNQTLVRQFQGHTDGASCIDISHDGTKLWTGGLDNTVRSWDLREGRQLQQHDFTSQIF
 10 SLGYCPTGEWLAVGMESNNVEVLHHTKPKDKYQLHLHESCVLSLKFAYCGKWFVSTGKDNL
 LNAWRTPYGASIFQSKESSSVLSCDISADDKYIVTGSGDKKATVYEVY

SEQ ID NO:22. Transducin-like enhancer protein 3 TLE3. UniProt ID Q04726-6. Isoform 6.
 >sp|Q04726-6|TLE3_HUMAN Isoform 6 of Transducin-like enhancer protein 3 OS=Homo sapiens
 OX=9606 GN=TLE3

15 MYPQGRHPAPHQPGQPGFKFTVAESCDRIKDEFQFLQAQYHSLKVEYDKLANEKTEMQRH
 YVMYYEMSYGLNIEMHKQTEIAKRLNTILAQIMPFLSQEHQQQVAQAVERAQVMTMELN
 AIIGQQQLQAQHLSHATHGPPVQLPPHPSGLQPPGIPPVTGSSSGLLALGALGSQAHLTV
 KDEKNHHELDHRERESSANNSVSPESLRASEKHRGSADYSMEAKKRKAEEKDSLRYDS
 DGDKSDDLVDVSNEDPATPRVSPAHSPPENGLDKARSLKKDAPTSPASVASSSSTPSSK
 20 TKDLGHNDKSSTPGLKSNTPTPRNDAPTPGTSTTPGLRSMGKPPGMDPIGIMASALRTP
 ISITSSYAAPFAMMSHHEMNGSLTSPGAYAGLHNIPPQMSAAAAAAAAAAYGRSPMVGFDP
 HPPMRATGLPSSLASIPGGKPAYSFHVSADGQMOPVFPDHALAGPGIPRHARQINTLSH
 GEVVCAVTISNPTRHVYTGKGKCVKIWDISQPGSKSPISQLDCLNRDNYIRSKLLPDGR
 TLIVGGEASTLTIWDLASPTPRIKAELTSSAPACYALAI SPDAKVCFSCCSDGNIAVWDL
 25 HNQTLVRQFQGHTDGASCIDISHDGTKLWTGGLDNTVRSWDLREGRQLQQHDFTSQIFSL
 GYCPTGEWLAVGMESNNVEVLHHTKPKDKYQLHLHESCVLSLKFAYCGKWFVSTGKDNLLN
 AWRTPYGASIFQSKESSSVLSCDISADDKYIVTGSGDKKATVYEVY

SEQ ID NO:23. Transducin-like enhancer protein 3 TLE3. UniProt ID Q04726-7. Isoform 7.

>sp|Q04726-7|TLE3_HUMAN Isoform 7 of Transducin-like enhancer protein 3 OS=Homo sapiens
 OX=9606 GN=TLE3

30 MAPHQPGQPGFKFTVAESCDRIKDEFQFLQAQYHSLKVEYDKLANEKTEMQRHYVMYYEM
 SYGLNIEMHKQTEIAKRLNTILAQIMPFLSQEHQQQVAQAVERAQVMTMELNAIIGQQQ
 LQAQHLSHATHGPPVQLPPHPSGLQPPGIPPVTGSSSGLLALGALGSQAHLTVKDEKNHH
 35 ELDHRERESSANNSVSPESLRASEKHRGSADYSMEAKKRKAEEKDSLRYDSGDGKSDD
 LVVDVSNEDPATPRVSPAHSPPENGLDKARSLKKDAPTSPASVASSSSTPSSKTKDLGHN
 DKSSTPGLKSNTPTPRNDAPTPGTSTTPGLRSMGKPPGMDPIASALRTPISITSSYAAP
 FAMMSHHEMNGSLTSPGAYAGLHNIPPQMSAAAAAAAAAAYGRSPMVSGAVGFDPPHPPMR
 ATGLPSSLASIPGGKPAYSFHVSADGQMOPVFPDHALAGPGIPRHARQINTLSHGEVVC
 40 AVTISNPTRHVYTGKGKCVKIWDISQPGSKSPISQLDCLNRDNYIRSKLLPDGRTLIVG
 GEASTLTIWDLASPTPRIKAELTSSAPACYALAI SPDAKVCFSCCSDGNIAVWDLHNQTL
 VRQFQGHTDGASCIDISHDGTKLWTGGLDNTVRSWDLREGRQLQQHDFTSQIFSLGYCPT
 GEWLAVGMESNNVEVLHHTKPKDKYQLHLHESCVLSLKFAYCGKWFVSTGKDNLLNAWRTP
 45 YGASIFQSKESSSVLSCDISADDKYIVTGSGDKKATVYEVY

SEQ ID NO:24. Oligophrenin-1 OPHN1. UniProt ID O60890. Isoform 1

>sp|O60890|OPHN1_HUMAN Oligophrenin-1 OS=Homo sapiens OX=9606 GN=OPHN1 PE=1 SV=1
 MGHPPLEFSDCYLDSPDFRERLKCYEQELERTNKFIDVIKDGNALISAMRNYSSAVQKF
 50 SQTLSQSFQDFIGDTLTDDEINIAESFKEFAELLNEVENERMMMVMHNASDLLIKPLENFR
 KEQIGFTKERKKKFEKDGERFYSLDRHLHLSSKKKESQLQEADLQVDKERHNFESSLD

YVYQIQEVQESKKFNIVEPVLAFHLSLFISNSLTVELTQDFLPYKQQLQLSLQNTRNHFS
 STREEMEELKKRMKEAPQTCKLPGQPTIEGYLYTQEKWALGISWVKYYCQYEKETKTLTM
 TPMEQKPGAQKQGPLDLTKYCVRRKTESIDKRFCDIETNERPGTITLQALSEANRRLWM
 EAMDGKEPIYHSPITKQQEMELNEVGFKFVRKCNIIETKGIKTEGLYRTVGSNIQVQKL
 5 LNAFFDPKCPGDVDFHNSDWDIKTITSSLKFYLRNLSEPVMTYRLHKELVSAAKSDNLDY
 RLGAHSLVYKLPEKNREMLELLIRHLVNVCEHSKENLMTPSNMGVIFGPTLMRAQEDTV
 AAMNIKFQNIIVVEILIEHFGKIYLGPPESAAPPVPPRVTARRHKPITISKRLLRERT
 VFYTSSLDESEDEIQHQTPTNGTITSSIEPPKPPQHPKLPIQRSGETDPGRKSPSRPILDG
 KLEPCPEVDVGKLVSRQLQDGGTKITPKATNGPMPGSGPTKTPSFHIKRPAPRPLAHHKEG
 10 DADFSFSKVRPPGEKPTIIRPPVRPPDPPCRAATPQKPEPKPDIVAGNAGEITSSVVASRT
 RFFETASRKTGSSQGRLPGDES

SEQ ID NO:25. Oligophrenin-1 OPHN1. UniProt ID O60890-2. Isoform 2

>sp|O60890-2|OPHN1_HUMAN Isoform 2 of Oligophrenin-1 OS=Homo sapiens OX=9606

GN=OPHN1

MGHPPLEFSDCYLDSPDFRERLKCYEQELERTNKFIKDVIKDGNALISAMRNYSSAVQKF
 SQTLSQSFQDFIGDITLDDDEINIAESFKEFAELLNEVENERMMMVMHNASDLLIKPLENFR
 KEQIGFTKERKKKFEKDGERFYSLDRHLHLSSKKKESQLQEADLQVDKERHNFESSLD
 YVYQIQEVQESKKFNIVEPVLAFHLSLFISNSLTVELTQDFLPYKQQLQLSLQNTRNHFS
 20 STREEMEELKKRMKEAPQTCKLPGQPTIEGYLYTQEKCVWGHGRGIHWVSISQELLPLVGC
 EFWAPLLFIDP

SEQ ID NO:26. Adhesion G-protein coupled receptor G1 AGRG1. UniProt ID Q9Y653. Isoform 1.

>sp|Q9Y653|AGRG1_HUMAN Adhesion G-protein coupled receptor G1 OS=Homo sapiens OX=9606

GN=ADGRG1 PE=1 SV=2

MTPQSLLQTTLFLLSLLFLVQGAHGRGHREDFRFCSQRNQTHRSSLHYKPTPDLRISIEN
 SEEALTVHAPFPAAHPASRSFPDPRGLYHFCLYWNRRHAGRLHLLYGKRDFLSDKASSLL
 CFQHQEESLAQGPELLATSVTSWWSPQNISLPSAASFTFSFHSPHTAAHNASVDMCELK
 RDLQLLSQFLKHPQKASRRPSAAPASQQQLQSLESKLTSVRFMGDMVSFEEDRINATVWKL
 30 QPTAGLQDLHIHSRQEEEQSEIMEYSVLLPRTLFRQTKGRSGEAEKRLLLVDFFSSQALFQ
 DKNSSQVLGEKVLGIVVQNTKVANLTPVVLTTFQHQLQPKNVTLCQVFWVEDPTLSSPGH
 WSSAGCETVRRETQTSCFCNHLTYFAVLMVSSVEVDVAVHKHYLSLLSYVGCVVVSALACLV
 TIAAYLCSRVPPLPCRRKPRDYTIKVHNMNLLAVFLLDTSFLLSEPVALTGSEAGCRASAI
 FLHFSLLTCLSWMGLEGYNLYRLVVEVFGTYVPGYLLKLSAMGWGFPIFLVTLVALVDVD
 35 NYGPIILAVHRTPEGVIYPSMCWIRDSLVSITNLGLFSLVFLFNMAMLATMVVQILRLR
 PHTQKWSHVLTLGLSLVLGLPWALIFFSFASGTFQLVVLYLFSIITSFQGLIFIWYWS
 MRLQARGGPSPLKSNDSARLPISSGSTSSRI

SEQ ID NO:27. Adhesion G-protein coupled receptor G1 AGRG1. UniProt ID Q9Y653-2. Isoform 2.

>sp|Q9Y653-2|AGRG1_HUMAN Isoform 2 of Adhesion G-protein coupled receptor G1 OS=Homo sapiens OX=9606 GN=ADGRG1

MTPQSLLQTTLFLLSLLFLVQGAHGRGHREDFRFCSQRNQTHRSSLHYKPTPDLRISIEN
 SEEALTVHAPFPAAHPASRSFPDPRGLYHFCLYWNRRHAGRLHLLYGKRDFLSDKASSLL
 CFQHQEESLAQGPELLATSVTSWWSPQNISLPSAASFTFSFHSPHTAAHNASVDMCELK
 45 RDLQLLSQFLKHPQKASRRPSAAPASQQQLQSLESKLTSVRFMGDMVSFEEDRINATVWKL
 QPTAGLQDLHIHSRQEEEQSEIMEYSVLLPRTLFRQTKGRSGEAEKRLLLVDFFSSQALFQ
 DKNSSQVLGEKVLGIVVQNTKVANLTPVVLTTFQHQLQPKNVTLCQVFWVEDPTLSSPGH
 WSSAGCETVRRETQTSCFCNHLTYFAVLMVSSVEVDVAVHKHYLSLLSYVGCVVVSALACLV
 TIAAYLCSRKPRDYTIKVHNMNLLAVFLLDTSFLLSEPVALTGSEAGCRASAI FLHFSL
 50 LTCLSWMGLEGYNLYRLVVEVFGTYVPGYLLKLSAMGWGFPIFLVTLVALVDVDNYGPII

LAVHRTPEGVIYPSMCWIRDSLVSYITNLGLFSLVFLFNMAMLATMVVQILRLRPHTQKW
SHVLTLLGLSLVLGLPWALIFFSFASGTFQLVVLYLFSIITSFQGFLIFIWYWSMRLQAR
GGPSPLKSNSDSARLPISSGSTSSRI

- 5 SEQ ID NO:28. Adhesion G-protein coupled receptor G1 AGRG1. UniProt ID Q9Y653-3. Isoform 3.
>sp|Q9Y653-3|AGRG1_HUMAN Isoform 3 of Adhesion G-protein coupled receptor G1 OS=Homo
sapiens OX=9606 GN=ADGRG1
MTPQSLLQTTLFLLSLLFLVQASASSGAHGRGHREDFRFCSQRNQTHRSSLHYKPTPDLR
ISIENSEEALTVHAPFAAHPASRSFPDPRGLYHFCLYWNRHAGRLHLLYGKRDFFLLSDK
10 ASSLLCFQHQEESLAQGPPLLATSVTSWWSPQNISLPSAASFTHSFHSPHTAAHNASVD
MCELKRDQLLSQFLKHPQKASRRPSAAPASQQLSLESKLTSVRFMGDMVSFEEDRINA
TVWKLQPTAGLQDLHIHSRQEEEQSEIMEYSVLLPRTLQRTKGRSGEAEKRLLLVDFSS
QALFQDKNSSQVLGEKVLGIVVQNTKVANLTEPVVLTQHQQLQPKNVTLCVFWVEDPTL
SSPGHWSSAGCETVRRETQTSCFCNHLTYFAVLMVSSVEVDVAVHKHYLSLLSYVGCVVSA
15 LACLVITIAAYLCSRRKPRDYTIKVMNLLAVFLLDTSFLLSEPVALTGSEAGCRASAI
LHFSLLTCLSWMGLEGYNLYRLVVEVFGTYVPGYLLKLSAMGWGFPIFLVTLVALVDVDN
YGPILAVHRTPEGVIYPSMCWIRDSLVSYITNLGLFSLVFLFNMAMLATMVVQILRLP
HTQKWSHVLTLGLSLVLGLPWALIFFSFASGTFQLVVLYLFSIITSFQGFLIFIWYWSM
RLQARGGPSPLKSNSDSARLPISSGSTSSRI

20

SEQ ID NO:29. Adhesion G-protein coupled receptor G1 AGRG1. UniProt ID Q9Y653-4. Isoform 4.
>sp|Q9Y653-4|AGRG1_HUMAN Isoform 4 of Adhesion G-protein coupled receptor G1 OS=Homo
sapiens OX=9606 GN=ADGRG1
MTPQSLLQTTLFLLSLLFLVQGAHGRGHREDFRFCSQQLSLESKLTSVRFMGDMVSFE
25 DRINATVWKLQPTAGLQDLHIHSRQEEEQSEIMEYSVLLPRTLQRTKGRSGEAEKRLLL
VDFSSQALFQDKNSSQVLGEKVLGIVVQNTKVANLTEPVVLTQHQQLQPKNVTLCVFW
EDPTLSSPGHWSSAGCETVRRETQTSCFCNHLTYFAVLMVSSVEVDVAVHKHYLSLLSYV
CVVSALACLVITIAAYLCSRVP LPCRKRPRDYTIKVMNLLAVFLLDTSFLLSEPVALTG
SEAGCRASAI FLHFSLLTCLSWMGLEGYNLYRLVVEVFGTYVPGYLLKLSAMGWGFPIFL
30 VTLVALVDVDNYGPILAVHRTPEGVIYPSMCWIRDSLVSYITNLGLFSLVFLFNMAMLA
TMVVQILRLRPHTQKWSHVLTLGLSLVLGLPWALIFFSFASGTFQLVVLYLFSIITSFQ
GFLIFIWYWSMRLQARGGPSPLKSNSDSARLPISSGSTSSRI

- 35 SEQ ID NO:30. Adhesion G-protein coupled receptor G1 AGRG1. UniProt ID Q9Y653-5. Isoform 5.
>sp|Q9Y653-5|AGRG1_HUMAN Isoform 5 of Adhesion G-protein coupled receptor G1 OS=Homo
sapiens OX=9606 GN=ADGRG1
MCELKRDQLLSQFLKHPQKASRRPSAAPASQQLSLESKLTSVRFMGDMVSFEEDRINA
TVWKLQPTAGLQDLHIHSRQEEEQSEIMEYSVLLPRTLQRTKGRSGEAEKRLLLVDFSS
40 QALFQDKNSSQVLGEKVLGIVVQNTKVANLTEPVVLTQHQQLQPKNVTLCVFWVEDPTL
SSPGHWSSAGCETVRRETQTSCFCNHLTYFAVLMVSSVEVDVAVHKHYLSLLSYVGCVVSA
LACLVITIAAYLCSRVP LPCRKRPRDYTIKVMNLLAVFLLDTSFLLSEPVALTGSEAGC
RASAI FLHFSLLTCLSWMGLEGYNLYRLVVEVFGTYVPGYLLKLSAMGWGFPIFLVTLVA
LVDVDNYGPILAVHRTPEGVIYPSMCWIRDSLVSYITNLGLFSLVFLFNMAMLATMVVQ
45 ILRLRPHTQKWSHVLTLGLSLVLGLPWALIFFSFASGTFQLVVLYLFSIITSFQGFLIF
IWYWSMRLQARGGPSPLKSNSDSARLPISSGSTSSRI

SEQ ID NO:31. Protein patched homolog 1 PTC1. UniProt ID Q13635. Isoform L.

>sp|Q13635|PTC1_HUMAN Protein patched homolog 1 OS=Homo sapiens OX=9606 GN=PTCH1
PE=1 SV=2

MASAGNAAEPQDRGGGGSGCIGAPGRPAGGGRRRRTGGLRRAAPDRDYLRPSYCDAAF
ALEQISKGKATGRKAPLWLRKAFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFVGLKAAN
5 LETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQHLD
SALQASRVHVYMYNRQWKLEHLCYKSGELITETGYMDQIIEYLYPCLITPLDCFWEAGAK
LQSGTAYLLGKPLRWTNFDPLEFLEELKKINYQVDSWEEMLNKAIEVGHGYMDRPNLPA
DPDCPATAPNKNSTKPLDMALVLNNGGCHGLSRKYMHWQEELIVGGTVKNSTGKLVSAHAL
QTMFQLMTPKQMYEHFKGYEYVSHINWNEDKAAAILEAWQRTYVEVVHQSV AQNSTQKVL
10 SFTTTTLDILKSFSDVSVIRVASGYLLMLAYACL TMLRWDCSKSQGAVGLAGVLLVALS
VAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAFSETGQNKRIPFEDRTGEC
LKRTGASVALTSISNVTAFMAALIPALRAFSLQAAVVVVFNFAMVLLIFPAILSMDL
YRREDRRLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYSPPPYSSHSFAHETQITMQS
TVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDTLSCQSPESTSSTRDLLSQFSDSSL
15 HCLEPPCTKWTLSFAEKHYAPFLPKAKVVFIFLFLGLLGVSLYGTTRVRDGLDLTDI
VPRETREYDFIAAQFKYFSFYNNYIVTQKADYPNIQHLLYDLHRSFSNVKYVMLEENKQL
PKMWLHYFRDWLQGLQDAFDSWETGKIMPNNYKNGSDDGVLAYKLLVQTGSRDKPIDIS
QLTKQRLVDADGIINPSAFYIYLTAWVSNPVAAY AASQANIRPHRPEWVHDKADYMPETR
LRIPAAEPIEYAQFPFYLNGLRDTSDFVEAIEKVRTICSNYTSLGLSSYPNGYPFLFWEQ
20 YIGLRHWLLL FSVVLACTFLVCAVFLNPNWTAGIIVMLALMTVELFGMMGLIGIKLSA
VPVVILIASVGIGVEFTVHVALAFLTAIGDKNRRRAVLAEHMFAPVLDGAVSTLLGVLM
AGSEFDIVRYFFAVLAILTILGVNLGLVLLPVLLSFFGPYPEVSPANGLNRLPTSPSEP
PPSVVRFAMPPGHTHSGSDSSDSEYSSQTTVSGLSEELRHYEAQQGAGGPAHQVIVEATE
NPVFAHSTVVHPESRHHPPSNPRQQPHLDSGSLPPGRQGGQPRRDPPREGLWPPPYRPRR
25 DAFEISTEGHSGPSNRARWGPRGARSHNPRNPASTAMGSSVPGYCQPITTVTASASVTVA
VHPPVPVGPGRNPRGGLCPGYPETDHGLFEDPHVPFHVRCERRDSKVEVIELQDVECEER
PRGSSSN

SEQ ID NO:32. Protein patched homolog 1 PTC1. UniProt ID Q13635-2. Isoform L'.

>sp|Q13635-2|PTC1_HUMAN Isoform L' of Protein patched homolog 1 OS=Homo sapiens OX=9606
GN=PTCH1

MELLNRNRLVIVSPRCTPPKASGGPARRGFYTFRSFCKDGGGGEEEEENGGEKDDRDK
ETRSDKGKATGRKAPLWLRKAFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFVGLKAANL
ETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQHLD
35 ALQASRVHVYMYNRQWKLEHLCYKSGELITETGYMDQIIEYLYPCLITPLDCFWEAGAKL
QSGTAYLLGKPLRWTNFDPLEFLEELKKINYQVDSWEEMLNKAIEVGHGYMDRPNLPA
PDCPATAPNKNSTKPLDMALVLNNGGCHGLSRKYMHWQEELIVGGTVKNSTGKLVSAHALQ
TMFQLMTPKQMYEHFKGYEYVSHINWNEDKAAAILEAWQRTYVEVVHQSV AQNSTQKVL
FTTTTLDILKSFSDVSVIRVASGYLLMLAYACL TMLRWDCSKSQGAVGLAGVLLVALSV
40 AAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAFSETGQNKRIPFEDRTGECL
KRTGASVALTSISNVTAFMAALIPALRAFSLQAAVVVVFNFAMVLLIFPAILSMDLY
RREDRRLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYSPPPYSSHSFAHETQITMQST
VQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDTLSCQSPESTSSTRDLLSQFSDSSLH
CLEPPCTKWTLSFAEKHYAPFLPKAKVVFIFLFLGLLGVSLYGTTRVRDGLDLTDIV
45 PRETREYDFIAAQFKYFSFYNNYIVTQKADYPNIQHLLYDLHRSFSNVKYVMLEENKQLP
KMWLHYFRDWLQGLQDAFDSWETGKIMPNNYKNGSDDGVLAYKLLVQTGSRDKPIDISQ
LTKQRLVDADGIINPSAFYIYLTAWVSNPVAAY AASQANIRPHRPEWVHDKADYMPETRL
RIPAAEPIEYAQFPFYLNGLRDTSDFVEAIEKVRTICSNYTSLGLSSYPNGYPFLFWEQY
IGLRHWLLL FSVVLACTFLVCAVFLNPNWTAGIIVMLALMTVELFGMMGLIGIKLSAV
50 PVVILIASVGIGVEFTVHVALAFLTAIGDKNRRRAVLAEHMFAPVLDGAVSTLLGVMLA

GSEFDFIVRYFFAVLAILTILGVLNGLVLLPVLLSFFGPYPEVSPANGLNRLPTPSPEPP
 PSVVRFAMPPGHTHSGSDSSDSEYSSQTTVSGLSEELRHYESAQAGGPAHQVIVEATEN
 PVFAHSTVVHPESRHHPPSNPRQQPHLDSGSLPPGRQGGQPRRDPPREGLWPPPYRPRRD
 AFEISTEGHSGPSNRARWGPRGARSHNPRNPASTAMGSSVPGYCQPITTTVTASASVTVAV
 5 HPPVPVPGPRNPRGGLCPGYPETDHGLFEDPHVPFHVRCERRDSKVEVIELQDVECEERP
 RGSSSN

SEQ ID NO:33. Protein patched homolog 1 PTC1. UniProt ID Q13635-3. Isoform M.

>sp|Q13635-3|PTC1_HUMAN Isoform M of Protein patched homolog 1 OS=Homo sapiens OX=9606
 GN=PTCH1

10 MGKATGRKAPLWLRAKFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFVGLKAANLETNVE
 ELWVEVGGRVSRELNYTRQKIGEEAMFNPLMIQTPKEEGANVLTTEALLQHLDLSALQAS
 RVHVYMYNRQWKLEHLCYKSGELITETGYMDQIIHYLYPCLITPLDCFWEAGAKLQSGTA
 YLLGKPPLRWTFNFDPLEFLEELKKINYQVDSWEEMLNKAEVGHGYMDRPNLNPADPDCPA
 15 TAPNKNSTKPLDMALVLNNGGCHGLSRKYMHWQEELIVGGTVKNSTGKLVSAHALQTMFQL
 MTPKQMYEHFKGYEYVSHINWNEDKAAALEAWQRTYVEVVHQSVAQNSTQKVLSTTTT
 LDDILKSFSFSDVSVIRVASGYLLMLAYACLTMLRWDCSKSQGAVGLAGVLLVALSVAAGLG
 LCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAFSETGQNKRIPFEDRTGECLKRTGA
 SVALTSISNVTAFMAALIPALRAFSLQAAVVVVFNFAMVLLIFPAILSMDLRREDR
 20 RLDIFCCFTSPCVSRVIQVEPQAYTDTHDNTRYSPPPYSSHSFAHETQITMQSTVQLRT
 EYDPHTHVYYTTAEPRSEISVQPVTVTQDTLSCQSPESTSSTRDLLSQFSDSSLHCLEPP
 CTKWTLSSFAEKHYAPFLLKPKAKVVVIFLFLGLLGVSLYGTTRVRDGLDLTDIVPRETR
 EYDFIAAQFKYFSFYNNMYIVTQKADYPNIQHLLYDLHRSFSNVKYVMLEENKQLPKMWLH
 YFRDWLQGLQDAFDSWETGKIMPNYKNGSDDGVLAYKLLVQTGSRDKPIDISQLTKQR
 25 LVDADGIINPSAFYIYLTAWVSNPVAAYASQANIRPHRPEWVHDKADYMPETRLRIPAA
 EPIEY AQFPFYLNGLRDTSDFVEAIEKVRTICSNYTSLGLSSYPNGYPFLFWEQYIGLRH
 WLLLFSVVLACTFLVCAVFLNPWTAGIIVMLALMTVELFGMMGLIGIKLSAVPVVIL
 IASVGIGVEFTVHVALAFLTAIGDKNRRAVLALEHMFAPVLDGAVSTLLGVLMLAGSEFD
 FIVRYFFAVLAILTILGVLNGLVLLPVLLSFFGPYPEVSPANGLNRLPTPSPEPPSVVR
 30 FAMPPGHTHSGSDSSDSEYSSQTTVSGLSEELRHYESAQAGGPAHQVIVEATENPVFAH
 STVVHPESRHHPPSNPRQQPHLDSGSLPPGRQGGQPRRDPPREGLWPPPYRPRRDAFEIS
 TEGHSGPSNRARWGPRGARSHNPRNPASTAMGSSVPGYCQPITTTVTASASVTVAVHPPPV
 PGPGRNPRGGLCPGYPETDHGLFEDPHVPFHVRCERRDSKVEVIELQDVECEERPRGSSSN

35 SEQ ID NO:34. Protein patched homolog 1 PTC1. UniProt ID Q13635-4. Isoform S.

>sp|Q13635-4|PTC1_HUMAN Isoform S of Protein patched homolog 1 OS=Homo sapiens OX=9606
 GN=PTCH1

MFNPQLMIQTPKEEGANVLTTEALLQHLDLSALQASRVHVYMYNRQWKLEHLCYKSGELIT
 ETGYMDQIIHYLYPCLITPLDCFWEAGAKLQSGTAYLLGKPPLRWTFNFDPLEFLEELKKI
 40 NYQVDSWEEMLNKAEVGHGYMDRPNLNPADPDCPATAPNKNSTKPLDMALVLNNGGCHGLS
 RKYMHWQEELIVGGTVKNSTGKLVSAHALQTMFQLMTPKQMYEHFKGYEYVSHINWNEDK
 AAILEAWQRTYVEVVHQSVAQNSTQKVLSTTTTLLDDILKSFSFSDVSVIRVASGYLLMLA
 YACLTMLRWDCSKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVG
 VDDVFLLAHAFSETGQNKRIPFEDRTGECLKRTGASVALTSISNVTAFMAALIPALR
 45 AFSLQAAVVVVFNFAMVLLIFPAILSMDLRREDRRLDIFCCFTSPCVSRVIQVEPQAYT
 DTHDNTRYSPPPYSSHSFAHETQITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPV
 VTQDTLSCQSPESTSSTRDLLSQFSDSSLHCLEPPCTKWTLSSFAEKHYAPFLLKPKAKV
 VVIFLFLGLLGVSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSFYNNMYIVTQKAD
 YPNIQHLLYDLHRSFSNVKYVMLEENKQLPKMWLHYFRDWLQGLQDAFDSWETGKIMPN
 50 NYKNGSDDGVLAYKLLVQTGSRDKPIDISQLTKQRLVDADGIINPSAFYIYLTAWVSNP

VAYAASQANIRPHRPEWVHDKADYMPETRLRIPAAEPIEYAQFPFYLNGLRDTSDFVEAI
 EKVRTICSNYTSLGLSSYPNGYPFLFWEQYIGLRHWLLLFISVVLACTFLVCAVFLNPNW
 TAGIIVMVLALMTVELFGMMGLIGIKLSAVPVVILIASVGIGVEFTVHVALAFLTAIGDK
 NRRAVLALAHMFAPVLDGAVSTLLGVLMLAGSEFDFIVRYFFAVLAILTILGVLNGLVLL
 5 PVLLSFFGPYPYEVSPANGLNRLPTPSPEPPPSVVRFAMPPGHTHSGSDSSDSEYSSQTTV
 SGLSEELRHYEAQQGAGGPAHQVIVEATENPVFAHSTVVHPESRHHPPSNPRQQPHLDSG
 SLPPGRQGQQPRRDPPREGLWPPPYRPRRDAFEISTEGHSGPSNRARWGPRGARSHNPRN
 PASTAMGSSVPGYCQPITTVTASASVTVAVHPPPVPGPGRNPRGGLCPGYPETDHGLFED
 PHVPFHVRCERRDSKVEVIELQDVECEERPRGSSSN

SEQ ID NO:35. Transducin-like enhancer protein 1 TLE1. UniProt ID Q04724.

>sp|Q04724|TLE1_HUMAN Transducin-like enhancer protein 1 OS=Homo sapiens OX=9606
 GN=TLE1 PE=1 SV=2

MFPQSRHPTPHQAAGQPFKFTIPESLDRIKEEFQFLQAQYHSLKLECEKLASEKTEMQRH
 15 YVMYYEMSYGLNIEMHKQTEIAKRLNTICAQVIPFLSQEHQQQVAQAVERAKQVTMAELN
 AIIGQQQLQAQHLSHGHGPPVPLTPHPSGLQPPGIPPLGGSAGLLALSSALSGQSHLAIK
 DDKKHHDAEHHRDREPGTNSNLLVPDSLRTGDKRRNGPEFSNDIKKRKVDDKDSSHYDSD
 GDKSDNLLVVDVSNEDPSSPRASPAHSPRENGIDKNRLLKKDASSPASTASSASSTSLK
 SKEMSLHEKASTPVLKSSSTPTPRSDMPTPGTSATPGLRPGLGKPPAIDPLVNQAAAGLRT
 20 PLAVPGPYPAFPGMVPHAGMNGELTSPGAAYASLHNMSQMSAAAAAAAVVAYGRSPMVG
 FDPPPHMRVPTIPPNLGIPGGKPAYSFHVTADGQMOPVFPFPDALIGPGIPRHARQINT
 LNHGEVVCVAVTISNPTRHVYTGGKGCVKVWDISHPGNKSPPVSQLDCLNRDNYIRSCKLLP
 DGCTLIVGGEASTLSIWDLAAPTPIKAELTSSAPACYALAI SPDSKVCFSCCSDGNIAV
 WDLHNQTLVRQFQGHDTGASCIDISNDGTKLWTGGLDNTVRSWDLREGRQLQQHDFTSQI
 25 FSLGYCPTGEWLAVGMESNNVEVLHVNKPKDYQLHLHESCVLSLKFAAYCGKWVSTGKDN
 LLNAWRPTYGASIFQSKESSSVLSCDISVDDKYIVTGSGDKKATVYEVIY

SEQ ID NO:36. Myosin-9 MYH9. UniProt ID P35579. Isoform 1.

>sp|P35579|MYH9_HUMAN Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4

MAQQAADKYLVDKNFINNPLAQADWAAKKLVWVPSDKSGFEPASLKEEVGEEAIVELVE
 30 NGKKVKVNKDDIQKMNPCKFSKVEDMAELTCLNEASVLHNLKERYYSGLIYTSGLFCVV
 INPYKNLPIYSEEIVEMYKGGKRHEMPPHIYAITDTAYRSMMQDREDQSILCTGESGAGK
 TENTKKVIQYLAYVASSHKSKKDQGELERQLLQANPILEAFGNAKTVKNDNSSRFGKFIR
 INFVNGYIVGANIETYLLEKSRAIRQAKEERTFHIFYLLSGAGEHLKTDLLLEPYNKY
 35 RFLSNGHVTIPGQQDKDMFQETMEAMRIMGIPEEEQMGLLRVISGVLQLGNIVFKKERN
 DQASMPDNTAAQKVSHLLGINVTDFTRGILTPRIKVGRDYVQKAQTKEQADFAIEALAKA
 TYERMFRWLVLRLINKALDKTKRQGASFIGILDIAFGFEIDLNSFEQLCINYTNEKLQQLF
 NHTMFILEQEEYQREGIEWNFIDFGLDLQPCIDLIEKPAGPPGILALLDEECWFPAKATDK
 SFVEKVMQEQQGTHPKFQKPKQLKDKADFCIIHYAGKVDYKADEWLMKNMDPLNDNIATLL
 40 HQSSDKFVSELWKDVDRIGLDQVAGMSETALPGAFKTRKGMFRTVGQLYKEQLAKLMAT
 LRNTNPNFVRCIIPNHEKKAGKLDPHLVLDQLRCNGVLEGIRICRQGFPNRVVFQEFRQR
 YEILTPNSIPKGFMDGKQACVLMIKALELDSNLYRIGQSKVFFRAGVLAHLEEERDLKIT
 DVIIGFQACCRGYLARKAFAKRQQQLTAMKVLQRNCAAYLKLRNWQWWRLFTKVKPLLQV
 SRQEEEMMAKEEELVKVREKQLAAENRLTEMETLQSQLMAEKLQLQEQLQAETELCAEAE
 45 ELRARLTAKKQEELEICHDLARVEEEEEERCQHLQAEKKKMQQNIQELEEQLEEEEESARQ
 KLQLEKVTTEAKLKKLEEEQIILEDQNCKLAKEKKLLEDRIAFTTNLTETEEKSKSLAK
 LKNKHEAMITDLEERLRREEKQRQELEKTRRKLEGDSTDLSQIAELQAQIAELKMQLAK
 KEEELQAALARVEEEAAQKNMALKKIRELESQISELQEDLESERASRNKAQKQRDLGEE
 LEALKTELEDTLSTAAQQELRSKREQEVNLLKKTLEEEAKTHEAQIQEMRQKHSQAVEE
 50 LAEQLEQTKRVKANLEKAKQTLENERGELANEVKVLLQKGKGDSEHKRKKVEAQLQELQVK

FNEGERVTELADKVTKLQVELDNVTGLLSQSDSKSSKLTKDFSALESQLODTQELLQEE
 NRQKLSLSTKLKQVEDEKNSFREQLSEEEEEAKHNLEKQIATLHAQVADMKKKMEDSVGCL
 ETAEEVKRKLQKDLGLSQRHEEKVAAAYDKLEKTKTRLQQELDDLLVDLDHQRQSACNLE
 KKQKKFDQLLAEKKTISAKYAEERDRAEAEAREKETKALSLARALEEAMEQKAELERLNK
 5 QFRTEMEDLMSSKDDVGKSVHELEKSKRALEQQVEEMKTQLEEELEDELQATEDAKLRLEV
 NLQAMKAQFERDLQGRDEQSEEKKKQLVRQVREMEAELEDERKQRSMAVAARKKLEMDLK
 DLEAHIDSANKNRDEAIKQLRKLQAQMKDCMRELD DTRASREEILAQAKENEKLLKSMEA
 EMIQLQEELAAAERAKRQAQQERDELADEIANSSGKGALALEEKRRLEARIAQLEEELEE
 EQGNTTELINDRLKKANLQIDQINTDLNLSHAQKNENARQQLERQNKELVKLQEMEGT
 10 VKSKYKASITALEAKIAQLEEQLDNETKERQAACKQVRRTEKKLKDVLQVDDERRNAEQ
 YKDQADKASTRLKQLKRQLEEAEEEAQRANASRRKLQRELEDATETADAMNREVSSLKNK
 LRRGDLPFVPPRRMARKGAGDGSDEEVDGKADGAEAKPAE

SEQ ID NO:37. Myosin-9 MYH9. UniProt ID P35579-2. Isoform 2.

15 >sp|P35579-2|MYH9_HUMAN Isoform 2 of Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9
 MYKGKKRHEMPPHIYAITDTAYRSMMDREDQSILCTGESGAGKTENTKKVIQYLAYVAS
 SHKSKKDQGELERQLLANPILEAFGNAKTVKNDNSSRFGKFIRINFDVNGYIVGANIET
 YLLEKSRAIRQAKEERTFHIFYLLSGAGEHLKTDLLLEPYNKYRFLSNGHVTIPGQQDK
 DMFQETMEAMRIMGIPEEEQMGLLRVISGVLQLGNIVFKKERN TDQASMPDNTAAQKVSH
 20 LLGINVTDFTRGILTPRIKVG RDYVQKAQTKEQADFAIEALAKATYERMFRWLVLRLINKA
 LDKTKRQGASFIGILDIAGFEIFDLNSFEQLCINYTNEKLQQLFNHTMFILEQEEYQREG
 IEWNFIDFGLDLQPCIDLIEKPAGPPGILALLDEECWFPAKTDKSFVEKVMQEQQTHPKF
 QKPKQLKDKADFCIIHYAGKVDYKADEWLMKNMDPLNDNIATLLHQSSDKFVSELWKDVD
 RIIGLDQVAGMSETALPGAFKTRKGMFRTVGQLYKEQLAKLMATLRNTNPNFVRCIIPNH
 25 EKKAGKLDPHLVLDQLRCNGVLEGIRICRQGFPNRVVFQEFRQRYEILTPNSIPKGFMDG
 KQACVLMIKALELDSNLYRIGQSKVFFRAGVLAHLEERDLKITDVIIGFQACCRGYLAR
 KAFARQQQLTAMKVLQRNCAAYLKLNRWQWWRLFTKVKPLLQVSRQEEEMMAKEEELVK
 VREKQLAAENRLTEMETLQSQLMAEKLQLQEQLQAETELCAEAEELRARLTAKKQEELEI
 CHDLEARVEEEERCQHLQAEKKKMQQNIQELEEQLSEESARQKLQLEKVTTEAKLKKL
 30 EEELDDLLVDLDHQRQSACNLEKKQKKFDQLLAEKKTISAKYAEERDRAEAEAREKETKA
 LSLARALEEAMEQKAELERLNKQFRTEMEDLMSSKDDVGKSVHELEKSKRALEQQVEEMK
 TQLEEELEDELQATEDAKLRLEVNLQAMKAQFERDLQGRDEQSEEKKKQLVRQVREMEAE
 EDERKQRSMAVAARKKLEMDLKDLEAHIDSANKNRDEAIKQLRKLQAQMKDCMRELD DTR
 ASREEILAQAKENEKLLKSMEAEMIQLQEELAAAERAKRQAQQERDELADEIANSSGKG
 35 LALEEKRRLEARIAQLEEELEEEQGNTTELINDRLKKANLQIDQINTDLNLSHAQKNEN
 ARQQLERQNKELVKLQEMEGTVKSKYKASITALEAKIAQLEEQLDNETKERQAACKQVR
 RTEKKLKDVLQVDDERRNAEQYKDQADKASTRLKQLKRQLEEAEEEAQRANASRRKLQ
 ELEDATETADAMNREVSSLKNKLRRGDLPFVPPRRMARKGAGDGSDEEVDGKADGAEAKPAE

40 SEQ ID NO:38. Ras GTPase-activating protein 1 RASA1. UniProt ID P20936. Isoform 1.

>sp|P20936|RASA1_HUMAN Ras GTPase-activating protein 1 OS=Homo sapiens OX=9606
 GN=RASA1 PE=1 SV=1

MMAAEAGSEEGGPVTAGAGGGGAAAGSSAYPAVCRVKIPAALPVAAAPYPGLVETGVAGT
 LGGGAALGSEFLGAGSVAGALGGAGLTGGGTAAGVAGAAAGVAGAAVAGPSGDMALTKLP
 45 TSLAETLGPGGGFPPLPPPPYLPPLGAGLGTVDGDSLDGPEYEEEEVAIPLTAPPTNQ
 WYHGKLDRTIAEERLRQAGKSGSYLIRESDRRPGSFVLSFLSQMNVVNHFRIIAMCGDYY
 IGGRRFSSSLDIGYYSHVSCLLKGEKLLYPVAPPEPVEDRRRVRAILPYTKVPDTDEIS
 FLKGDMFIVHNELEDGWMWVTNLRTDEQGLIVEDLVEEVGREEDPHEGKIWFHGKISKQE
 AYNLLMTVGQVCSFLVRPSDNTPGDYSLYFRTNENIQRFKICPTPNNQFMMGGRYNSIG
 50 DIIDHYRKEQIVEGYLLKEPVPMQDQEQLNDTVDGKEIYNTIRRKTKDAFYKNIVKKGY

LLKKGKGKRWKNLYFILEGSDAQLIFYESEKRA TKPKGLIDLSVCSVYVVHDSLFG RPNCFQIVVQHFSEEHYIFYFAGETPEQAEDWMKGLQAF CNLRKSSPGTSNKRLRQVSSLV LHI EEAHKLPVKHFTNPYCN IYLN SVQVAKTHAREGQNPVWSEEFVFD DLPPDINRFEITLSN KTKKSKDPDILFMRCQLSRLQKGHATDEWFLSSHIPLKGIEPGSLRVRARYSMEKIMPE
 5 EEFSEFKELILQKELHV VYALSHVCGQDR TLLASILLRIFLHEKLESLL LCTLNDR EISM EDEATTLFRATTLASTLMEQYMKATATQFVHHALKDSILKIMESKQSCELSPSKLEK NED VNTNLTHLLNLS ELVEKIFMASEILPPTLR IYIYGCLQKSVQHKWPTNTTMRTRVVS GFV FLRLICPAILNPRMFNIISDSPSIAARTLILVAKSVQNLANLVEFGAKEPYMEGVN PFI KSNKHRMIMFLDELGNVPELPDTTEHSRTDLSRDLAALHEICVAHSDELRTLSNERGAQQ
 10 HVLKLLAITELLQKQKNQYTKTNDVR

SEQ ID NO:39. Ras GTPase-activating protein 1 RASA1. UniProt ID P20936-2. Isoform 2.
 >sp|P20936-2|RASA1_HUMAN Isoform 2 of Ras GTPase-activating protein 1 OS=Homo sapiens
 OX=9606 GN=RASA1

MKGWYH GKLDRTIAEERLRQAGKSGSYLIRESDRRPGSFVLSF LSQMNVVNHFR IIAMCG
 15 DYYIGGRRFSSLSDLIGYYSHV SCLLKGEKLLYPVAPPEPVEDRRRVRAILPYTKVPD TD EISFLKGDMFIVHNELEDGWMWVTNLRTDEQGLIVEDLVEEVGREEDPHEGKIWFHGKIS KQEAYNLLMTVGQVCSFLVRPSDNTPGDYSLYFRTNENIQRFKICPTPNNQFMMGG RYYN SIGDIIDHYRKEQIVEGYYLKEPVPMQDQEQLNDTVDGKEIYNTIRRKT KDAFYKNIVK
 20 KGYLLKKGKGKRWKNLYFILEGSDAQLIFYESEKRA TKPKGLIDLSVCSVYVVHDSLFG RPNCFQIVVQHFSEEHYIFYFAGETPEQAEDWMKGLQAF CNLRKSSPGTSNKRLRQVSSLV LHI EEAHKLPVKHFTNPYCN IYLN SVQVAKTHAREGQNPVWSEEFVFD DLPPDINRFEIT LSNKTKKSKDPDILFMRCQLSRLQKGHATDEWFLSSHIPLKGIEPGSLRVRARYSMEKI MPEEEYSEFKELILQKELHV VYALSHVCGQDR TLLASILLRIFLHEKLESLL LCTLNDR E
 25 ISMEDEATTLFRATTLASTLMEQYMKATATQFVHHALKDSILKIMESKQSCELSPSKLEK NEDVNTNLTHLLNLS ELVEKIFMASEILPPTLR IYIYGCLQKSVQHKWPTNTTMRTRVVS GFVFLRLICPAILNPRMFNIISDSPSIAARTLILVAKSVQNLANLVEFGAKEPYMEGVN PFI KSNKHRMIMFLDELGNVPELPDTTEHSRTDLSRDLAALHEICVAHSDELRTLSNERG AQQHVLKLLAITELLQKQKNQYTKTNDVR
 30

SEQ ID NO:40. Ras GTPase-activating protein 1 RASA1. UniProt ID P20936-3. Isoform 3.
 >sp|P20936-3|RASA1_HUMAN Isoform 3 of Ras GTPase-activating protein 1 OS=Homo sapiens
 OX=9606 GN=RASA1

MMAAEAGSEEGGPVTAGAGGGGAAAGSSAYPAVCRVKIPAALPVAAAPYPGLVETGVAGT
 35 LGGGAALGSEFLGAGSVAGALGGAGLTGGGTAAGVAGAAAGVAGAAVAGPSGDMALTKLP TSLLAETLPGGGFPPLPPPPYLPPLGAGLGTVDGDSLDGPEYEEEEVAIPLTAPPTNQ WYH GKLDRTIAEERLRQAGKSGSYLIRESDRRPGSFVLSF LSQMNVVNHFR IIAMCGDYY IGGRRFSSLSDLIGYYSHV SCLLKGEKLLYPVAPPEPVEDRRRVRAILPYTKVPD TDEIS
 40 FLKGDMFIVHNELEDGWMWVTNLRTDEQGLIVEDLVEEVGREEDPHEGKIWFHGKISKQE AYNLLMTVGQVCSFLVRPSDNTPGDYSLYFRTNENIQRFKICPTPNNQFMMGG RYYNSIG DIIDHYRKEQIVEGYYLKEPVPMQDQEQLNDTVDGKEIYNTIRRKT KDAFYKNIVKKGY LLKKGKGKRWKNLYFILEGSDAQLIFYESEKRA TKPKGLIDLSVCSVYVVHDSLFG RCS

SEQ ID NO:41. Ras GTPase-activating protein 1 RASA1. UniProt ID P20936-4. Isoform 4.
 >sp|P20936-4|RASA1_HUMAN Isoform 4 of Ras GTPase-activating protein 1 OS=Homo sapiens
 OX=9606 GN=RASA1

MRTGYSSVPSKLRWYH GKLDRTIAEERLRQAGKSGSYLIRESDRRPGSFVLSF LSQMNVV
 45 NHFR IIAMCGDYYIGGRRFSSLSDLIGYYSHV SCLLKGEKLLYPVAPPEPVEDRRRVRAI LPYTKVPD TDEISFLKGDMFIVHNELEDGWMWVTNLRTDEQGLIVEDLVEEVGREEDPHE GKIWFHGKISKQEAYNLLMTVGQVCSFLVRPSDNTPGDYSLYFRTNENIQRFKICPTPNN

QFMMGGRYYSIGDIIDHYRKEQIVEGYLKEPVPMQDQEQVLNDTVDGKEIYNTIRRKTKDAFYKNIVKKGYYLLKKGKGRWKNLYFILEGSDAQLIYFESEKRA TKPKGLIDLSVCSVYVVHDSLFGPNCQFQIVVQHFSEEHYIFYFAGETPEQAEDWMKGLQAFCNLRKSSPGTSN
 5 KRLRQVSSSLVLHIEEAHKLPVKHFTNPYCNIYLSVQVAKTHAREGQNPVWSEEFVFDLPPDINRFEITLSNKT KSKDPDILFMRCQLSRLQKGHATDEWFLLSSHIPLKGIPEGSLRVRARYSMEKIMPEEEYSEFKELILQKELHVVYALSHVCGQDRTLLASILLRIFLHEKLES
 LLLCTLNDREISMEDEATTLFRATTLASTLMEQYMKATATQFVHHALKDSILKIMESKQS
 CELSPSKLEKNEDVNTNLTHLLNLSLSELVEKIFMASEILPPTLRYIYGCLQKSVQHKWPT
 10 NTTMRTRVVS GFVFLRLICPAILNPRMFNIISDPSPIAARTLILVAKSVQNLANLVEFG
 AKEPYMEGVNPFIKSNKHRMIMFLDELGNVPELPDTTEHSRTDLSRDLAALHEICVAHSD
 ELRTLSNERGAQQHVLLKKLLAITELLQQKQNKQYTKTNDVR

SEQ ID NO:42. Hairy/enhancer-of-split related with YRPW motif protein 1 HEY1. UniProt ID Q9Y5J3. Isoform 1.

>sp|Q9Y5J3|HEY1_HUMAN Hairy/enhancer-of-split related with YRPW motif protein 1 OS=Homo sapiens OX=9606 GN=HEY1 PE=1 SV=1
 MKRAHPEYSSSDSELDETIEVEKESADENGNLSSALGSMSPPTSSQILARKRRRGIIIEKR
 RRDRINNSLSELRRRLVPSAFEKQGS AKLEKAEILQMTVDHLKMLHTAGGKGYFDAHALAM
 DYRSLGFRECLAEVARYLSIIEGLDASDPLRVRLVSHLNNYASQREAASGAHAGLGHIPW
 20 GTVFGHHPHIAHPLLLPQNGHGNAGTTASPTEPHHQGR LGSAHPEAPALRAPPSGSLGPV
 LPVVTASAKLSPLLSSVASLSAFPFSFGSFHLLSPNALSPSAPTQAANLGKPYRPWGTE IGAF

SEQ ID NO:43. Hairy/enhancer-of-split related with YRPW motif protein 1 HEY1. UniProt ID Q9Y5J3-2. Isoform 2.

>sp|Q9Y5J3-2|HEY1_HUMAN Isoform 2 of Hairy/enhancer-of-split related with YRPW motif protein 1 OS=Homo sapiens OX=9606 GN=HEY1
 MKRAHPEYSSSDSELDETIEVEKESADENGNLSSALGSMSPPTSSQILARKRRRGIIIEKR
 RRDRINNSLSELRRRLVPSAFEKQVMEQGS AKLEKAEILQMTVDHLKMLHTAGGKGYFDAH
 ALAMDYRSLGFRECLAEVARYLSIIEGLDASDPLRVRLVSHLNNYASQREAASGAHAGLG
 30 HIPWGTVFGHHPHIAHPLLLPQNGHGNAGTTASPTEPHHQGR LGSAHPEAPALRAPPSGS
 LGPVLPVVTASAKLSPLLSSVASLSAFPFSFGSFHLLSPNALSPSAPTQAANLGKPYRP
 WGTEIGAF

SEQ ID NO:44. Protein C-ets-2 ETS2. UniProt ID P15036.

>sp|P15036|ETS2_HUMAN Protein C-ets-2 OS=Homo sapiens OX=9606 GN=ETS2 PE=1 SV=1
 MNDFGIKNMDQVAPVANSYRGTLKRQPAFDTFDGS LFAVFPSLNEEQTLQEVPTGLDSIS
 HDSANCELPLLT PCSKAVMSQALKATFSGFKKEQRR LGIPKNPWLWSEQQVCQWLLWATN
 EFSLVNVNLQRFGMNGQMLCNLGKERFLELAPDFVGDILWEHLEQMIKENQEKTEDQYEE
 NSHLTSVPHWINSNTLGFGEQAPYGMQTQNYPKGGLLD SMCPASTPSVLSSEQEFQMFP
 40 KSRLSSVSVTYCSVSQDFPGSNLNL TNNSGTPKDHDSPENGADSFESSDLLQSWNSQS
 SLLDVQRVPSFESFEDDCS QSLCLNKPTMSFKDYI QERSDPVEQGKPVIPAAVL AGFTGS
 GPIQLWQFLLELLSDKSCQSFISWTGDGWEFKLADPDEVARRWGKRKNPKMNYEKL SRG
 LRYYYDKNIIHKTSGKRYVYRFVCDLQNL LGFTPEELHAILGVQPDTE

SEQ ID NO:45. Hairy/enhancer-of-split related with YRPW motif protein 2 HEY2. UniProt ID Q9UBP5.

>sp|Q9UBP5|HEY2_HUMAN Hairy/enhancer-of-split related with YRPW motif protein 2 OS=Homo sapiens OX=9606 GN=HEY2 PE=1 SV=1
 MKRPCEETTSESDMDETIDVGSENNYSGQSTSSVIRLNSPTTTSQIMARKKRRRGIIIEKR
 RRDRINNSLSELRRLVPTAFEKQGS AKLEKAEILQMTVDHLKMLQATGGKGYFDAHALAMD
 50 FMSIGFRECLTEVARYLSSVEGLDSSDPLRVRLVSHLSTCATQREAAAMTSSMAHHHHL

HPHHWAAAFHHLPAALLQPNGLHASESTPCRLSTTSEVPPAHGSALLTATFAHADALSALRM
 PSTGSAVPCVPPLSTSLLSATVHAAAAAATAAAHSFPLSFAGAFMPLPPNAAAATAAA
 TAISPPLSVSATSSPQQTSSGTNNKPYRPWGTEVGAF

- 5 SEQ ID NO:46. LIM domain-binding protein 1 LDB1. UniProt ID Q86U70. Isoform 1.
 >sp|Q86U70|LDB1_HUMAN LIM domain-binding protein 1 OS=Homo sapiens OX=9606 GN=LDB1
 PE=1 SV=2

MSVGCACPGCSSKSFKLYSPKEPPNGNAFPPFHPGTMLDRDVGPTPMYPPTYLEPGIGRH
 TPYGNQTDYRIFELNKRLQNWTEECDNLWWDAFTTEFFEDDAMLTITFCLEDGPKRYTIG
 10 RTLIPRYFRSIFEGGATELYYVLKHPKEAFHSNFVSLDCDQGSMTQHGKPMFTQVCVEG
 RLYLEFMFDDMMRIKTWHFSIRQHRELIPRSILAMHAQDPQMLDQLSKNITRCGLSNSTL
 NYLRLCVILEPMQELMSRHKTYSLSPRDCLKTCLFQKWQRMVAPPAEPTQQPSKRRKRK
 MSGGSTMSGGGNTNNSNSKKKSPASTFALSSQVPDVMVVGEPITLMGGEFGDEDERLITR
 LENTQFDAANGIDDEDSFNNSPALGANSPPSSQESKSENPTSQASQ

- 15 SEQ ID NO:47. LIM domain-binding protein 1 LDB1. UniProt ID Q86U70-3. Isoform 2.
 >sp|Q86U70-3|LDB1_HUMAN Isoform 2 of LIM domain-binding protein 1 OS=Homo sapiens
 OX=9606 GN=LDB1

MLDRDVGPTPMYPPTYLEPGIGRHTPYGNQTDYRIFELNKRLQNWTEECDNLWWDAFTTE
 20 FFEDDAMLTITFCLEDGPKRYTIGRTLIPRYFRSIFEGGATELYYVLKHPKEAFHSNFVS
 LDCDQGSMTQHGKPMFTQVCVEGRLYLEFMFDDMMRIKTWHFSIRQHRELIPRSILAMH
 AQDPQMLDQLSKNITRCGLSNSTLNYLRLCVILEPMQELMSRHKTYSLSPRDCLKTCLFQ
 KWQRMVAPPAEPTQQPSKRRKRKMSGGSTMSGGGNTNNSNSKKKSPASTFALSSQVPV
 SISAFFSLLGCPTTHP

- 25 SEQ ID NO:48. LIM domain-binding protein 1 LDB1. UniProt ID Q86U70-2. Isoform 3.
 >sp|Q86U70-2|LDB1_HUMAN Isoform 3 of LIM domain-binding protein 1 OS=Homo sapiens
 OX=9606 GN=LDB1

MLDRDVGPTPMYPPTYLEPGIGRHTPYGNQTDYRIFELNKRLQNWTEECDNLWWDAFTTE
 30 FFEDDAMLTITFCLEDGPKRYTIGRTLIPRYFRSIFEGGATELYYVLKHPKEAFHSNFVS
 LDCDQGSMTQHGKPMFTQVCVEGRLYLEFMFDDMMRIKTWHFSIRQHRELIPRSILAMH
 AQDPQMLDQLSKNITRCGLSNSTLNYLRLCVILEPMQELMSRHKTYSLSPRDCLKTCLFQ
 KWQRMVAPPAEPTQQPSKRRKRKMSGGSTMSGGGNTNNSNSKKKSPASTFALSSQVPD
 VMVVGEPITLMGGEFGDEDERLITRLENTQFDAANGIDDEDSFNNSPALGANSPPSS
 35 SQESKSENPTSQASQ

SEQ ID NO:49. Netrin receptor UNC5C. UniProt ID O95185. Isoform 1.
 >sp|O95185|UNC5C_HUMAN Netrin receptor UNC5C OS=Homo sapiens OX=9606 GN=UNC5C
 PE=1 SV=2

- 40 MRKGLRATAARCGGLGYLLQMLVLPALALLSASGTGSAAQDDDDFFHELPETFPSPDPPEP
 LPHFLIEPEEAYIVKNKPNLYCKASPATQIYFKCNSEVWHQKDHIVDERVDETSGLIVR
 EVSIEISRQQVEELFGPEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSL
 EQEVLLQCRPPEGIPVAEVEWLKNEDIIDPVEDRNFYITIDHNLIKQARLSDTANYTCV
 AKNIVAKRKSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRCTNPAPLNGGAFCE
 45 GQSVQKIACTTLCVPDGRWTPWSKWSTCGTECTHWRRECTAPAPKNGGKDCDGLVLQSK
 NCTDGLCMQTAPDSDDVALYVGIVIAVIVCLAISVVVALFVYRKNHRDFESDIIDSSALN
 GGFQPVNIKAARQDLLAVPPDLTSAAAMYRGPVYALHDVSDKIPMTNSPILDPLPNLKIK
 VYNTSGAVTPQDDLSEFTSKLSPQMTQSLENEALS LKNQSLARQTDPSCTAFGSFNSLG
 GHLIVPNSGVSLIPAGAIPQGRVYEMYVTVHRKETMRPPMDDSQTLLTPVWSCGPPGAL
 50 LTRPVVLTMHHCADPNTEDWKILLKNQAAQGWEDVVVVGEENFTTPCYIQLDAEACHIL
 TENLSTYALVGHSTTKAAAKRLKLAIFGPLCCSSLEYSIRVYCLDDTDQDALKEILHLERQ

MGGQLLEPKALHFKGSTHNLRLSIHDIASLWWSKLLAKYQEIPFYHVWSGSQRNLHCT
FTLERFSLNTVELVCKLCVRQVEGEGQIFQLNCTVSEETGIDLPLLDPAANTITVTGPGS
AFSIPLPIRQKLCSSLDAPQTRGHDWRMLAHKLNLDRLNYFATKSSPTGVILDLWEAQN
FPDGNLSMLAAVLEEMGRHETVVSALAEQGY

5

SEQ ID NO:50. Netrin receptor UNC5C. UniProt ID O95185-2. Isoform 2.

>sp|O95185-2|UNC5C_HUMAN Isoform 2 of Netrin receptor UNC5C OS=Homo sapiens OX=9606
GN=UNC5C

MRKGLRATAARCGGLGYLLQMLVLPALALLSASGTGSAAQDDDFHHELPETFPSPDPEP
10 LPHFLIEPEEAYIVKNKPNLYCKASPATQIYFKCNSEVWHQKDHIVDERVDETSGLIVR
EVSIEISRQQVEELFGPEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSL
EQEVLLQCRPEGIPVAEVEWLKNEIDIIPVEDRNFYITIDHNLIIKQARLSDTANYTCV
AKNIVAKRKSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRTCTNPAPLNGGAFCE
GQSVQKIACTTLCVPDGRWTPWSKWSTCGTECTHWRRRECTAPAPKNGGKDCDGLVLQSK
15 NCTDGLCMQSFYIPISTEQRTQNEYGFSSAPDSDDVALYVGIVIAVIVCLAISVVVALFV
YRKNHRDFESDIIDSSALNGGFQPVNIKAARQDLLAVPPDLTSAAAMYRGPVYALHDVSD
KIPMTNSPILDPLPNLKIKVYNTSGAVTPQDDLSEFTSKLSPQMTQSLENEALS LKNQS
LARQTDPSCTAFGSFNSLGGHLIVPNSGVSLIPAGAIPQGRVYEMYVTVHRKETMR

20

SEQ ID NO:51. Neurofibromin NF1. UniProt ID P21359. Isoform 2.

>sp|P21359|NF1_HUMAN Neurofibromin OS=Homo sapiens OX=9606 GN=NF1 PE=1 SV=2

MAAHRPVEWVQAVVSRFDEQLPIKTGQQNTHTKVSTEHNKECLINISKYKFSLVISGLTT
ILKNVNNMRIFGEAAEKNLYLSQLIILDTLEKCLAGQPKDTMRLDETMVLVKQLLPEICHF
LHTCREGNQHAELRNSASGVLFSLSCNNFNNAVFSRISTRQLQELTVCESDNVDVHDIELL
25 QYINVDCAKLKRLKETAFAFKALKKVAQLAVINSLEKAFWNWVENYPDEFTKLYQIPQT
DMAECAEKLFDLVDGFAESTKRKAADVWPLQIILLILCPEIIQDISKDVVDENNMNKKLFL
DSLRKALAGHGGSRQLTESAAIACVKLCKASTYINWEDNSVIFLLVQSMVVDLKNLLFNP
SKPFSRGSQPADVDLMIDCLVSCFRISPHNNQHFKICLAQNSPSTFHYVLVNSLHRIITN
SALDWWPKIDAVYCHSVELRNMFGETLHKAVQGC GAHPAIRMAPSLTFKEKVTSLKFKEK
30 PTDLETRSYYKYL LLSMVKLIHADPKLLCNPRKQGPETQGSTAELITGLVQLVPQSHMPE
IAQEAMEALLVLHQLDSIDLWNPDAVETFWISSQMLFYICKKLTSHQMLSSTEILKWL
REILICRNKFLKKNKQADRSSCHFLFYGVGCDIPSSGNTSQMSMDHEELLRTPGASLRK
GKGNSSMDSAAGCSGTPPICRQAQTKLEVALYMF LWNPDTEAVLVAMSCFRHLCEEADIR
CGVDEVSVHNLLPNYNTFMEFASVSNNMSTGRAALQKRV MALLRRIEHP TAGNTEAWEDT
35 HAKWEQATKLILNYPKAKMEDGQAESLHKTIVKRRMSHVS GGGSIDLSDTDSLQEWINM
TGFLCALGGVCLQQRNSGLATYSPPMGPVSEKSGSMISVMSSEGNADTPVSKFMDRLLS
LMVCNHEKVGLQIRTNVKDLVGLLESPALYPMLFNKLKNTISKFFDSQGQVLLTDTNTQF
VEQTIAIMKNLLDNHTEGSSEHLGQASIETMMLNLVRYVRVLGNMVHAIQIKTKLCQLVE
VMMARRDDL SFCQEMKFRNKMVEYLT DWVMGTSNQAADDDVKCLTRDL DQASMEAVVSL
40 AGLPLQPEEGDGVELMEAKSQLFLKYFTLFMNLLNDCSEVEDESAQTGGRKRGMSRRLAS
LRHCTVLAMSNLLNANVDSGLMHSIGLYHKDLQTRATFMEVLT KILQQGTEFDTLAETV
LADRFERLVELVTMMGDQGELPIAMALANVVPCSQWDELARVLVTLFDSRHLLYQLLWNM
FSKEVELADSMQTLFRGNSLASKIMTFCFKVYGATY LQKLLDPLL RIVITSSDWQHVSFE
VDPTRLEPSESLEENQRNLLQMTEKFFHAISSSSEFP PQLRSVCHCLYQATCHSLLNKA
45 TVKEKKENKKS VVSQRFPQNSIGAVGSAMFLRFINPAIVSPYEAGILDKKPPPRIERGLK
LMSKILQSIANHVLF TKEEHMRPFNDFVKS NFDAARRFFLDIASDCPTSDAVNHSLSFIS
DGNVLALHRLLWNNQE KIGQYLSSNRDHKA VGRRPFDKMATLLAYLGPPEHKPVADTHWS
SLNLTSSKFEEFMTRHQVHEKEEFKALKTLSIFYQAGTSKAGNPIFYVARRFKTGQING
DLLIYHVLLTLKPYYAKPYEIVVDLTHTGPSNRFKTDFLSKWFVVPFGFAYDNVSAVYIY
50 NCNSWVREYTKYHERLLTGLKGSKRLVFIDCPGKLAEHIEHEQQKLPAATLALEEDLKVF
HNALKLAHKD TKVSIKVGSTAVQVTS AERTKVLGQSVFLNDIYYASEIEEICLV DENQFT

- LTIANQGTPLTFMHQECEAIVQSIHIRTWELSQPDSIPQHTKIRPKDVPGTLLNIAL
 NLGSSDPSLRSAAYNLLCALTCTFNLKIEGQLLETSGLCIPANNTLFIVSISKTAAANEP
 HLTLEFLEECISGFSKSSIELKHLCLCYMTPWLSNLVRFCKHNDDAKRQRVTAILEDKLIT
 MTINEKQMYPSIQAKIWGSLGQITDLLDVVLDSFIKTSATGGLGSIKAEVMADTAVAS
 5 GNVKLVSSKVIGRMCKIIDKTCLSPPTLEQHLMWDDIAILARYMLMLSFNNSLDVA AHL
 PYLFHVVTFLVATGPLSLRASTHGLVINIHSCTCSQLHFSEETKQVLRSLTEFSLPK
 FYLLFGISKVKSAAVIAFRSSYRDRSFSPGSYERETFALTSLETVTEALLEIMEACMRDI
 PTCKWLDQWTELAQRFAFQYNPSLQPRALVVFSGISKRVSHGQIKQIIRILSKALESCLK
 GPDYNSQVLIEATVIALTKLQPLLKDSPLHKALFWVAVAVLQLDEVNLYSAGTALLEQ
 10 NLHTLDSLRFNDKSPEEVFMAIRNPLEWHCKQMDHFVGLNFNSNFNFALVGHLLKGYRH
 PSPAIVARTVRILHTLLTLVNKHRNCDKFEVNTQSVAYLAALLTVSEEVRSRCCLKHRKS
 LLLTDISMENVPMDTYPIHHGDPSTYTLKETQPWSSPKGSEGYLAATYPTVGQTSRARK
 SMSLDMGQPSQANTKKLLGTRKSFHDLISDTKAPKRQEMESGITTPPKMRRVAETDYEME
 TQRISSSQQHPHLRKVSSESNNVLLDEEVLTDPKIQALLTLVATLVKYTTDEFDQRIY
 15 EYLAEASVVFVKVFPVHNLLDSKINTLLSLCQDPNLLNPIHGIVQSVVYHEESPPQYQT
 SYLQSFNGFLWRFAGPFSKQTQIPDYAELIVKFLDALIDTYLPGIDEETSEESLLTPTS
 PYPPALQSLSITANLNSNSMTSLATSQHSPGIDKENVELSPTTGHCSNGRTRHGSASQ
 VQKQRSAGSFKRNSIKKIV
- 20 SEQ ID NO:52. Neurofibromin NF1. UniProt ID P21359-2. Isoform 1.
 >sp|P21359-2|NF1_HUMAN Isoform 1 of Neurofibromin OS=Homo sapiens OX=9606 GN=NF1
 MAAHRPVEWVQAVVSRFDEQLPIKTGQQNTHTKVSTEHNKECLINISKYKFSLVISGLTT
 ILKNVNNMRIFGEAAEKNLYLSQLIILDTLEKCLAGQPKDTMRLDETMVLKQLLPEICHF
 LHTCREGNQHAAELRNSASGVFLSLSCNNFNAVFSRISTRQLQELTVCSNVDVVDIELL
 25 QYINVDCAKLKRLKETAFKFKALKKVAQLAVINSLEKAFWNWVENYPDEFTKLYQIPQT
 DMAECAEKLFDLVDGFAESTKRKAADVWPLQIILLILCPEIIQDISKDVVDENNMNKKLFL
 DSLRKALAGHGGSRLTESAAIACVKLCKASTYINWEDNSVIFLLVQSMVVDLKNLLFNP
 SKPFSRGSQPADVDLMIDCLVSCFRISPHNNQHFKICLAQNSPSTFHYVLVNSLHRIITN
 SALDWWPKIDAVYCHSVELRNMFGETLHKAVQGC GAHPAIRMAPSLTFKEKVTSLKFKKEK
 30 PTDLETRSYKYLLLSMVKLIHADPKLLLCNPRKQGPETQGSTAELITGLVQLVPQSHMPE
 IAQEAMEALLVLHQLDSIDLWNPDAVETFWISSQMLFYICKKLTSHQMLSSTEILKWL
 REILICRNKFLKKNKQADRSSCHFLFYGVGCDIPSSGNTSQMSMDHEELLRTPGASLRK
 GKGNSMDSAAGCSGTPPICRQAQTKLEVALYMFLLWNPDEAVLVAMSCFRHLCEEADIR
 CGVDESVHNLLPNYNTFMEFASVSNNMSTGRAALQKRVMA LLRRIEHTAGNTEAWEDT
 35 HAKWEQATKLILNYPKAKMEDGQAESLHKTIVKRRMSHVSGGGSIDLSDTDSLQEWINM
 TGFLCALGGVCLQQRNSGLATYSPPMGPVSEKSGSMISVMSSEGNADTPVSKFMDRLLS
 LMVCNHEKVGLQIRTNVKDLVGLLESPALYPMLFNKLKNTISKFFDSQGQVLLTDTNTQF
 VEQTIAIMKNLLDNHTEGSSEHLGQASLETMMLNLVRYVRVLGNMVAHQIKTKLCQLVE
 VMMARRDDL SFCQEMKFRNKMVEYLTDWVMGTSNQAADDDVKCLTRDL DQASMEAVVSL
 40 AGLPLQPEEGDGVELMEAKSQLFLKYFTLFMNLLNDCSEVEDESAQTGGRKRGMSRRLAS
 LRHCTVLAMSNLLNANVDSGLMHSIGLGYHKDLQTRATFMEVLT KILQQGTEFDTLAETV
 LADRFERLVELVTMMGDQGELPIAMALANVVPSCSQWDELARVLVTLFDSRHLLYQLLWNM
 FSKEVELADSMQTLFRGNSLASKIMTFCFKVYGATY LQKLLDPLL RIVITSSDWQHVSFE
 VDPTRELPSESLEENQRNLLQMTEKFFHAISSSSEFPPLRSVCHCLYQVVSQRFPQNS
 45 IGAVGSAMFLRFINPAIVSPYEAGILDKPPPRIERGLKLSKILQSIANHVLF TKEEHM
 RPFNDFVKS NFDAARRFFLDIASDCPTSDAVNHSLSFISDGNVLALHRL LWNQEKIGQY
 LSSNRDHKAVGRRPFDKMATLLAYLGPPEHKPVADTHWSSLNLTSSKFEEFMTRHQVHEK
 EEFKALKTLSIFYQAGTSKAGNPIFYVARRFKTGQINGDLLIYHVLLTLKPYYAKPYEI
 VVDLTHTGPSNRFKTDFLSKWFVFPGFAYDNVSAVYIYNCNSWVREYTKYHERLLTGLK
 50 GSKRLVFIDCPGKLAHIEHEQKLPATLAL EEDLKVFHNALKLAHKDTKVSIVGSTA

VQVTS AERTKVLGQSVFLNDIYYASEIEEICLV DENQFTLTIANQGTPLTFMHQECEAIV
 QSIIHIRTRWELS QPDSIPQHTKIRPKDVP GTLLNIALNLGSSDPSLRSAAYNLLCALT
 CTFNLKIEGQLLETSGLCIPANNTLFIVSISKTLAANEPHLTLEFLEECISGFSKSSIEL
 KHLCLEYMTWPWLSNLVRFCKHNDDAKRQRV TAILDKLITMTINEKQMPYSIQAKIWGSLG
 5 QITDLLDVVLDSFIKTSATGGLGSIKAEVMADTAVALASGNV KLVSSKVIGRMCKIIDKT
 CLSPTPTLEQHLMWDDIAILARYMLMLSFN NSLDVAAHLPYLFHVVTFLVATGPLSLRAS
 THGLVINIIHSLCTCSQLHFSEETKQVLR LSLTEFSLPKFYLLFGISKVKSAAVIAFRSS
 YRDRSFSPGSIYERETFALTSLETVT EALLEIMEACMRDIPTCKWLDQWTELAQRFAFQYN
 PSLQPRALVVF GCISKRVSHGQIKQIIRILSKALESCLKGPDTYNSQVLI EATVIALTKL
 10 QPLLNKDSPLHKALFWVAVAVLQLDEVNLYSAGTALLEQNLHTLDSL RIFNDKSPEEVFM
 AIRNPLEWHCKQMDHFVGLNFNSNFNFALVGHLLKGYRHPSPAIVARTVRILHTLLTLVN
 KHRNCDKFEVNTQSVAYLAALLTVSEE VRSRCSLKHRKSLLLTDISMENVPM DTYPIHHG
 DPSYRTLKETQPWSSPKGSEG YLAATYPTVGGTSPRARKSMSLDMGQPSQANTKKLLGTR
 KSF DHLISDTKAPKRQEMESGITTPPKMRRVAETDYEMETQRISSSQ QHPLRKVSSES
 15 NVLLDEEVLTPDKIQALLTVLATLVKYTTDEFDQRILYEYLAEASV VFPKVPV VHNLL
 DSKINTLLSLCQDPNLLNPIHGIVQSVVYHEESP PQYQTSYLSQSGFNGLWRFAGPFSKQ
 TQIPDYAELIVKFLDALIDTYLP GIDEETSEESLLTPTSPYPPALQS QLSITANLNSNS
 MTSLATSQHSPGIDKENVELSPTTGHCNSGRTRHGSASQVQKQRSAGSFKRNSIKKIV

20 SEQ ID NO:53. Neurofibromin NF1. UniProt ID P21359-3. Isoform 3.
 >sp|P21359-3|NF1_HUMAN Isoform 3 of Neurofibromin OS=Homo sapiens OX=9606 GN=NF1
 MAAHRPVEWVQAVVSRFDEQLPIKTGQQNTHTKVSTEHNKECLINISKYKFSLVISGLTT
 ILKNVNNMRIFGEAAEKNLYLSQLIILDTLEKCLAGQPKDTMRLDETM LVKQLLPEICHF
 LHTCREGNQHAAELRNSASGVLFSLSCNNFNAVFSRISTR LQELTVCS EDNVDVHDIELL
 25 QYINVDCAKLRLLKETAFKFKALKKVAQLAVINSLEKAFWNWVENYPDEFTKLYQIPQT
 DMAECAEKLFDLVDGFAESTKRKA AVWPLQIILLILCPEIIQDISKDVVDENNMNKKLFL
 DSLRKALAGHGGS RQLTESAAIACVKLCKASTYINWEDNSVIFLLVQSMVVDLKNLLFNP
 SKPFSRGSQPADVDLMIDCLVSCFRISPHNNQHFKICLAQNSPSTFH YVLVNSLHRIITN
 SALDWWPKIDAVYCHSVELRNMFGETLHKAVQGC GAHPAIRMAPSLTFKEKVTSLKFKEK
 30 PTDLETRSYKYLLLSMVKLIHADPKLLLCNPRKQGPETQGSTAELITGLVQLVPQSHMPE
 IAQEAMEVRGK

SEQ ID NO:54. Neurofibromin NF1. UniProt ID P21359-4. Isoform 4.
 >sp|P21359-4|NF1_HUMAN Isoform 4 of Neurofibromin OS=Homo sapiens OX=9606 GN=NF1
 35 MAAHRPVEWVQAVVSRFDEQLPIKTGQQNTHTKVSTEHNKECLINISKYKFSLVISGLTT
 ILKNVNNMRIFGEAAEKNLYLSQLIILDTLEKCLAGQPKDTMRLDETM LVKQLLPEICHF
 LHTCREGNQHAAELRNSASGVLFSLSCNNFNAVFSRISTR LQELTVCS EDNVDVHDIELL
 QYINVDCAKLRLLKETAFKFKALKKVAQLAVINSLEKAFWNWVENYPDEFTKLYQIPQT
 DMAECAEKLFDLVDGFAESTKRKA AVWPLQIILLILCPEIIQDISKDVVDENNMNKKLFL
 40 DSLRKALAGHGGS RQLTESAAIACVKLCKASTYINWEDNSVIFLLVQSMVVDLKNLLFNP
 SKPFSRGSQPADVDLMIDCLVSCFRISPHNNQHFKICLAQNSPSTFH YVLVNSLHRIITN
 SALDWWPKIDAVYCHSVELRNMFGETLHKAVQGC GAHPAIRMAPSLTFKEKVTSLKFKEK
 PTDLETRSYKYLLLSMVKLIHADPKLLLCNPRKQGPETQGSTAELITGLVQLVPQSHMPE
 IAQEAMEALLVLHQLDSIDLWNPDPVETFW EISSQMLFYICKKLTSHQMLSSTEILKWL
 45 REILICRNKFLKKNKQADRSSCHFLLFYGVGCDIPSSGNTS QMSMDHEELLRTPGASLRK
 GKGNSSMDSAAGCSGTPPICRQAQTKLEVALY MFLWNPDEAVLVAMSCFRHLCEEADIR
 CGVDEVSVHNLLPNYNTFMEFASVSNMMSTGRAALQKRV MALLRRIEHTAGNTEAWEDT
 HAKWEQATKLILNYPKAKMEDGQAAESLHKTIVKRRMSHVS GGSIDLSDTDSLQEWINM
 TGFLCALGGVCLQQRNSGLATYSPPMGPV SERKGS MISVMSSEGNADTPVSKFMDRLLS
 50 LMVCNHEKVGLQIRTNVKDLVGLELSPALY PMLFNKLKNTISKFFDSQGVLLTDTNTQF

VEQTIAIMKNLLDNHTEGSSEHLGQASMETMMLNLVRYVRVLGNMVHAIQIKTKLCQLVE
 VMMARRDDL SFCQEMKFRNKMVEYLT DWVMTSNQAADDDVKCLTRDL DQASMEAVVSL
 AGLPLQPEEGDGV LMEAKSQLFLKYFTLFMNLLNDCSEVEDESAQTGGRKRGMSRRLAS
 LRHCTVLAMSNLLNANVDSGLMHSIGLGYHKDLQTRATFMEVLT KILQQGTEFDTLAETV
 5 LADRFERLVELVTMMGDQ GELPIAMALANVVPCSQWDELARVLVTLFDSRHLLYQLLWNM
 FSKEVELADSMQTLFRGNSLASKIMTFCFKVYGATY LQKLLDPLL RIVITSSDWQHVSFE
 VDPTRELPESLEENQRNLLQMTEKFFHAISSSSEFP PQLRSVCHCLYQATCHSLLNKA
 TVKEKKENKKS VVSQRFPQNSIGAVGSAMFLRFINPAIVSPYEAGILDKKPPPRIERGLK
 10 LMSKILQSIANHVLF TKEEHMRPFNDFVKS NFDAARRFFLDIASDCPTSDAVNHSLSFIS
 DGNVLALHRL LWNQEKIGQYLSSNRDHKAVGRRPFDK MATLLAYLGPPEHKPVADTHWS
 SLNLTSSKFEF MTRHQVHEKEEFKALKTLTPPEPET

SEQ ID NO:55. Neurofibromin NF1. UniProt ID P21359-5. Isoform 5.

>sp|P21359-5|NF1_HUMAN Isoform 5 of Neurofibromin OS=Homo sapiens OX=9606 GN=NF1
 15 MAAHRPVEWVQAVVSRFDEQLPIKTGQQNTHTKVSTEHNKECLINISKYKFSLVISGLTT
 ILKNVNNMRIFGEAAEKNLYLSQLIILDTLEKCLAGQPKDTMRLDETMLVKQLLPEICHF
 LHTCREGNQHAAELRNSASGVLFSLSCNNFNAVFSRISTR LQELTVCS EDNVDVHDIELL
 QYINVDCAKLRLLKETAFKFKALKKVAQLAVINSLEKAFWNWVENYPDEFTKLYQIPQT
 DMAECAEKLFDLVDGFAESTKRKA AVWPLQIILLILCPEIIQDISKDVVDENNMKKLFL
 20 DSLRKALAGHGGS RQLTESAAIACVKLCKASTYINWEDNSVIFLLVQSMVVDLKNLLFNP
 SKPFSRGSQPADVDLMIDCLVSCFRISPHNNQHFKICLAQNSPSTFHYVLVNSLHRIITN
 SALDWWPKIDAVYCHSVELRNMFGETLHKA VQGC GAHPAIRMAPSLTFKEKVTSLKFKEK
 PTDLETRSYKYLLLSMVKLIHADPKLLLCNPRKQGPETQGSTAELITGLVQLVPQSHMPE
 25 IAQEAMEALLVLHQLDSIDLWNP DAPVETFW EIRYMYFYFLNSTFKFYFVFLS

SEQ ID NO:56. Neurofibromin NF1. UniProt ID P21359-6. Isoform 6.

>sp|P21359-6|NF1_HUMAN Isoform 6 of Neurofibromin OS=Homo sapiens OX=9606 GN=NF1
 MAAHRPVEWVQAVVSRFDEQLPIKTGQQNTHTKVSTEHNKECLINISKYKFSLVISGLTT
 30 ILKNVNNMRIFGEAAEKNLYLSQLIILDTLEKCLAGQPKDTMRLDETMLVKQLLPEICHF
 LHTCREGNQHAAELRNSASGVLFSLSCNNFNAVFSRISTR LQELTVCS EDNVDVHDIELL
 QYINVDCAKLRLLKETAFKFKALKKVAQLAVINSLEKAFWNWVENYPDEFTKLYQIPQT
 DMAECAEKLFDLVDGFAESTKRKA AVWPLQIILLILCPEIIQDISKDVVDENNMKKLFL
 DSLRKALAGHGGS RQLTESAAIACVKLCKASTYINWEDNSVIFLLVQSMVVDLKNLLFNP
 SKPFSRGSQPADVDLMIDCLVSCFRISPHNNQHFKICLAQNSPSTFHYVLVNSLHRIITN
 35 SALDWWPKIDAVYCHSVELRNMFGETLHKA VQGC GAHPAIRMAPSLTFKEKVTSLKFKEK
 PTDLETRSYKYLLLSMVKLIHADPKLLLCNPRKQGPETQGSTAELITGLVQLVPQSHMPE
 IAQEAMEALLVLHQLDSIDLWNP DAPVETFW EISSQMLFYICKKLTSHQMLSSTEILKWL
 REILICRNKFL LKNKQADRSSCHFL LFYGVGCDIPSSGNTSQMSMDHEELLRTPGASLRK
 GKGNSSMDSAAGCSGTPPICRQAQTKLEVALY MFLWNP DTEAVLVAMSCFRHLCEEADIR
 40 CGVDEVSVHNLLPNYNTFMEFASVSNMMSTGRAALQKRVMALLRRIEHTAGNTEAWEDT
 HAKWEQATKILNYPKAKMEDGQA AESLHK TIVKRRMSHVS GGGSIDLSDTDSLQEWINM
 TGFLCALGGVCLQQRNSGLATYSPPMGPV SERKGS MISVMSSEGNADTPVSKFMDRLLS
 LMVCNHEKVGLQIRTNVKDLVGLELSPALY PMLFNKLKNTISKFFDSQGQVLLTDTNTQF
 VEQTIAIMKNLLDNHTEGSSEHLGQASMETMMLNLVRYVRVLGNMVHAIQIKTKLCQLVE
 45 VMMARRDDL SFCQEMKFRNKMVEYLT DWVMTSNQAADDDVKCLTRDL DQASMEAVVSL
 AGLPLQPEEGDGV LMEAKSQLFLKYFTLFMNLLNDCSEVEDESAQTGGRKRGMSRRLAS
 LRHCTVLAMSNLLNANVDSGLMHSIGLGYHKDLQTRATFMEVLT KILQQGTEFDTLAETV
 LADRFERLVELVTMMGDQ GELPIAMALANVVPCSQWDELARVLVTLFDSRHLLYQLLWNM
 FSKEVELADSMQTLFRGNSLASKIMTFCFKVYGATY LQKLLDPLL RIVITSSDWQHVSFE
 50 VDPTRELPESLEENQRNLLQMTEKFFHAISSSSEFP PQLRSVCHCLYQVVSQRFPQNS
 IGAVGSAMFLRFINPAIVSPYEAGILDKKPPPRIERGLKLMSKILQSIANHVLF TKEEHM

RPFNDFVKSNFDAARRFFLDIASDCPTSDAVNHSLSFISDGNVLALHRLWLNNQEKIGQY
 LSSNRDHKAVGRRPFDKMATLLAYLGPPEHKPVADTHWSSLNLTSKFEEFMTRHQVHEK
 EEFKALKTLSIFYQAGTSKAGNPIFYVARRFKTGQINGDLLIYHVLLTLKPYYAKPYEI
 VVDLTHTGPSNRFKTDFLSKWFVFPGFAYDNVSAVYIYNCNSWVREYTKYHERLLTGLK
 5 GSKRLVFIDCPGKLAEHIEHEQKLPAATLAEEDLKVFHNALKLAHKDTKVSIVGSTA
 VQVTSARTKVLGQSVFLNDIYYASEIEEICLVDENQFTLTIANQGTPLTFMHQECEAIV
 QSIIHIRTRWELSQPDSIPQHTKIRPKDVP GTLLNIALNLGSSDPSLRSAAYNLLCALT
 CTFNLKIEGQLLETSGLCIPANNTLFIVSISKTLAANEPHLLTEFLEECISGFSKSSIEL
 KHLCLEYMTWPWLSNLVRFCKHNDDAKRQRTAILDKLITMTINEKQMYPSIQAKIWGSLG
 10 QITDLLDVVLDSFIKTSATGGLGSIKAEVMADTAVALASGNVCLVSSKVIGRMCKIIDKT
 CLSPTPTLEQHLMWDDIAILARYMLMLSFNNSLDVAAHLPYLFHVVTFLVATGPLSLRAS
 THGLVINIHSCTCSQLHFSEETKQVLRSLTEFSLPKFYLLFGISKVKSAAVIAFRSS
 YRDRSFSPGSYERETFALTSLETVTALLEIMEACMRDIPTCKWLDQWTELAQRFAFYQN
 PSLQPRALVVFSGICKRVSHGQIKQIIRILSKALESCLKGPDTYNSQVLEATVIALTKL
 15 QPLLNKDSPLHKALFWVAVAVLQLDEVNLYSAGTALLEQNLHTLDSLRFNDKSPREEVFM
 AIRNPLEWHCKQMDHFVGLNFNSNFNFALVGHLLKGYRHPSPAIVARTVRILHTLLTLVN
 KHRNCDKFVNTQSVAYLAALLTVSEEVSRCSLKHRSLLLTDISMENVPMDTYPIHGG
 DPSYRTLKETQPWSSPKGSEGYLAATYPTVGQTSRARKSMSLDMGQPSQANTKLLGTR
 KSFDHLISDTKAPKRQEMESGITTPPKMRRVAETDYEMETQRISSSQHPLRKVSSES
 20 NVLLDEEVLTPDKIQALLTVLATLVKYTTDEFDQRILYEYLAESVFPKVPVHNL
 DSKINTLLSLCQDPNLLNPIHGIVQSVVYHEESPQYQTSYLSFGFNGLWRFAGPFSKQ
 TQIPDYAELIVKFLDALIDTYLPGIDEETSEESLLTPTSPYPPALQSLSITANLNSNS
 MTSLATSQHSPASLPCNSAVFMQLFPHQGIDKENVELSPTTGHCNSGRTRHGSASQVQK
 25 QRSAGSFKRNSIKKIV

SEQ ID NO:57. Cyclin-dependent kinase 6 CDK6. UniProt ID Q00534.

>sp|Q00534|CDK6_HUMAN Cyclin-dependent kinase 6 OS=Homo sapiens OX=9606 GN=CDK6 PE=1 SV=1

30 MEKDGLCRADQYECVAEIGEGAYGKVFKAARDLKNNGRFVALKRVRVQTGEEGMPLSTIR
 EVAVLRHLETFEHPNVVRLFDVCTVSRTDRETKLTLVFEHVDQDLTTYLDKVPEPGVPTE
 TIKDMMFQLLRGLDFLHSHRVVHRDLKPQNILVTSSGQIKLADFGGLARIYSFQMALTSVV
 VTLWYRAPEVLLQSSYATPVDLWSVGCIFAEMFRRKPLFRGSSDQDLGKILDVIGLPGE
 EDWPRDVALPRQAFHKSQAQPIEFVTDIDELGKDLLLKCLTFNPAKRISAYSALSHPYF
 35 QDLERCKENLDLPSQNTSELNTA

SEQ ID NO:58. Cyclin-Very low-density lipoprotein receptor VLDLR. UniProt ID P98155. Isoform Long.

>sp|P98155|VLDLR_HUMAN Very low-density lipoprotein receptor OS=Homo sapiens OX=9606 GN=VLDLR PE=1 SV=1

40 MGTSALWALWLLLALCWAPRESGATGTGRKAKCEPSQFQCTNGRCITLLWKCDGDEDCVD
 GSDEKNCVKKTCAESDFVCNNGQCVPSRWKCDGDPDCEDGSDESPEQCHMRTCRIHEISC
 GAHSTQCIPVSWRCGENDCDSGEDEENCNITCSPDEFTCSSGRCSIRNFVCNGQDDCS
 45 DGSDDELDCAPPTCGAHEFCSTSSCIPISWVCDDADADCSDQSDLESLEQCGRQPVHTKCP
 ASEIQCGSGECIHKKWRCDGDPCKDGSDEVNCPSTRCPDQFECEDGSCIHGSRQCNGI
 RDCVDGSDDEVNCKNVNQCLGPGKFKCRSGECIDISKVCNQEQQDCRDWSDEPLKECHINEC
 LVNNGGCSHICKDLVIGYECDAAGFELIDRKTCGDIDECQNPICISQICINLKGKYKCE
 CSRGYQMDLATGVCKAVGKEPSLIFTNRRDIRKIGLERKEYIQLVEQLRNTVALDADIAA
 50 QKLFWADLSQKAIFSASIDDKVGRHVKMDNVYNPAIAVDWVYKTIYWTDAASKTISVA
 TLDGTRKRLFLNSDLREPASIAVDPLSGFVYWSWGEPAKIEKAGMNGFDRRPLVTADIQ

WPNGITLDLIKSRLYWLDSKLHMLSSVDLNGQDRRIVLKSLEFLAHPLALTIFEDRVYWI
 DGENEAVYGANKFTGSELATLVNNLND AQDIIIVYHEL VQPSGKNWCEEDMENG GCEYLCL
 PAPQINDHSPKYTCSCPSGYNVEENGRDCQSTATTVTYSETKDTNTTEISATSGLVPGGI
 NVT TAVSEVSVPPKGTSAAWAILPLLLL VMAAVGGYLMWRNWQHKNMKSMNFDNPVYLKT
 5 TEEDLSIDIGRHSASVGHTYPAISVVSTDDDLA

SEQ ID NO:59. Cyclin-Very low-density lipoprotein receptor VLDLR. UniProt ID P98155-2. Isoform Short.

>sp|P98155-2|VLDLR_HUMAN Isoform Short of Very low-density lipoprotein receptor OS=Homo sapiens OX=9606 GN=VLDLR

10 MGTSALWALWLLLALCWAPRESGATGTGRKAKCEPSQFQCTNGRCITLLWKCDGDEDCVD
 GSDEKNCVKKTCAESDFVCNNGQCVPSRWKCDGDPDCEDGSDESPEQCHMRTCRIHEISC
 GAHSTQCIPVSWRCGENDCDSGEDEENCGNITCSPDEFTCSSGRCISRNFCNGQDDCS
 DGSDDELDCAPPTCGAHEFQCSTSSCIPISWVCDDADADCSDQSDLESLEQCGRQPVHTKCP
 15 ASEIQCGSGECIHKKWRCGDGDPCKDGSDEVNCPSTRCPDQFECEDGSCIHGSRQCNGI
 RDCVDGSDEVNCKNVNQC LGPGKFKCRSGECIDISKVCNQE QDCRDWSDEPLKECHINEC
 LVNNGGCSHICKDLVIGYECDAAGFELIDRKT CGDIDECQNP GICSQICINLKGGYKCE
 CSRGYQMDLATGVCKAVGKEPSLIFTNRRDIRKIGLERKEYIQLVEQLRNTVALDADIAA
 QKLFWADLSQKAIFSASIDDKVGRHV K MIDNVYNPAIAI AVDWVYKTIYWTDAASKTISVA
 20 TLDGTRKRLFN S D L REPASIAVDPLSGFVYWS DWGEPAKIEKAGMNGFDRRPLVTADIQ
 WPNGITLDLIKSRLYWLDSKLHMLSSVDLNGQDRRIVLKSLEFLAHPLALTIFEDRVYWI
 DGENEAVYGANKFTGSELATLVNNLND AQDIIIVYHEL VQPSGKNWCEEDMENG GCEYLCL
 PAPQINDHSPKYTCSCPSGYNVEENGRDCQ RINVT TAVSEVSVPPKGTSAAWAILPLLLL
 VMAAVGGYLMWRNWQHKNMKSMNFDNPVYLKTTEEDLSIDIGRHSASVGHTYPAISVVST
 25 DDDLA

SEQ ID NO:60. Neuropilin-2 NRP2. UniProt ID O60462. Isoform A22.

>sp|O60462|NRP2_HUMAN Neuropilin-2 OS=Homo sapiens OX=9606 GN=NRP2 PE=1 SV=3

30 MDMFPLTWVFLALYFSRHQVRGQPDPPCGRLNSKDAGYITSPGYPDYPSHQNCEWIVY
 APEPNQKIVLNFNPHFEIEKHDCKYDFIEIRDGDSEADLLGKHCGNIAPPTIISGSM L
 YIKFTSDYARQGAGFSRLYEIFKTGSEDCSKNFTSPNGTIESPGFPEKYPHNLDCFTIL
 AKPKMEIILQFLIFDLEHDPLQVGEGDCKYDWLDIWDGIPHVGPLIGKYCGTKTPSELRS
 STGILSLTFHTDMAVAKDGFSARYYL VHQEPLNFQC NVPLGMESGRIANEQISASSTYS
 DGRWTPQQSRLHGDDNGWTPNLD SNKEYLQVDLRFLTMLTAIATQGAISRETQNGYYVKS
 35 YKLEVSTNGEDWMVYRHGKNHKVFQANNDATEVVLNKLHAPLLTRFVRIRPQTWHSGIAL
 RLELFGCRVTDAPCSNMLGMLSGLIADSQISASSTQEYLWSPSAARLVSSRSGWFPRIPQ
 AQPGEEWLQVDLGT PKTVKGVIIQGARGGDSITAVEARAFVRKFKVSYSLNGKDWEYIQD
 PRTQQPKLFEGNMHYDTPDIRRFDPIPAQYVRVYPERWSPAGIGMRLEV LGCDWTD SKPT
 VETLGPTVKSEETTTPTPT EEEATECGENCSFEDDKDLQLPSGFNCNFD FLEEPCGW MYD
 40 HAKWLRTTWASSSSPNDRTFPDDRNFLRLQSDS QREGQYARLISPPVHLPRSPVCM EFQY
 QATGGRGVALQVVREASQESKLLWVIREDDQGG EWKHGRIILPSYDMEYQIVFEGVIGKGR
 SGEIAIDDIRISTDVPLENCMEPISAFAGENFKVDIPEIHEREGYEDEIDDEYEVDWSNS
 SSATSGSGAPSTDKEKSWLYTLDPILITIIAMSSLG VLLGATCAGLLLYCTCSYSGLSSR
 SCTTLENYNFELYDGLKHKVKMNHQKCCSEA
 45

SEQ ID NO:61. Neuropilin-2 NRP2. UniProt ID O60462-2. Isoform A0.

>sp|O60462-2|NRP2_HUMAN Isoform A0 of Neuropilin-2 OS=Homo sapiens OX=9606 GN=NRP2

MDMFPLTWVFLALYFSRHQVRGQPDPPCGRLNSKDAGYITSPGYPDYPSHQNCEWIVY
 APEPNQKIVLNFNPHFEIEKHDCKYDFIEIRDGDSEADLLGKHCGNIAPPTIISGSM L
 50 YIKFTSDYARQGAGFSRLYEIFKTGSEDCSKNFTSPNGTIESPGFPEKYPHNLDCFTIL

AKPKMEIILQFLIFDLEHDPLQVGECDCKYDWLDIWDGIPHVGPLIGKYCGTKTPSELRS
 STGILSLTFHTDMAVAKDGFSARYYL VHQEPLNFQCNVPLGMESGRIANEQISASSTYS
 DGRWTPQQSRLHGDDNGWTPNLDNKEYLQVDLRFLTMLTAIATQGAISRETQNGYYVKS
 YKLEVSTNGEDWMVYRHGKNHKVFQANNDATEVVLNKLHAPLLTRFVRIRPQTWHSGIAL
 5 RLELFGCRVTDAPCSNMLGMLSGLIADSQISASSTQEYLWSPSAARLVSSRSGWFPRIPQ
 AQPGEEWLQVDLGTPTKTVKGVIIQGARGGDSITAVEARAFVRKFKVSYSLNGKDEYIQD
 PRTQQPKLFEGNMHYDTPDIRRFDPIPAQYVRVYPERWSPAGIGMRLEVLGCDWTDKPT
 VETLGPTVKSEETTTTPYTEEEATECGENCSEFEDDKDLQLPSGFNCNDFLEEPCGWMYD
 HAKWLRTTWASSSSPNDRTPDDRNLRLQSDSQREGQYARLISPPVHLPRSPVCMFQY
 10 QATGGRGVALQVREASQESKLLWVIREDDQGGGEWKHGRIILPSYDMEYQIVFEGVIGKGR
 SGEIAIDDIRISTDVPLENCMEPISAFADYEYVDWSNSSSATSGSGAPSTDKEKSWLYTL
 DPILITIAMSSSLGVLLGATCAGLLLYCTCSYSGLSRSSCTTLENYNFELYDGLKHKVKM
 NHQKCCSEA

15 SEQ ID NO:62. Neuropilin-2 NRP2. UniProt ID O60462-3. Isoform A17.

>sp|O60462-3|NRP2_HUMAN Isoform A17 of Neuropilin-2 OS=Homo sapiens OX=9606 GN=NRP2
 MDMFPLTWVFLALYFSRHQVRGQPDPPCGRLNSKDAGYITSPGYPDYPSHQNCEWIVY
 APEPNQKIVLNFNPHFEIEKHDCKYDFIEIRDGDSEADLLGKHCGNIAPPTIISGSM
 YIKFTSDYARQGAGFSLRYEIFKTGSEDCSKNFTSPNGTIESPGFPEKYPHNLDCTFTIL
 20 AKPKMEIILQFLIFDLEHDPLQVGECDCKYDWLDIWDGIPHVGPLIGKYCGTKTPSELRS
 STGILSLTFHTDMAVAKDGFSARYYL VHQEPLNFQCNVPLGMESGRIANEQISASSTYS
 DGRWTPQQSRLHGDDNGWTPNLDNKEYLQVDLRFLTMLTAIATQGAISRETQNGYYVKS
 YKLEVSTNGEDWMVYRHGKNHKVFQANNDATEVVLNKLHAPLLTRFVRIRPQTWHSGIAL
 RLELFGCRVTDAPCSNMLGMLSGLIADSQISASSTQEYLWSPSAARLVSSRSGWFPRIPQ
 25 AQPGEEWLQVDLGTPTKTVKGVIIQGARGGDSITAVEARAFVRKFKVSYSLNGKDEYIQD
 PRTQQPKLFEGNMHYDTPDIRRFDPIPAQYVRVYPERWSPAGIGMRLEVLGCDWTDKPT
 VETLGPTVKSEETTTTPYTEEEATECGENCSEFEDDKDLQLPSGFNCNDFLEEPCGWMYD
 HAKWLRTTWASSSSPNDRTPDDRNLRLQSDSQREGQYARLISPPVHLPRSPVCMFQY
 QATGGRGVALQVREASQESKLLWVIREDDQGGGEWKHGRIILPSYDMEYQIVFEGVIGKGR
 30 SGEIAIDDIRISTDVPLENCMEPISAFADYEYVDWSNSSSATSGSGAPSTDKEKSWLYTL
 DPILITIAMSSSLGVLLGATCAGLLLYCTCSYSGLSRSSCTTLENYNFELYDGLKHKVKM
 NHQKCCSEA

35 SEQ ID NO:63. Neuropilin-2 NRP2. UniProt ID O60462-4. Isoform B0.

>sp|O60462-4|NRP2_HUMAN Isoform B0 of Neuropilin-2 OS=Homo sapiens OX=9606 GN=NRP2
 MDMFPLTWVFLALYFSRHQVRGQPDPPCGRLNSKDAGYITSPGYPDYPSHQNCEWIVY
 APEPNQKIVLNFNPHFEIEKHDCKYDFIEIRDGDSEADLLGKHCGNIAPPTIISGSM
 YIKFTSDYARQGAGFSLRYEIFKTGSEDCSKNFTSPNGTIESPGFPEKYPHNLDCTFTIL
 40 AKPKMEIILQFLIFDLEHDPLQVGECDCKYDWLDIWDGIPHVGPLIGKYCGTKTPSELRS
 STGILSLTFHTDMAVAKDGFSARYYL VHQEPLNFQCNVPLGMESGRIANEQISASSTYS
 DGRWTPQQSRLHGDDNGWTPNLDNKEYLQVDLRFLTMLTAIATQGAISRETQNGYYVKS
 YKLEVSTNGEDWMVYRHGKNHKVFQANNDATEVVLNKLHAPLLTRFVRIRPQTWHSGIAL
 RLELFGCRVTDAPCSNMLGMLSGLIADSQISASSTQEYLWSPSAARLVSSRSGWFPRIPQ
 AQPGEEWLQVDLGTPTKTVKGVIIQGARGGDSITAVEARAFVRKFKVSYSLNGKDEYIQD
 45 PRTQQPKLFEGNMHYDTPDIRRFDPIPAQYVRVYPERWSPAGIGMRLEVLGCDWTDKPT
 VETLGPTVKSEETTTTPYTEEEATECGENCSEFEDDKDLQLPSGFNCNDFLEEPCGWMYD
 HAKWLRTTWASSSSPNDRTPDDRNLRLQSDSQREGQYARLISPPVHLPRSPVCMFQY
 QATGGRGVALQVREASQESKLLWVIREDDQGGGEWKHGRIILPSYDMEYQIVFEGVIGKGR
 SGEIAIDDIRISTDVPLENCMEPISAFAGGTLLPGTEPTVDTVPMQPIPAYWYVMAAGG
 50 AVLVLVSVALALVLHYHFRFYAAKKTDSITYKTSHTNGAPLAVEPTLTIKLEQDRGSH C

SEQ ID NO:64. Neuropilin-2 NRP2. UniProt ID O60462-5. Isoform B5.

>sp|O60462-5|NRP2_HUMAN Isoform B5 of Neuropilin-2 OS=Homo sapiens OX=9606 GN=NRP2
MDMFPLTWVFLALYFSRHQVRGQPDPPCGGRLNSKDAGYITSPGYPDYPSHQNCEWIVY
5 APEPNQKIVLNFNPHFEIEKHDCKYDFIEIRDGDSEADLLGKHCGNIAPPTIISGSML
YIKFTSDYARQGAGFSLRYEIFKTGSEDCSKNFTSPNGTIESPGFPEKYPHNLDCTFTIL
AKPKMEIILQFLIFDLEHDPLQVGECDCKYDWLDIWDGIPHVGPLIGKYCGTKTPSELRS
STGILSLTFHTDMAVAKDGFSAARYLVHQEPLNFQCNVPLGMESGRIANEQISASSTYS
10 DGRWTPQQSRLHGDDNGWTPNLDNKEYLQVDLRFLTMLTAIATQGAISRETQNGYYVKS
YKLEVSTNGEDWMVYRHGKNHKVFQANNDATEVVLNKLHAPLLTRFVRIRPQTWHSIAL
RLELFGCRVTDAPCSNMLGMLSGLIADSQISASSTQEYLWSPSAARLVSSRSGWFPRIPQ
AQPGEWLQVDLGTPTKTVKGVIQARGGDSITAVEARAFVRKFKVSYSLNGKDWWEYIQD
PRTQQPKLFEGNMHYDTPDIRRFDPIAQYVRVYPERWSPAGIGMRLEVLGCDWTDKPT
15 VETLGPTVKSEETTPYPTEEEATECGENCSEFDDKDLQLPSGFNCNFDLFLEPCGWMYD
HAKWLRTTWASSSSPNDRTPDDRNLRLQSDSQREGQYARLISPPVHLPRSPVCMFQY
QATGGRGVALQVVREASQESKLLWVIREDDQGEWKHGRILPSYDMEYQIVFEGVIGKGR
SGEIAIDDIRISTDVPLENCMEPISAFAGENFKGGTLLPGTEPTVDTVPMQPIPAYWYYV
MAAGGAVLVLSVALALVLHYHFRFYAAKKTDSITYKTSHYTNGAPLAVEPTLTIKLEQ
20 DRGSHC

SEQ ID NO:65. Neuropilin-2 NRP2. UniProt ID O60462-6. Isoform s9.

>sp|O60462-6|NRP2_HUMAN Isoform s9 of Neuropilin-2 OS=Homo sapiens OX=9606 GN=NRP2
MDMFPLTWVFLALYFSRHQVRGQPDPPCGGRLNSKDAGYITSPGYPDYPSHQNCEWIVY
25 APEPNQKIVLNFNPHFEIEKHDCKYDFIEIRDGDSEADLLGKHCGNIAPPTIISGSML
YIKFTSDYARQGAGFSLRYEIFKTGSEDCSKNFTSPNGTIESPGFPEKYPHNLDCTFTIL
AKPKMEIILQFLIFDLEHDPLQVGECDCKYDWLDIWDGIPHVGPLIGKYCGTKTPSELRS
STGILSLTFHTDMAVAKDGFSAARYLVHQEPLNFQCNVPLGMESGRIANEQISASSTYS
DGRWTPQQSRLHGDDNGWTPNLDNKEYLQVDLRFLTMLTAIATQGAISRETQNGYYVKS
YKLEVSTNGEDWMVYRHGKNHKVFQANNDATEVVLNKLHAPLLTRFVRIRPQTWHSIAL
30 RLELFGCRVTDAPCSNMLGMLSGLIADSQISASSTQEYLWSPSAARLVSSRSGWFPRIPQ
AQPGEWLQVDLGTPTKTVKGVIQARGGDSITAVEARAFVRKFKVSYSLNGKDWWEYIQD
PRTQQPKVGCSSWRPL

SEQ ID NO:66. Dihydropyrimidinase-related protein 2 DPYL2. UniProt ID Q16555. Isoform 1.

>sp|Q16555|DPYL2_HUMAN Dihydropyrimidinase-related protein 2 OS=Homo sapiens OX=9606
GN=DPYSL2 PE=1 SV=1
MSYQGKKNIPRITSRLLIKGGKIVNDDQSFYADIYMEDGLIKQIGENLIVPGGVKTIEA
HSRMVIPGGIDVHTRFQMPDQGMTSADDDFFQGTKAALAGGTTMIDHVVPEPGTSLAFAF
40 DQWREWADSKSCCDYSLHVDISEWHKGIQEEMEALVKDHGVNSFLVYMAFKDRFQLTDCQ
IYEVLSVIRDIGAIQVHAENGDIIEEQQRILDLGITGPEGHVLSRPEEVEAEAVNRAI
TIANQTNCPLYITKVMSSSAEVIAQARKKGTVVYGEPIASLGTDGSHYWSKNWAKAAA
FVTSPLSPDPTTPDFLNSLLSCGDLQVTGSAHCTFNATAQKAVGKDNFTLIPEGTNGTEE
RMSVIWDKAVVTGKMNDENQFVAVTSTNAAKVFNLPRKGRIAVGSDADLVIWDPDPSVKTI
SAKTHNSSLEYNIFEGMECRGSPLVVISQKIVLEDGTLHVTEGSGRYIPRKPFDFVYK
45 RIKARSRLAELRGVPRGLYDGPVCEVSVTPKTVTPASSAKTSPAKQAPPVRNLHQSGFS
LSGAQIDDNIPRRTTQRIVAPPGGRANITSLG

SEQ ID NO:67. Dihydropyrimidinase-related protein 2 DPYL2. UniProt ID Q16555-2. Isoform 2.

>sp|Q16555-2|DPYL2_HUMAN Isoform 2 of Dihydropyrimidinase-related protein 2 OS=Homo sapiens
OX=9606 GN=DPYSL2

MEDGLIKQIGENLIVPGGVKTIEAHSRMVIPGGIDVHTRFQMPDQGMTSADDDFFQGTKAA
 LAGGTTMIIDHVVPEPGTSLAAFDQWREWADSKSCCDYSLHVDISEWHKGIQEEMEALV
 KDHGVNSFLVYMAFKDRFQLTDCQIYEVLSVIRDIGAIQVHAENGDIIEEQQRILDLG
 ITGPEGHVLSRPEEVEAEAVNRAITIANQTNCLYITKVMSSSAEVIAQARKKGTVVYVY
 5 EPITASLGTDGSHYWSKNWAKAAAFVTSPPLSPDPTTPDFLNSLLSCGDLQVTGSAHCTF
 NTAQKAVGKDNFTLIPEGTNGTEERMSVIWDKAVVTGKMDENQFVAVTSTNAAKVFNLYP
 RKGRIAVGSDADLVIWDPDSVKTISAKTHNSSLEYNIFEGMECRGSPLVVISQGKIVLED
 GTLHVTEGSGRYIPRKPFDFVYKRIKARSRLAELRGVPRGLYDGPVCEVSVTPKTVTPA
 SSAKTSPAKQQAPPVRNLHQSGFSLSGAQIDDNIPRRTTQRIVAPPGGRANITSLG

SEQ ID NO:68. Neuropilin-1 NRP1. UniProt ID O14786. Isoform 1.

>sp|O14786|NRP1_HUMAN Neuropilin-1 OS=Homo sapiens OX=9606 GN=NRP1 PE=1 SV=3
 MERGLPLLCVLAALVLAAPAGAFRNDKCGDTIKIESPGYLTSPGYPHSYHPSEKCEWLIQA
 PDPYQRIMINFNPHFDLEDRCCKYDYVEVFDGENENGHFRGKFCGKIAPPPVVSSGPFLF
 15 IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTTSPGVKSPGFPEKYPNSLECTYIVFVP
 KMSEIILEFESFDLEPDSNPPGGMFRCYDRLEIWDGFPDVGPHIGRYCGQKTPGRIRSSS
 GILSMVFYTDIAIAKEGFSANYSVLQSSVSEDFKCMEALGMESGEIHSQDITASSQYSTN
 WSAERSRLNYPENGWTPGEDSYREWIQVDLGLLRFTAVGTQGAISKETKKKYVVKTYKI
 DVSSNGEDWITIKENKPVLFQGNTPDVTVAVFVKPLITRFVRIKPTWETGISMRFE
 20 VYGCKITDYPSCGMLGMVSGGLISDSQITSSNQGNRWNMPENIRLVTSRSGWALPPAPHSY
 INEWLQIDLGEKIVRGIIIQGGKHRENKVFMRKFKIGYSNNGSDWKMMDDSKRKAKSF
 EGNNNYDTPELRTFPALSTRFIRIYPERATHGGLGLRMELLGCEVEAPTAGPTTPNGNLV
 DECDDDQANCHSGTGDDFQLTGGTTVLATEKPTVIDSTIQSEFPTYGFNCEFGWGSHTKF
 CHWEHDNHVQLKWSVLTSKTGPIQDHTGDGNFIYSQADENQKKGKVARLVSPVVYSQNSAH
 25 CMTFWYHMSGSHVGTLRVKLRYQKPEEYDQLVWMAIGHQGDHWKEGRVLLHKSLLKLYQVI
 FEIGEIGKGNLGGIAVDDISNNHISQEDCAKPADLDDKNPEIKIDETGSTPGYEGEGEGD
 KNISRKPGNVLKTLDPILITHIAMSALGVLLGAVCGVVLYCACWHNGMSENNLSALENNYN
 FELVDGVKLLKDKLNTQSTYSEA

SEQ ID NO:69. Neuropilin-1 NRP1. UniProt ID O14786-2. Isoform 2.

>sp|O14786-2|NRP1_HUMAN Isoform 2 of Neuropilin-1 OS=Homo sapiens OX=9606 GN=NRP1
 MERGLPLLCVLAALVLAAPAGAFRNDKCGDTIKIESPGYLTSPGYPHSYHPSEKCEWLIQA
 PDPYQRIMINFNPHFDLEDRCCKYDYVEVFDGENENGHFRGKFCGKIAPPPVVSSGPFLF
 IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTTSPGVKSPGFPEKYPNSLECTYIVFVP
 35 KMSEIILEFESFDLEPDSNPPGGMFRCYDRLEIWDGFPDVGPHIGRYCGQKTPGRIRSSS
 GILSMVFYTDIAIAKEGFSANYSVLQSSVSEDFKCMEALGMESGEIHSQDITASSQYSTN
 WSAERSRLNYPENGWTPGEDSYREWIQVDLGLLRFTAVGTQGAISKETKKKYVVKTYKI
 DVSSNGEDWITIKENKPVLFQGNTPDVTVAVFVKPLITRFVRIKPTWETGISMRFE
 VYGCKITDYPSCGMLGMVSGGLISDSQITSSNQGNRWNMPENIRLVTSRSGWALPPAPHSY
 40 INEWLQIDLGEKIVRGIIIQGGKHRENKVFMRKFKIGYSNNGSDWKMMDDSKRKAKSF
 EGNNNYDTPELRTFPALSTRFIRIYPERATHGGLGLRMELLGCEVEAPTAGPTTPNGNLV
 DECDDDQANCHSGTGDDFQLTGGTTVLATEKPTVIDSTIQSGIK

SEQ ID NO:70. Neuropilin-1 NRP1. UniProt ID O14786-3. Isoform 3.

>sp|O14786-3|NRP1_HUMAN Isoform 3 of Neuropilin-1 OS=Homo sapiens OX=9606 GN=NRP1
 MERGLPLLCVLAALVLAAPAGAFRNDKCGDTIKIESPGYLTSPGYPHSYHPSEKCEWLIQA
 PDPYQRIMINFNPHFDLEDRCCKYDYVEVFDGENENGHFRGKFCGKIAPPPVVSSGPFLF
 IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTTSPGVKSPGFPEKYPNSLECTYIVFVP
 KMSEIILEFESFDLEPDSNPPGGMFRCYDRLEIWDGFPDVGPHIGRYCGQKTPGRIRSSS
 50 GILSMVFYTDIAIAKEGFSANYSVLQSSVSEDFKCMEALGMESGEIHSQDITASSQYSTN

WSAERSRLNYPENGWTPGEDSYREWIQVDLGLLRFTAVGTQGAISKETKKKYYVKTYKI
DVSSNGEDWITIKEGNKPVLFQGNTNPTDVVAVFPKPLITRFVRIKPATWETGISMRFE
VYGCKITDYPCSGMLGMVSGLISDSQITSSNQGDRNWMPENIRLVTSRSGWALPPAPHSY
5 INEWLQIDLGEEKIVRGIIIQGGKHRENKVFMRKFKIGYSNNGSDWKMIMDDSKRKAKSF
EGNNNYDTPELRTFPALSTRFIRIYPERATHGGLGLRMELLGCEVEGGTTVLATEKPTVI
DSTIQSGIK

WHAT IS CLAIMED IS:

1 1. A method for treating a tumor in a subject with cancer, the method
2 comprising administering an effective amount of ultra-high-dose-rate (FLASH) radiation and a
3 therapeutic agent to the tumor.

1 2. The method of claim 1, wherein the method reduces damage to normal
2 tissue when compared to administering conventional radiation at a dose of 0.5 Gy/sec to the
3 tumor.

1 3. The method of claim 1, wherein the radiation is administered at a dose rate
2 equal to or greater than 40 Gy/sec, or the dose is administered in 1 second or less, and the
3 radiation is administered in a single pulse or in multiple pulses.

1 4. The method of claim 1, wherein the radiation comprises or consists of
2 protons.

1 5. The method of claim 1, wherein the therapeutic agent is an immune
2 modulator, a senolytic agent, a radiosensitizer, or a nanoparticle.

1 6. The method of claim 1, wherein the therapeutic agent is a mitotic spindle
2 inhibitor, a DNA damage repair and response inhibitor, a MAPK pathway inhibitor, an epithelial
3 to mesenchymal (EMT) inhibitor, an activator of T helper type 1 (TH1) lymphocytes, an
4 activator of the PTEN pathway, and inhibitor of the TGF-beta pathway, an activator of the type-1
5 interferon signaling pathway, an activator of dendritic cell maturation, an inhibitor of
6 CD47/SIRP-alpha, or an inhibitor of the Aryl Hydrocarbon Receptor (ahR).

1 7. The method of claim 6, wherein the mitotic spindle inhibitor is selected
2 from a CDK4/6 inhibitor, an AURKA inhibitor, a TPX2-AURKA complex inhibitor, or a taxane.

1 8. The method of claim 6, wherein the DNA damage repair and response
2 inhibitor is selected from a PARP inhibitor, a RAD51 inhibitor, or an inhibitor of a DNA damage
3 response kinase selected from CHCK1, ATM, or ATR.

1 9. The method of claim 6, wherein the MAPK pathway inhibitor is an
2 inhibitor of EGFR, MEK, BRAF, or ERK.

1 10. The method of claim 6, wherein the EMT inhibitor is a TGF β -pathway
2 inhibitor selected from a compound, small molecule, antibodies or fragments thereof that bind
3 TGF-beta.

1 11. The method of claim 6, wherein the activator of T helper type 1 (TH1)
2 lymphocytes is a cytokine, a toll-like receptor agonist, a STAT3 modulator, compounds derived
3 from inactivated bacteria or parasites or their derivatives that trigger interferon gamma or IL-12
4 production, staphylococcus enterotoxin B, unmethylated CpG nucleotides, or bacterial or virus
5 based gene expression systems that lead to production of IL2, IL-12 and IFN-gamma when
6 injected at the tumor site.

1 12. The method of claim 6, wherein the activator of the PTEN pathway is
2 selected from an mTOR inhibitor selected from rapamycin, temsirolimus, everolimus, sirolimus
3 or AP-2357; Ublituximab, Rituximab, Sunitinib, Trastuzumab, Pertuzumab, Resistin,
4 Simvastatin, Lovastatin, Rosiglitazone, NVP-AEW541, an Src inhibitor, or PP1 Herbimycin.

1 13. The method of claim 6, wherein the activator of the type-1 interferon
2 signaling pathway is a STING agonist, an Toll-like receptor (TLR) agonist, or a MAVS agonist.

1 14. The method of claim 6, wherein the activator of dendritic cell maturation
2 is a synthetic peptide vaccine, and the inhibitor of CD47/SIRP-alpha is selected from an antibody
3 or fragment thereof, or a small molecule compound that inhibits the CD47/DSIRP-alpha
4 interaction.

1 15. The method of claim 6, wherein the nanoparticle has a high effective
2 atomic number, or comprises gold or gadolinium.

1 16. The method of claim 6, wherein the ahR inhibitor is SR1, CH-223191,
2 UM729, or Galangin.

1 17. The method of claim 2, wherein dermatitis, lung fibrosis or lymphocyte
2 apoptosis are decreased compared to administering conventional radiation.

1 18. A therapeutic agent for use in a method of treating a tumor in a subject
2 with cancer, characterized in that the method comprises administering FLASH radiation and the
3 therapeutic agent to the tumor.

1 19. A therapeutic agent for use in a method of treating a tumor in a subject
2 with cancer, characterized in that the therapeutic agent is administered in combination with
3 FLASH radiation.

1 20. A therapeutic agent for use in a method of treating a tumor in a subject
2 with cancer according to claim 19, wherein the therapeutic agent is administered to a heightened
3 immune competent tumor environment caused by said FLASH radiation.

1 21. A hedgehog antagonist for use in a method of treating a tumor in a subject
2 with cancer, characterized in that the hedgehog antagonist is administered in combination with
3 FLASH radiation.

1 22. A non-transitory computer-readable storage medium having computer-
2 executable instructions for causing a computing system to perform a method of ultra-high-dose-
3 rate (FLASH) radiation treatment planning for treating a tumor in combination with a therapeutic
4 agent; wherein said method comprises adjusting beam parameters using information about
5 treatment of the tumor with a therapeutic agent.

1 23. The non-transitory computer-readable storage medium of claim 22, the
2 method comprising:
3 accessing values of parameters from memory of the computing system, wherein
4 the parameters comprise directions of beams to be directed into sub-volumes in a target and
5 beam energies for the beams;
6 accessing information that specifies limits for the radiation treatment plan,
7 wherein the limits are based on a dose threshold and comprise a limit on irradiation time for each
8 sub-volume outside the target;

9 wherein the information that specifies limits comprises information about
10 treatment of the tumor with a therapeutic agent; and

11 adjusting the values of the parameters that affect a calculated amount of dose to
12 be delivered by the beams until differences between respective total values for the sub-volumes
13 satisfy a threshold value.

1 24. The non-transitory computer-readable storage medium of claim 23,
2 wherein each portion of the beams that is in the target is represented as a respective set of
3 longitudinal beam regions, and wherein the method further comprises:

4 for each of the beam regions, computing an amount of dose to be delivered by a
5 beam region and assigning a value to the beam region corresponding to the amount; and

6 for each of the sub-volumes, computing a total value for the sub-volume by
7 adding together the value for each beam region of each beam that reaches the sub-volume;

8 wherein said adjusting further comprises adjusting the parameters that affect the
9 calculated amounts of dose to be delivered by the beam regions until differences between
10 respective total values for the sub-volumes satisfy the threshold value.

1 25. The non-transitory computer-readable medium of claim 24, wherein said
2 adjusting further comprises:

3 determining whether a beam overlaps any other beams outside the target; and

4 weighting beam intensities for beam segments of the beam according to how
5 many other beams are overlapped by the beam outside the target.

1 26. The non-transitory computer-readable medium of claim 25 wherein the
2 method further comprises performing a dose calculation for an outside-the-target sub-volume,
3 wherein said performing a dose calculation comprises:

4 accessing a value for a dose calculation factor for the outside-the-target sub-
5 volume, wherein the value for the dose calculation factor is determined according to how many
6 beams reach the outside-the-target sub-volume;

7 calculating a dose for the outside-the-target sub-volume; and

8 applying the value of the dose calculation factor to the dose calculated for the
9 outside-the-target sub-volume.

1 27. The non-transitory computer-readable medium of claim 26 wherein the
2 dose calculation factor reduces the dose calculated for the outside-the-target sub-volume if only
3 one beam reaches the outside-target sub-volume.

1 28. The non-transitory computer-readable medium of claim 27 wherein the
2 limits are selected from the group consisting of: a limit on irradiation time for each sub-volume
3 in the target; a limit on dose rate for each sub-volume in the target; and a limit on dose rate for
4 each sub-volume outside the target.

1 29. The non-transitory computer-readable medium of claim 28, wherein the
2 dose threshold is further dependent on tissue type.

1 30. A computer-implemented method of radiation treatment planning for
2 treating a tumor in combination with an immune modulator, the method comprising determining
3 beam parameters for ultra-high-dose-rate (FLASH) radiation, the method using a prescribed dose
4 based on a response of the tumor to a therapeutic agent.

1 31. The computer-implemented method of claim 30, the method comprising:
2 determining a prescribed dose of FLASH radiation to be delivered into and across
3 a tumor target, wherein the prescribed dose is determined based on a response of the tumor to a
4 therapeutic agent;

5 accessing values of parameters comprising a number of beams in a plurality of
6 beams to be directed into sub-volumes in the target, directions of the plurality of beams, and
7 beam energies for the plurality of beams, wherein each of the beams comprises a plurality of
8 beam segments;

9 identifying any overlapping beams in the plurality of beams that have respective
10 beam paths that overlap outside the target;

11 for each beam in the plurality of beams, determining a maximum beam energy for
12 the beam and determining beam energies for the beam segments of the beam as a percentage of
13 the maximum beam energy for the beam; and

14 for each overlapping beam of the overlapping beams that overlap outside the
15 target, reducing the beam intensities for the beam segments of the overlapping beam by a dose
16 calculation factor, wherein the beam intensities for the beam segments for the plurality of beams
17 are determined such that a cumulative dose delivered to the target satisfies the prescribed dose.

1 32. The computer-implemented method of claim 31, wherein the method
2 further comprises:

3 representing each of the beams in the target as a respective set of longitudinal
4 beam regions, wherein each beam region in the set has a value corresponding to a calculated
5 amount of dose to be delivered by the beam region;

6 for each sub-volume in the target, adding together the value for each beam region
7 of each beam that reaches the sub-volume to determine a total value for the sub-volume, to
8 produce respective total values for the sub-volumes in the target; and

9 adjusting the values of the parameters that affect the calculated amounts of dose
10 to be delivered by the beam regions until differences between the total values for the sub-
11 volumes satisfy a threshold value.

1 33. The computer-implemented method of claim 32 wherein the method
2 further comprises:

3 accessing a value for the dose calculation factor for an outside-the-target sub-
4 volume, wherein the value for the dose calculation factor is determined according to how many
5 beams reach the outside-the-target sub-volume;

6 calculating a dose for the outside-the-target sub-volume; and

7 applying the value of the dose calculation factor to the dose calculated for the
8 outside-the-target sub-volume, wherein the dose calculation factor reduces the dose calculated
9 for the outside-the-target sub-volume if only one beam reaches the outside-target sub-volume.

1 34. The computer-implemented method of claim 33 wherein the method
2 comprises using a dose threshold to specify limits for the radiation treatment plan, wherein the
3 limits are selected from the group consisting of: a limit on irradiation time for each sub-volume
4 in the target; a limit on irradiation time for each sub-volume outside the target; a limit on dose

rate for each sub-volume in the target; and a limit on dose rate for each sub-volume outside the target.

35. An in-vitro method of identifying a subject with cancer as a candidate for treatment comprising ionizing FLASH radiation and an immune modulator, the method including:

(a) determining an expression level of one or more biomarkers in a tumor sample from the subject;

(b) comparing the expression level of the one or more biomarkers to an expression level of the one or more biomarkers in a normal tissue sample; and

(c) classifying the subject as a candidate for treatment comprising ionizing FLASH radiation and the immune modulator if the expression level of the one or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample.

36. An in-vitro method of selecting a treatment for a subject with cancer, the method comprising:

(a) determining an expression level of one or more biomarkers in a tumor sample from the subject,

(b) comparing the expression level of the one or more biomarkers to an expression level of the one or more biomarkers in a normal tissue sample; and

(c) selecting a treatment comprising ionizing FLASH radiation and an immune modulator if the expression level of the one or more biomarkers in the tumor sample is modified compared to the expression level in the normal tissue sample.

37. The in-vitro method of claim 36 wherein:

treatment comprising ionizing FLASH radiation and an immune modulator is selected if the expression level of CD44 is increased and/or the expression level of MFG-E8 is decreased relative to the expression level in a normal or control sample;

the amount of ionizing FLASH radiation and/or the amount of an immune modulator selected is increased if the expression level of CD44 is increased and/or the expression level of MFG-E8 is decreased relative to the expression level in a normal or control sample; and

9 the amount of ionizing FLASH radiation and/or the amount of an immune
10 modulator selected is decreased if the expression level of CD44 is decreased and/or the
11 expression level of MFG-E8 is increased relative to the expression level in a normal or control
12 tissue sample.

1 38. The in-vitro method of claim 37 wherein:
2 treatment comprising ionizing FLASH radiation and an immune modulator is
3 selected if the expression level of CD68 is increased relative to the expression level in a normal
4 or control tissue sample;

5 the amount of ionizing FLASH radiation and/or the amount of an immune
6 modulator selected is increased if the expression level of CD68 is increased relative to the
7 expression level in a normal or control tissue sample;

8 the amount of ionizing FLASH radiation and/or the amount of an immune
9 modulator selected can be decreased if the expression level of CD68 is decreased relative to the
10 expression level in a normal or control tissue sample.

1 39. The in-vitro method of claim 38 wherein:
2 the treatment is selected using a computer implemented algorithm that analyses
3 the expression level of the biomarkers in the tumor sample relative to the level in the normal
4 sample;

5 wherein the algorithm comprises one or more of:

6 a linear regression algorithm that includes the biomarker expression levels
7 and coefficients for combining the expression levels;

8 a least squares fit to calculate the coefficients; and

9 a nonparametric regression tree;

10 wherein treatment comprising FLASH radiation and an immune modulator is
11 selected in dependence on the algorithm determining that the expression level of the biomarkers
12 in the tumor sample is increased or decreased relative to the normal sample.

1 40. The in-vitro methods of claims 35 to 39 wherein:
2 the biomarker is one or more of CD44, MMP9, ALDH1A1, Vimentin,
3 hyaluronan, beta-catenin, MFG-E8, CD68, TGF β , a TGF β -pathway related biomarker; and/or

the expression levels of one or more biomarkers is ranked or weighted.

41. The use claims 18-21, the medium claims 22-29 and the method claims 30-40, wherein the radiation is administered at a dose rate equal to or greater than 40 Gy/sec, or the dose is administered in 1 second or less, and the radiation is administered in a single pulse or in multiple pulses.

42. The use claims 18-21, the medium claims 22-29 and the method claims 30-40, wherein the radiation comprises or consists of protons.

43. The use claims 18-21, the medium claims 22-29 and the method claims 30-40, wherein the therapeutic agent is an immune modulator, a senolytic agent, a radiosensitizer, or a nanoparticle.

44. The use claims 18-21, the medium claims 22-29 and the method claims 30-40, wherein the therapeutic agent is a mitotic spindle inhibitor, a DNA damage repair and response inhibitor, a MAPK pathway inhibitor, an epithelial to mesenchymal (EMT) inhibitor, an activator of T helper type 1 (TH1) lymphocytes, an activator of the PTEN pathway, and inhibitor of the TGF-beta pathway, an activator of the type-1 interferon signaling pathway, an activator of dendritic cell maturation, an inhibitor of CD47/SIRP-alpha, or an inhibitor of the Aryl Hydrocarbon Receptor (ahR).

45. The use, medium and methods of claim 44, wherein the mitotic spindle inhibitor is selected from a CDK4/6 inhibitor, an AURKA inhibitor, a TPX2-AURKA complex inhibitor, or a taxane.

46. The use, medium and methods of claim 44, wherein the DNA damage repair and response inhibitor is selected from a PARP inhibitor, a RAD51 inhibitor, or an inhibitor of a DNA damage response kinase selected from CHCK1, ATM, or ATR.

47. The use, medium and methods of claim 44, wherein the MAPK pathway inhibitor is an inhibitor of EGFR, MEK, BRAF, or ERK.

1 48. The use, medium and methods of claim 44, wherein the EMT inhibitor is a
2 TGFβ-pathway inhibitor selected from a compound, small molecule, antibodies or fragments
3 thereof that bind TGF-beta.

1 49. The use, medium and methods of claim 44, wherein the activator of T
2 helper type 1 (TH1) lymphocytes is a cytokine, a toll-like receptor agonist, a STAT3 modulator,
3 compounds derived from inactivated bacteria or parasites or their derivatives that trigger interferon
4 gamma or IL-12 production, staphylococcus enterotoxin B, unmethylated CpG nucleotides, or
5 bacterial or virus based gene expression systems that lead to production of IL2, IL-12 and IFN-
6 gamma when injected at the tumor site.

1 50. The use, medium and methods of claim 44, wherein the activator of the
2 PTEN pathway is selected from an mTOR inhibitor selected from rapamycin, temsirolimus,
3 everolimus, sirolimus or AP-2357; Ublituximab, Rituximab, Sunitinib, Trastuzumab,
4 Pertuzumab, Resistin, Simvastatin, Lovastatin, Rosiglitazone, NVP-AEW541, an Src inhibitor,
5 or PP1 Herbimycin.

1 51. The use, medium and methods of claim 44, wherein the activator of the
2 type-1 interferon signaling pathway is a STING agonist, an Toll-like receptor (TLR) agonist, or a
3 MAVS agonist.

1 52. The use, medium and methods of claim 44, wherein the activator of
2 dendritic cell maturation is a synthetic peptide vaccine, and the inhibitor of CD47/SIRP-alpha is
3 selected from an antibody or fragment thereof, or a small molecule compound that inhibits the
4 CD47/DSIRP-alpha interaction.

1 53. The use, medium and methods of claim 44, wherein the nanoparticle has a
2 high effective atomic number, or comprises gold or gadolinium.

1 54. The use, medium and methods of claim 44, wherein the ahR inhibitor is
2 SR1, CH-223191, UM729, or Galangin.

1 55. A method for reducing activation of the of the hedgehog signaling
2 pathway in a subject, comprising administering an effective amount of ultra-high-dose-rate

(FLASH) radiation to the subject, where activation of the hedgehog signaling pathway is reduced compared to administering conventional proton radiation to the subject.

56. The method of claim 55, wherein the expression of genes activated by the hedgehog signaling pathway is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy.

57. The method of claim 56, wherein the expression of one or more genes selected from CELSR1, TLE3, OPHN1, GPR56, PTCH1, TLE1, MYH9, RASA1, HEY1, ETS2, HEY2, LDB1, UNC5C, NF1, CDK6, VLDLR, NRP2, DPYSL2, and NRP1 is decreased when FLASH radiation is administered to the subject compared to administering conventional proton radiation therapy.

58. The method of claim 55, further comprising administering a hedgehog antagonist to the subject.

59. The method of claim 58, where the hedgehog antagonist is selected from a Smo antagonist, a PTCH1 inhibitor, Cyclopamine, Vismodegib, LDE 225, saridegib, BMS 833923, LEQ 506, PF- 04449913 , PF-5274857, GANT61, SANT-1, Glasdegib (PF-04449913), Taladegib (LY2940680) or TAK-441.

60. The method of claims 55-59, where the FLASH radiation comprises or consists of protons.

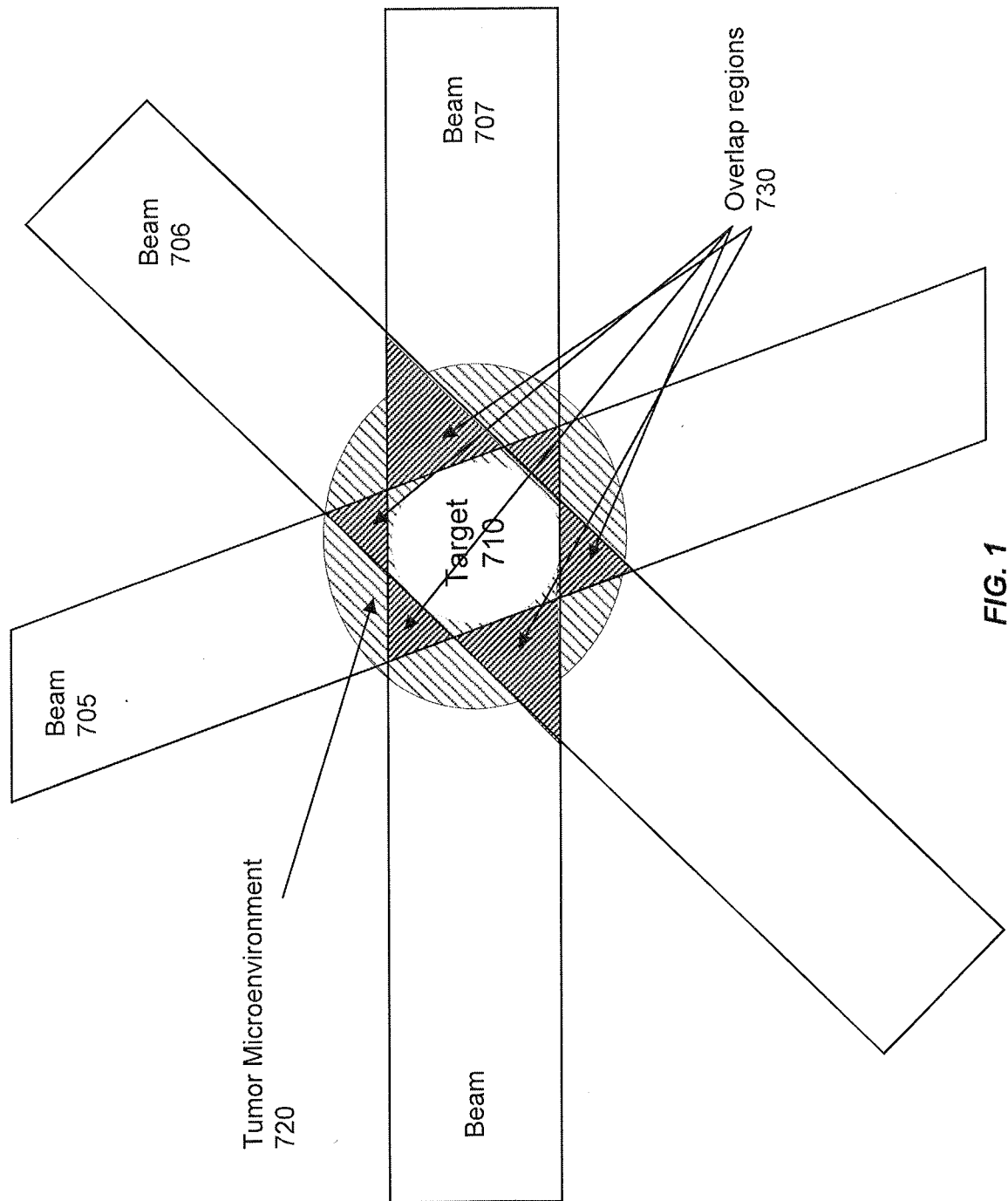


FIG. 1

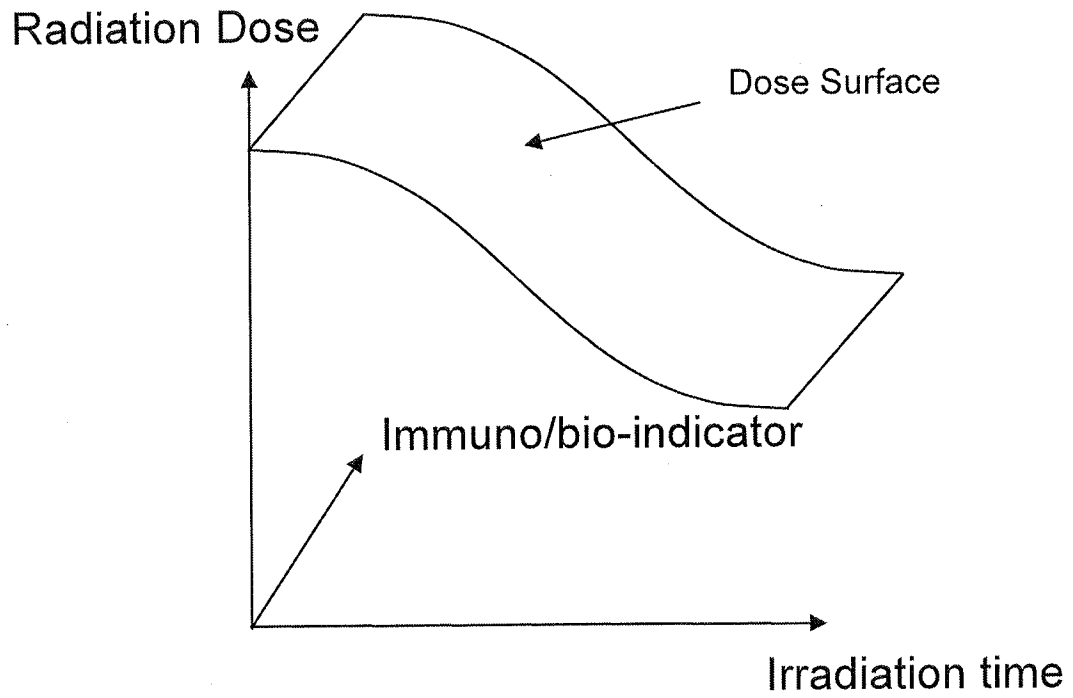
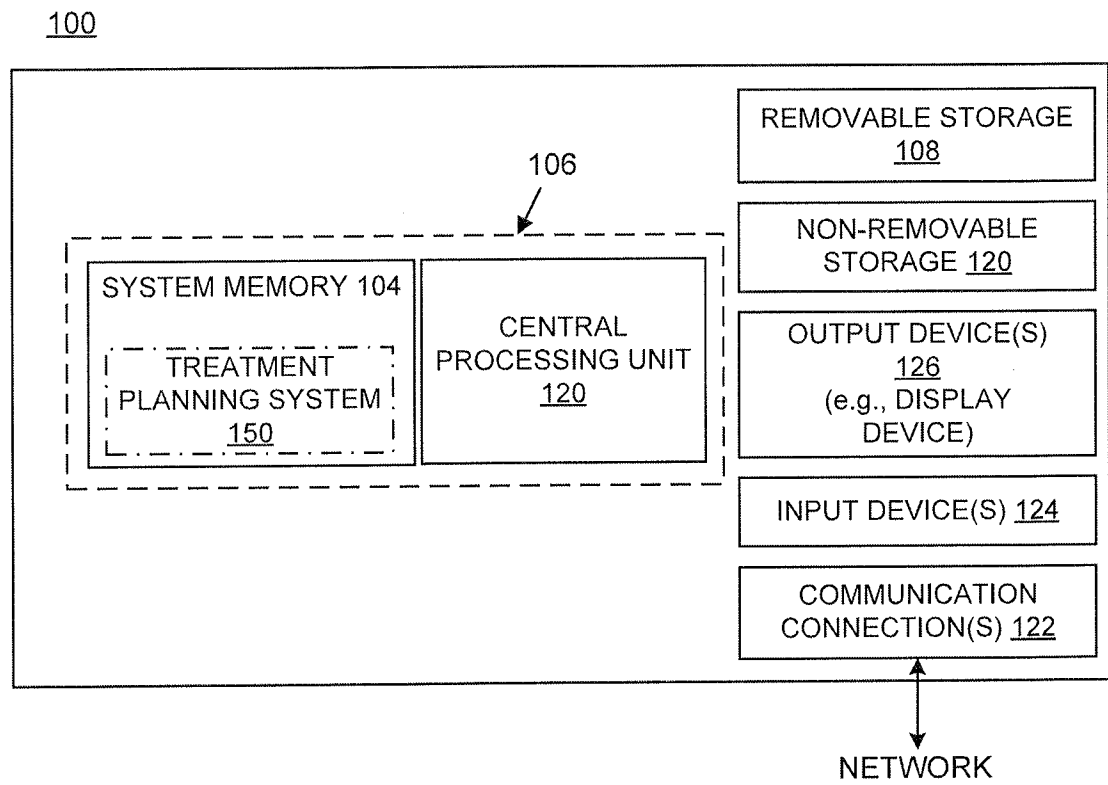
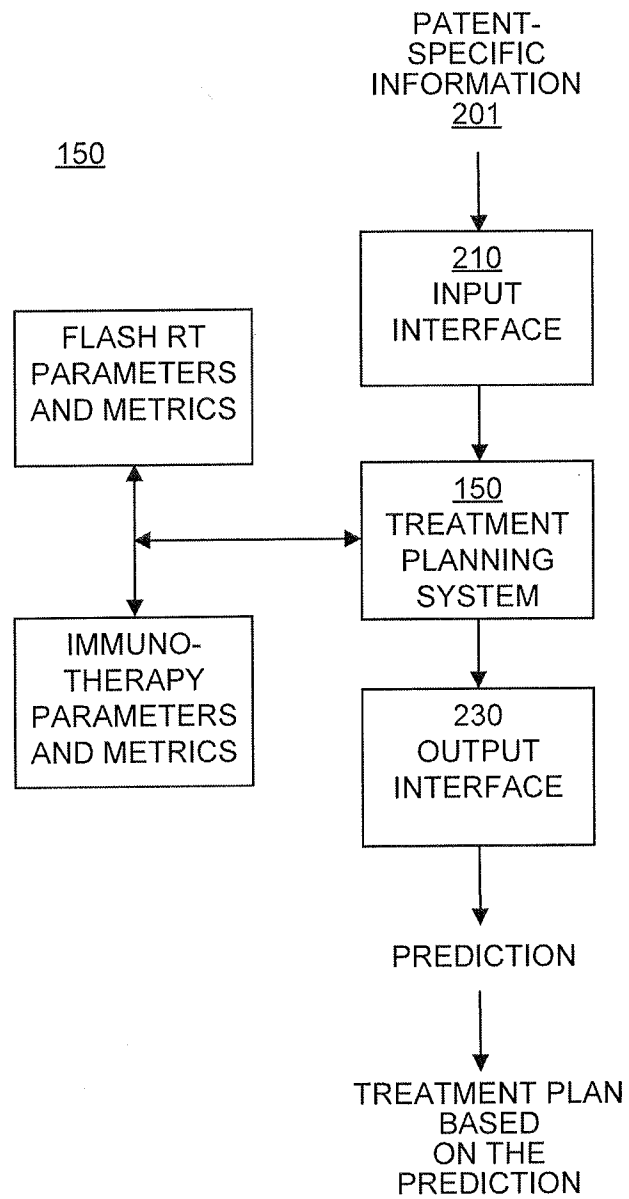


FIG. 2

**FIG. 3**

**FIG. 4**

Normal Tissue Toxicity Studies

TIME POINT	DOSE (Gy)	CONV	FLASH	SP FLASH	SHAM	TOTAL	392 age and sex matched mice
1 HOUR	0 15	10	10	10	6	6 30 36	
24 HOUR	0 15	10	10	10	6	6 30 36	6 Cohorts: Sacrifice 1hr-36 wks after lung irradiation 4 Groups in each cohort • Shame: no radiation • Conventional: 1Gy/s • FLASH: 40Gy/s beam on • Split-FLASH: 4Gy/0.1 sec, pulsed delivery
8 WEEK	0 15	10	10	10	6	6 30 36	
16 WEEK	0 15	20	20	20	6	6 60 66	
24 WEEK	0 15	20	20	20	6	6 60 66	
36 WEEK	0 15 17.5 20	20 20 20	20 20 20	20	12	12 60 40 40 152	
		130	130	90	42	392	GRAND TOTAL

FIG. 5

Lung Function

Penh (Typical Values):

Baseline:	100%
Mildly Challenged:	250%
Moderately Challenged:	400%
Severely Challenged:	1000%

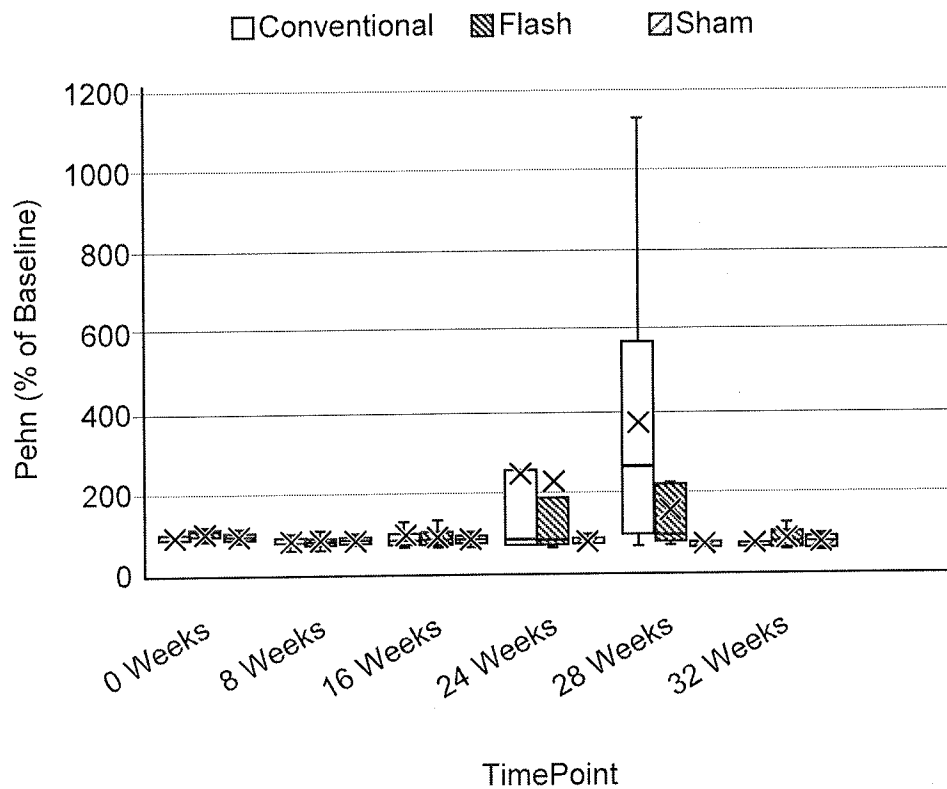


FIG. 6

Survival Study

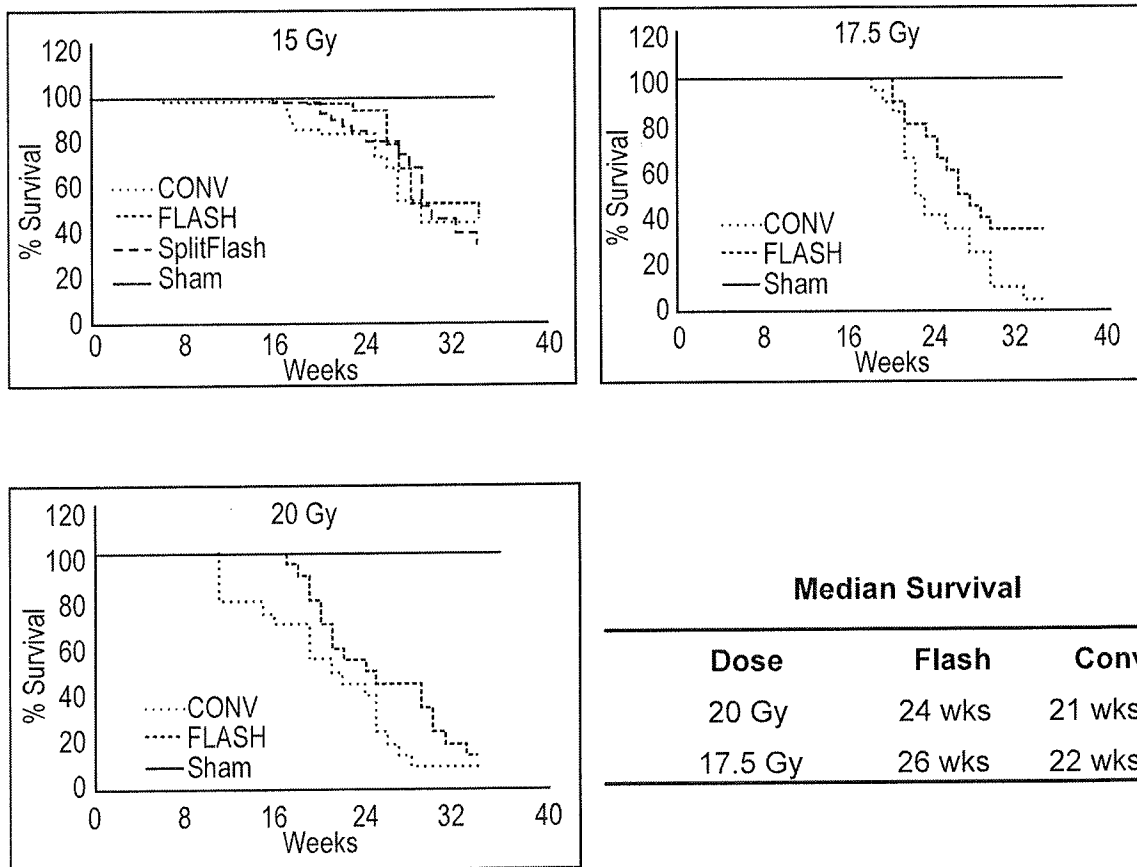
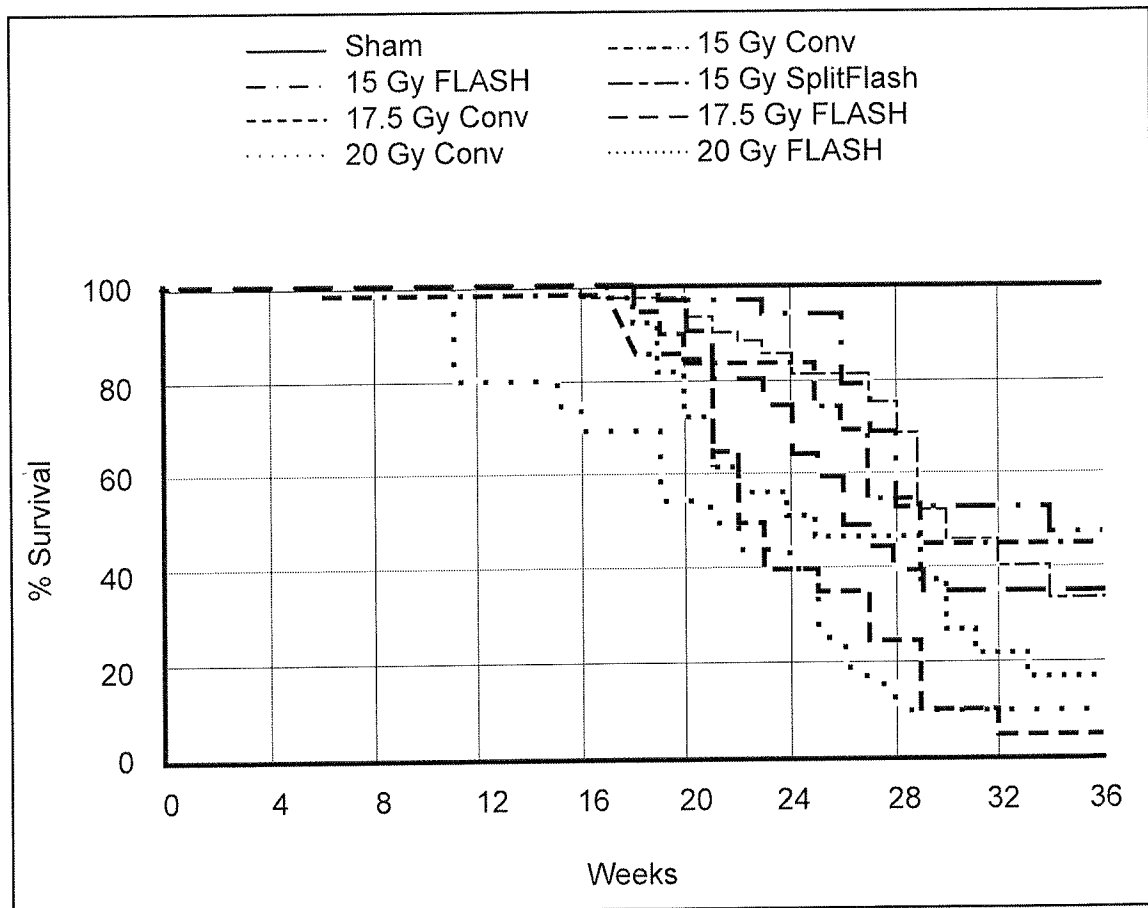
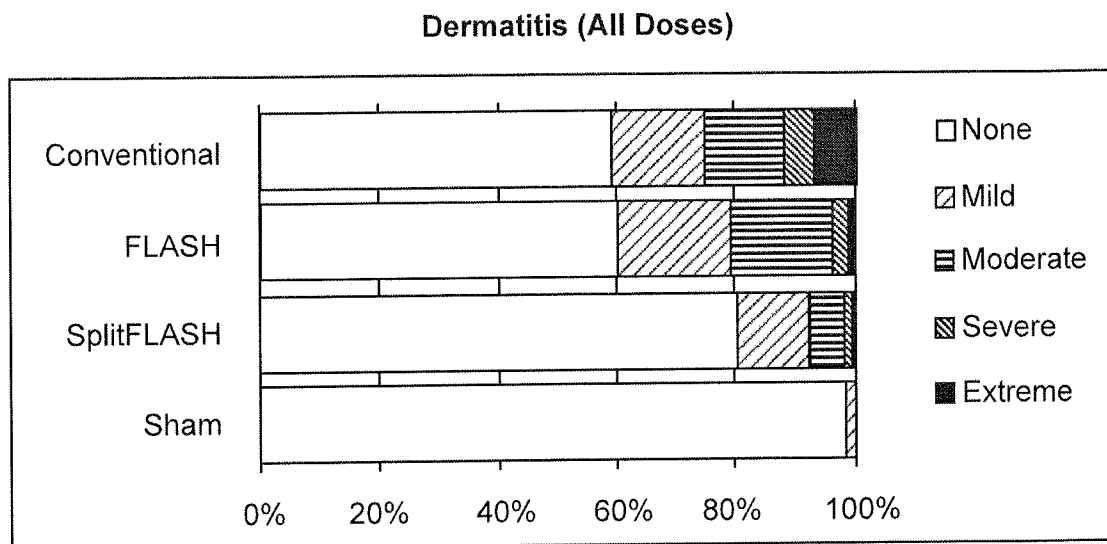


FIG. 7

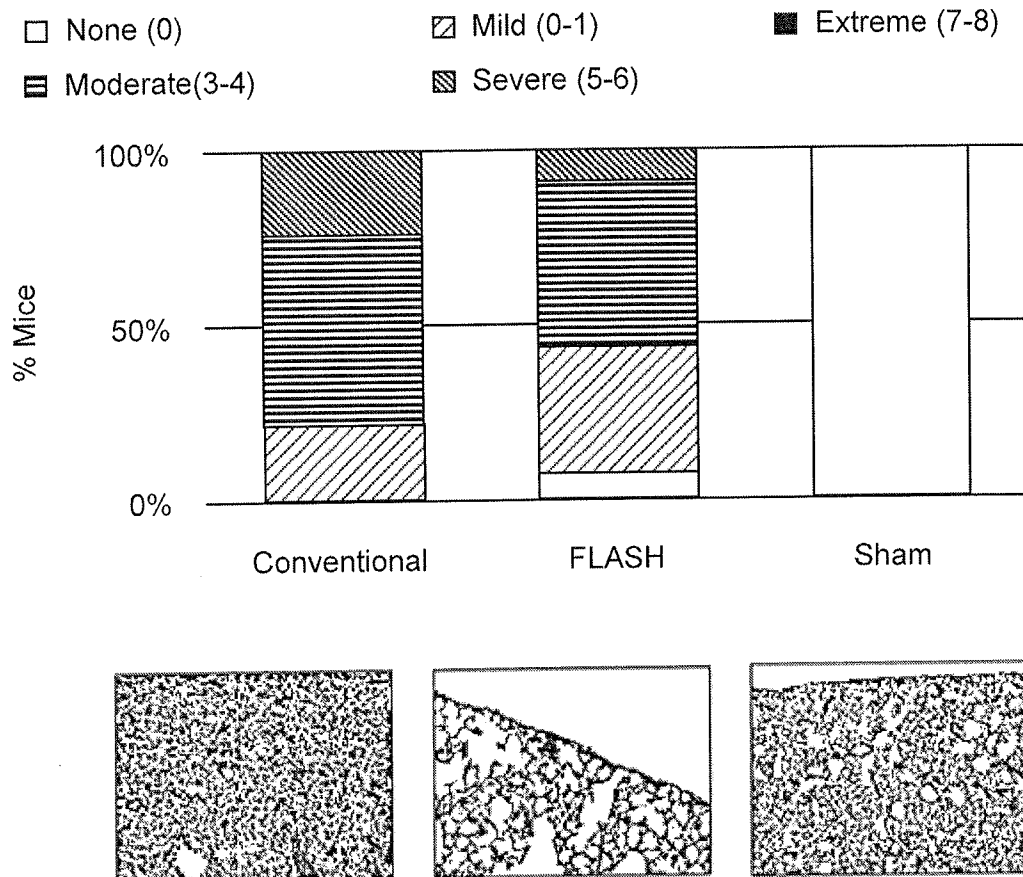
Survival Study**FIG. 8**

Dermatitis



ActualTreatment	Severe	Extreme
SplitFLASH	1	1
FLASH	4	2
Conventional	7	10

FIG. 9

Lung Fibrosis, 36 wks cohort**FIG. 10**

Lung Weight

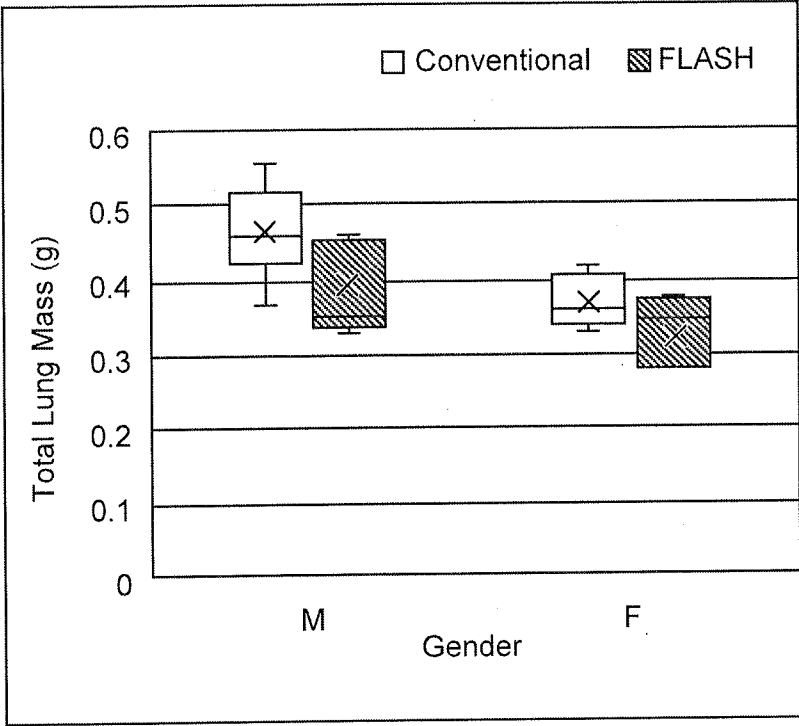


FIG. 11

FIG 12A

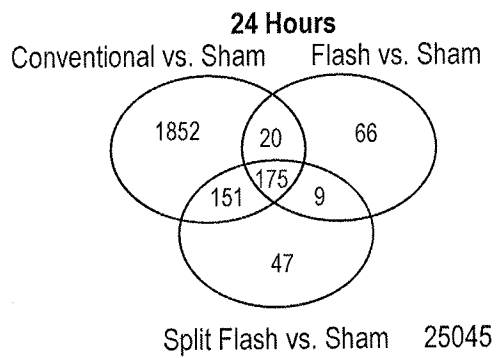


FIG 12B

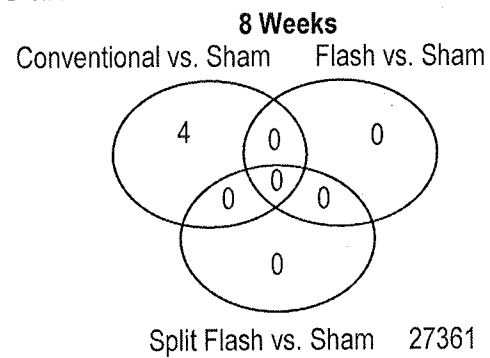


FIG 12C

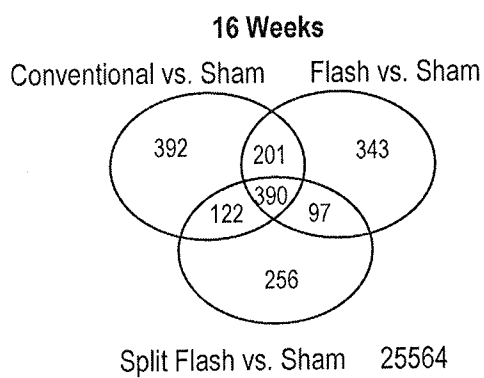


FIG 12D

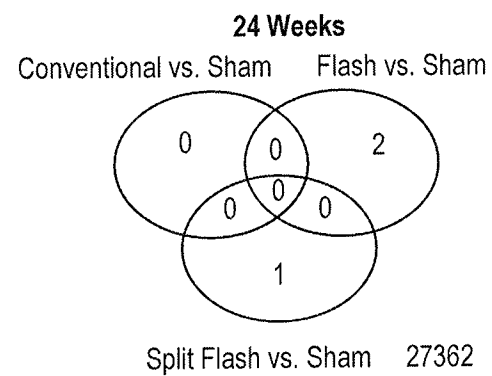


FIG. 12

FIG 13A

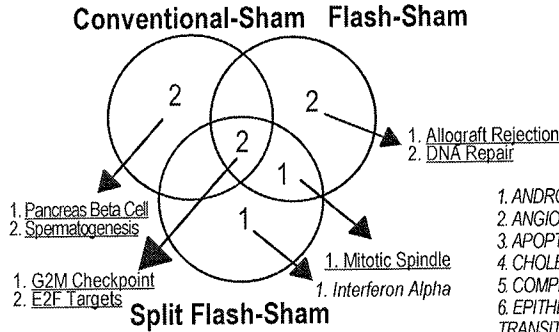


FIG 13B

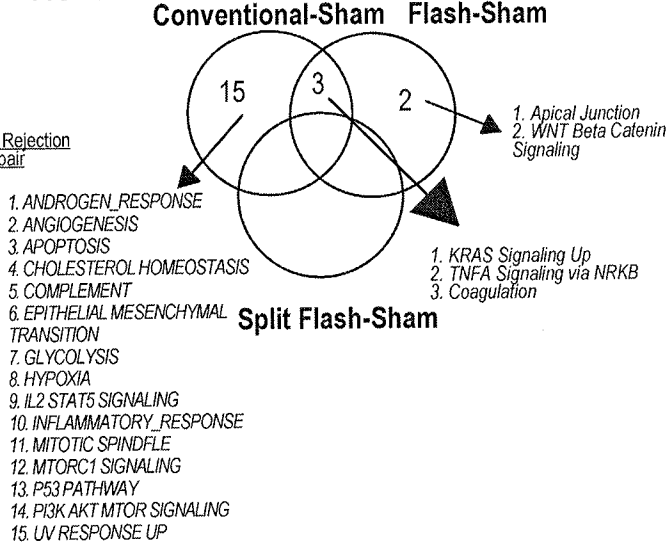


FIG 13C

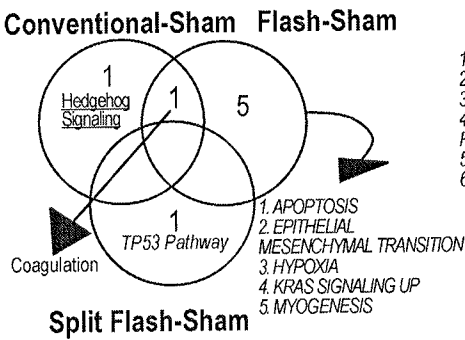


FIG 13D

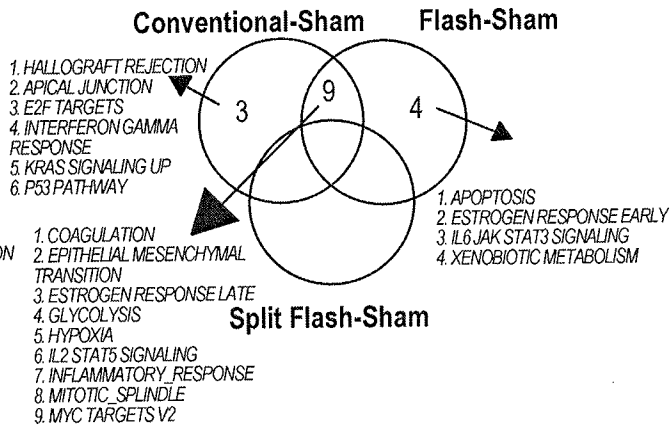


FIG. 13

FIG 14A

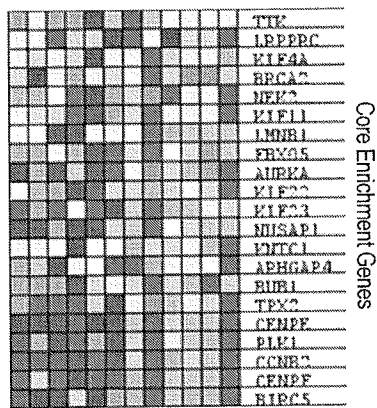
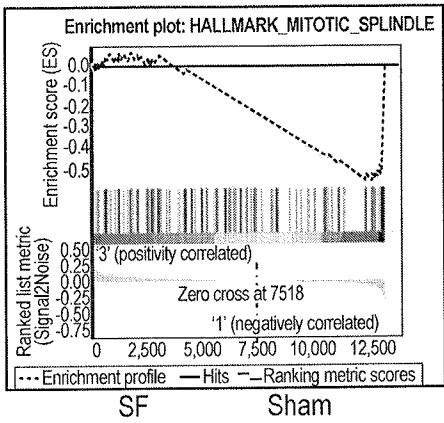


FIG 14B

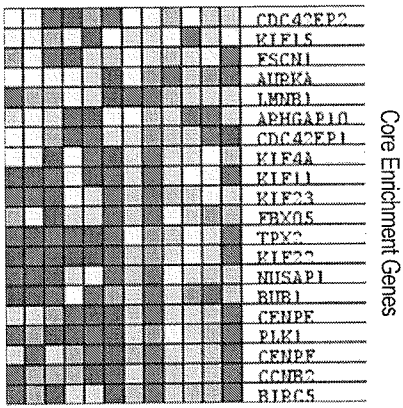
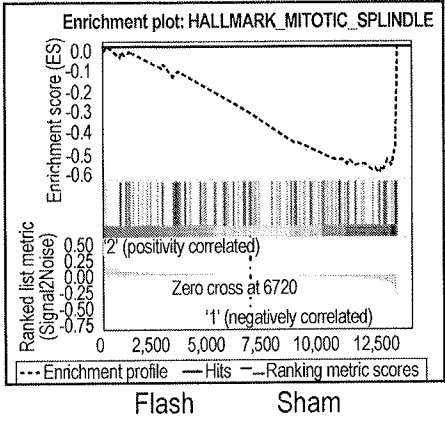


FIG 14C

Flash-Core-Genes Split Flash-Core-Genes

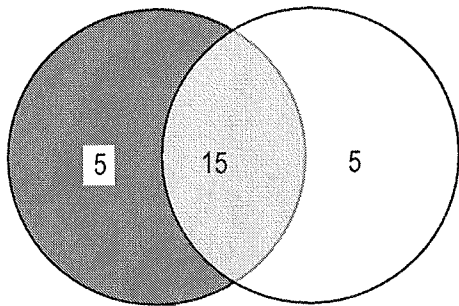


FIG 14D

Flash ^a	Overlap ^a	Split-Flash ^a	^a
ARHGAP10 ^a	AURKA ^a	ARHGAP4 ^a	^a
CDC42EP1 ^a	BIRC5 ^a	BRCA2 ^a	^a
CDC42EP2 ^a	BUB1 ^a	KNTC1 ^a	^a
FSCN1 ^a	CCNB2 ^a	LPPPRC ^a	^a
KIF15 ^a	CENPE ^a	NEK2 ^a	^a
^a	CENPF ^a	^a	^a
^a	FBXO5 ^a	^a	^a
^a	KIF11 ^a	^a	^a
^a	KIF22 ^a	^a	^a
^a	KIF23 ^a	^a	^a
^a	KIF4A ^a	^a	^a
^a	LMNB1 ^a	^a	^a
^a	NUSAP1 ^a	^a	^a
^a	PLK1 ^a	^a	^a
^a	TPX2 ^a	^a	^a

FIG. 14

FIG 15A

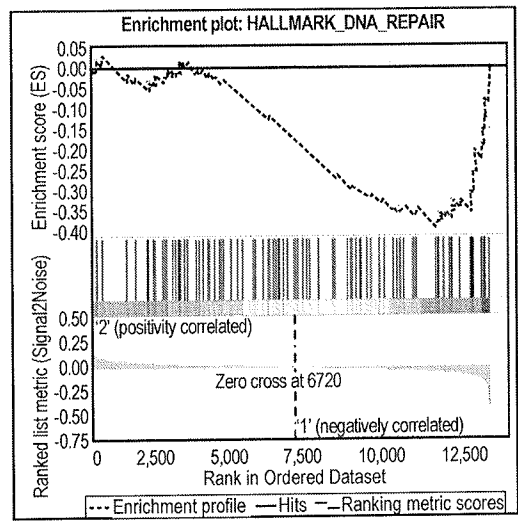
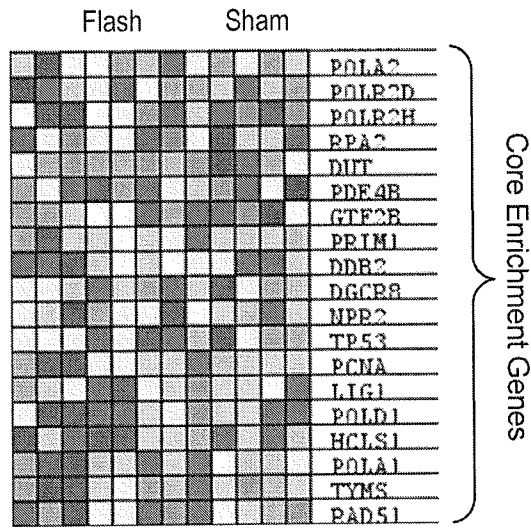


FIG 15B

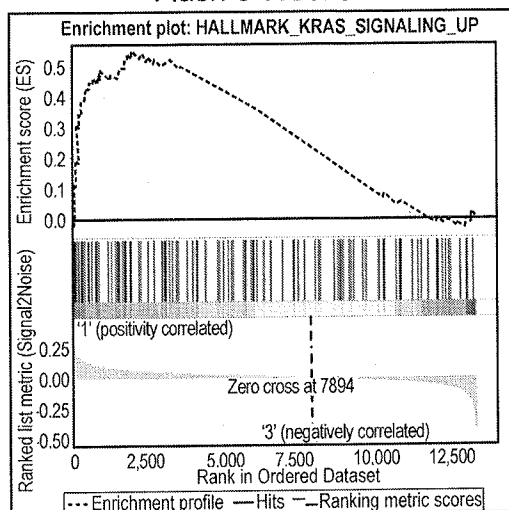


GeneSet	DNA Repair
Norm. Enrichment Score	-1.50316
Nominal p-value	0.041016
FDR q-value	0.208294

FIG. 15

FIG 16A

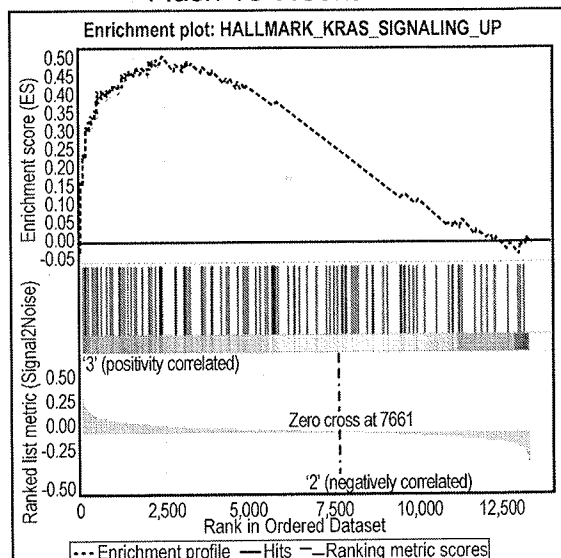
Flash 8 Weeks



NES	1.351275
Nominal p-value	0.043561
FDR q-value	0.228315

FIG 16B

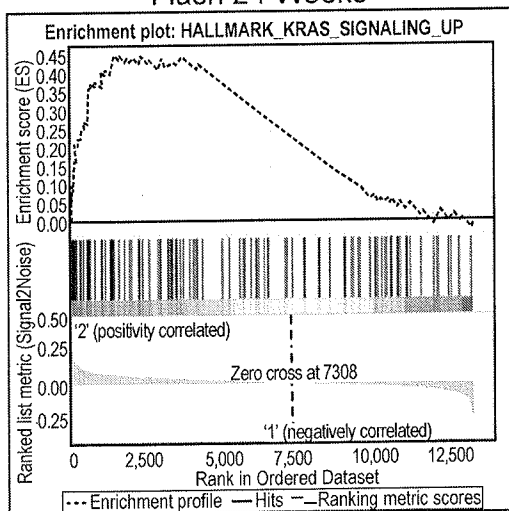
Flash 16 Weeks



NES	1.438819
Nominal p-value	0.0233463
FDR q-value	0.22549081

FIG 16C

Flash 24 Weeks

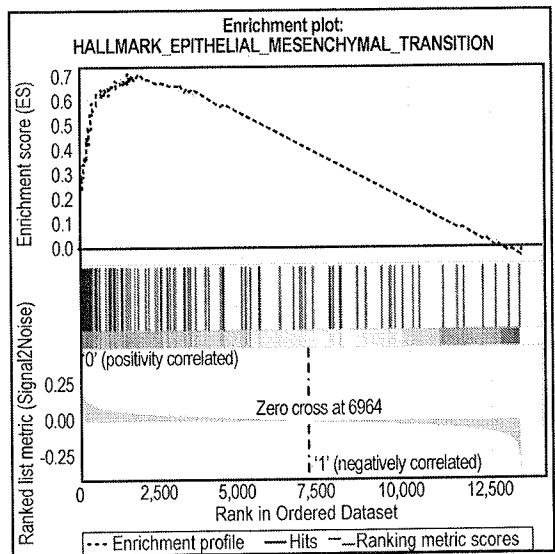


NES	1.438819
Nominal p-value	0.0233463
FDR q-value	0.22549081

FIG. 16

FIG 17A

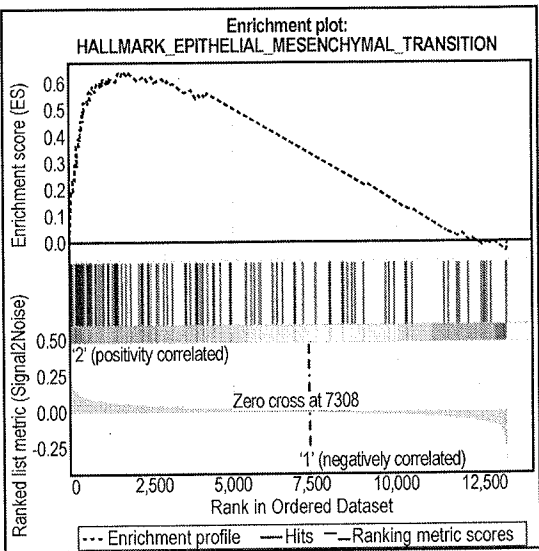
Conventional 24 Weeks



GeneSet	EMT
NES	1.715518
Nominal p-value	0.001
FDR q-value	0.066558

FIG 17B

Flash 24 Weeks



GeneSet	EMT
NES	1.743082
Nominal p-value	0.002066
FDR q-value	0.017006

FIG 17C

Conv EMT Core Flash EMT Core

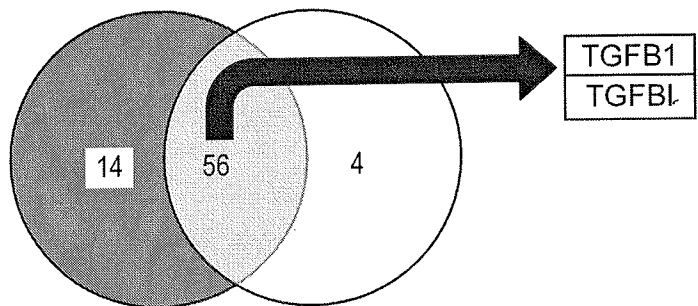
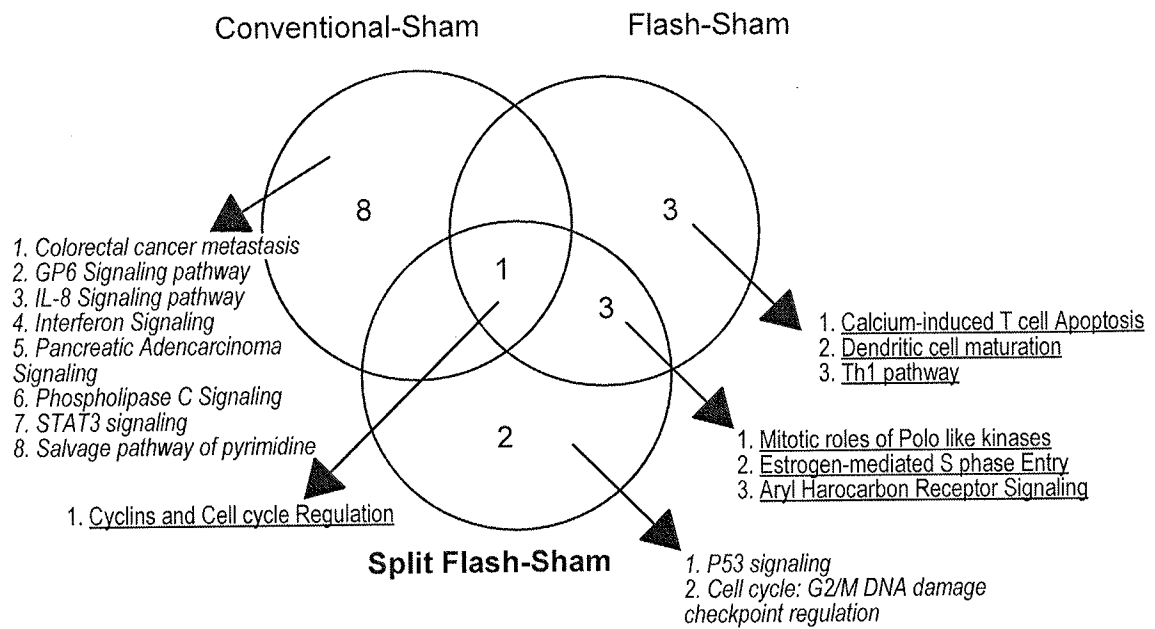


FIG. 17

FIG 18A 24 Hr IPA data**FIG. 18**

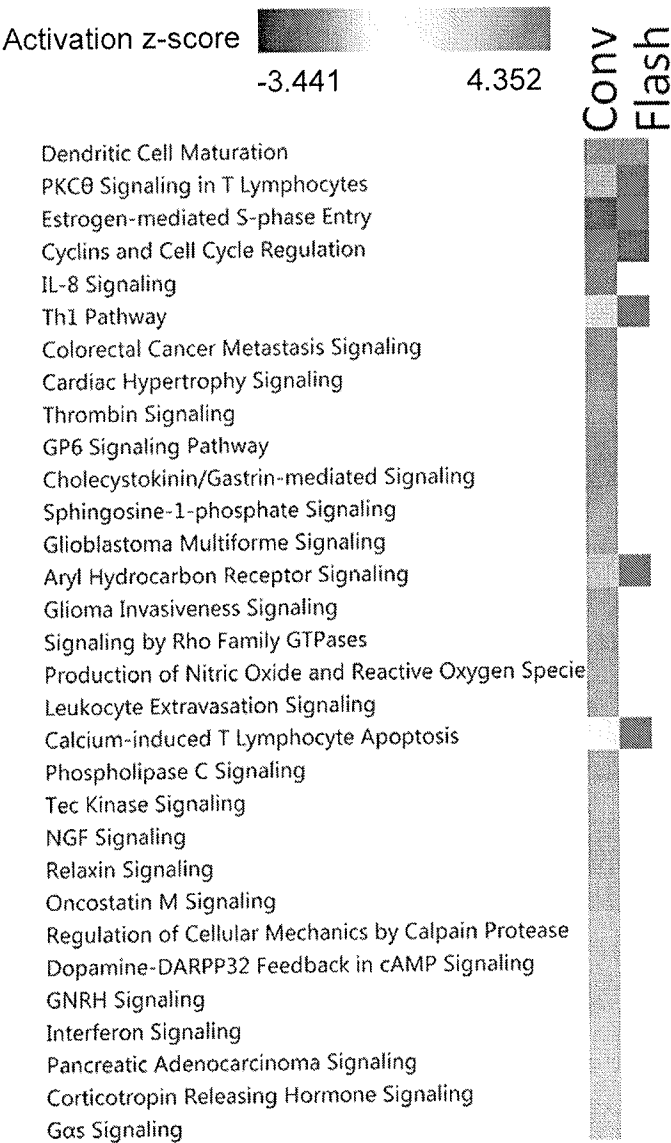


FIG. 19

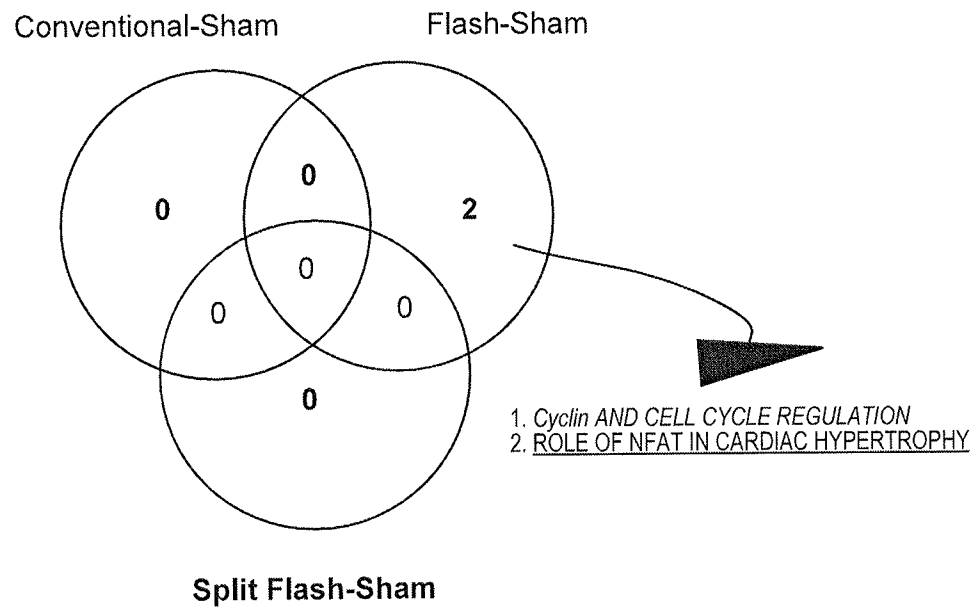
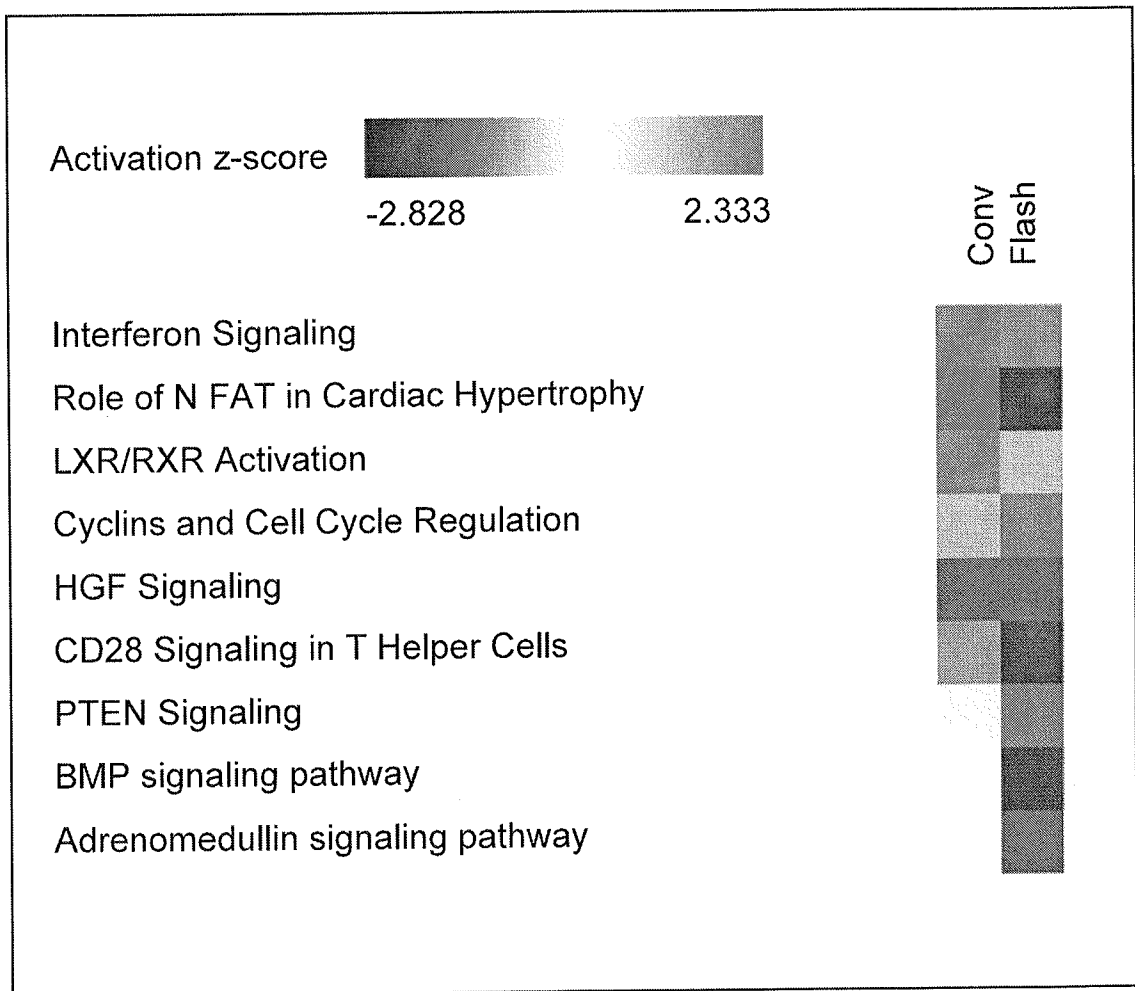


FIG. 20

**FIG. 21**

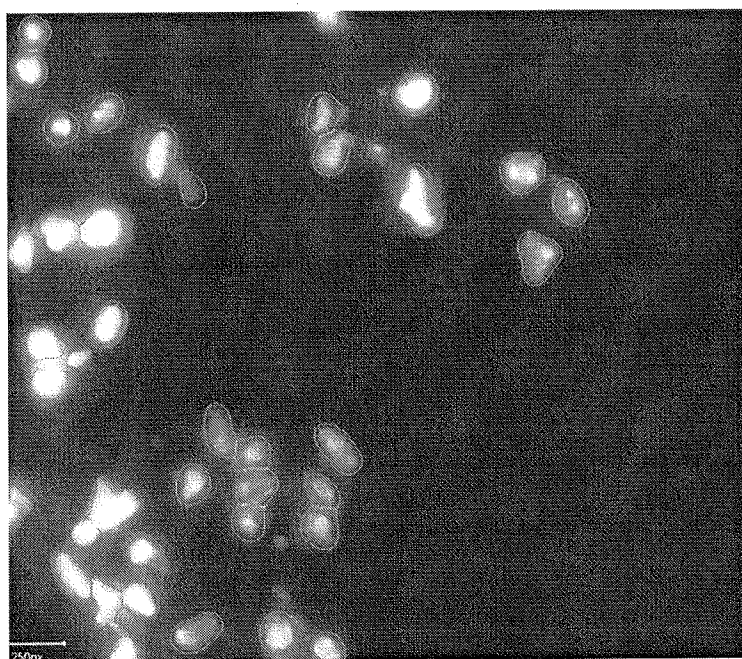


FIG. 22

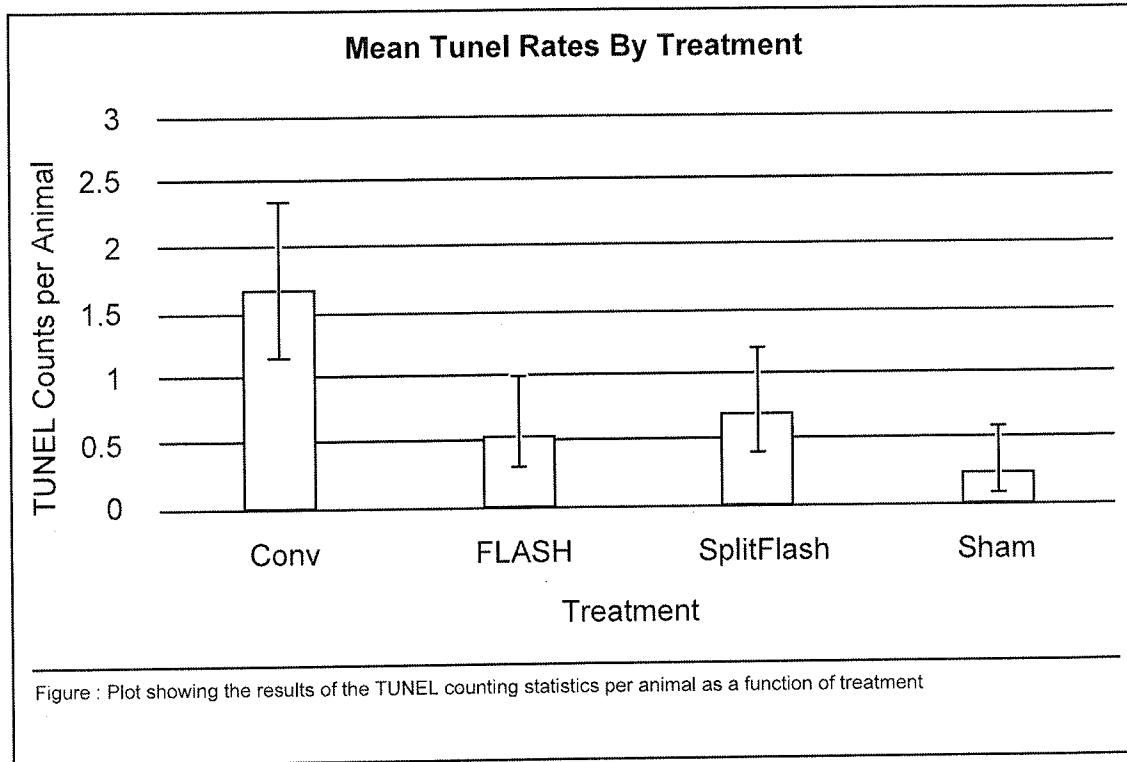


FIG. 23A



FIG. 23B

FIG. 23

**FIG. 24**

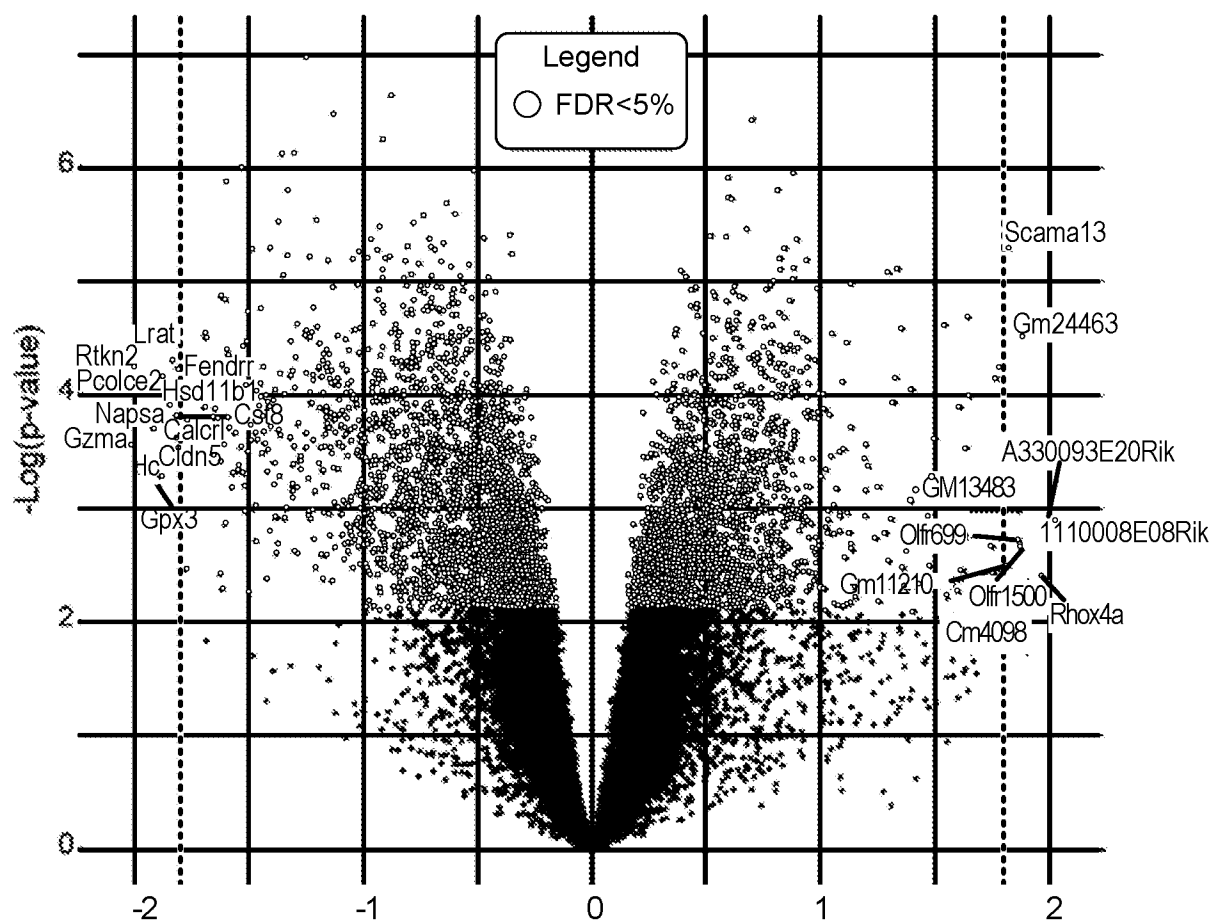


FIG. 25A

HALLMARK Signatures	SIZE	NES	NOM p-val
UV RESPONSE DOWN	87	-1.44	0.013592233
HEDGEHOG SIGNALING	19	-1.40	0.021235522
INFLAMMATORY RESPONSE	116	-1.40	0.04699248
APOPTOSIS	106	-1.39	0.027777778
MITOTIC SPINDLE	111	-1.38	0.018796992
WNT BETA CATENIN SIGNALING	25	-1.38	0.047034767

FIG. 25B

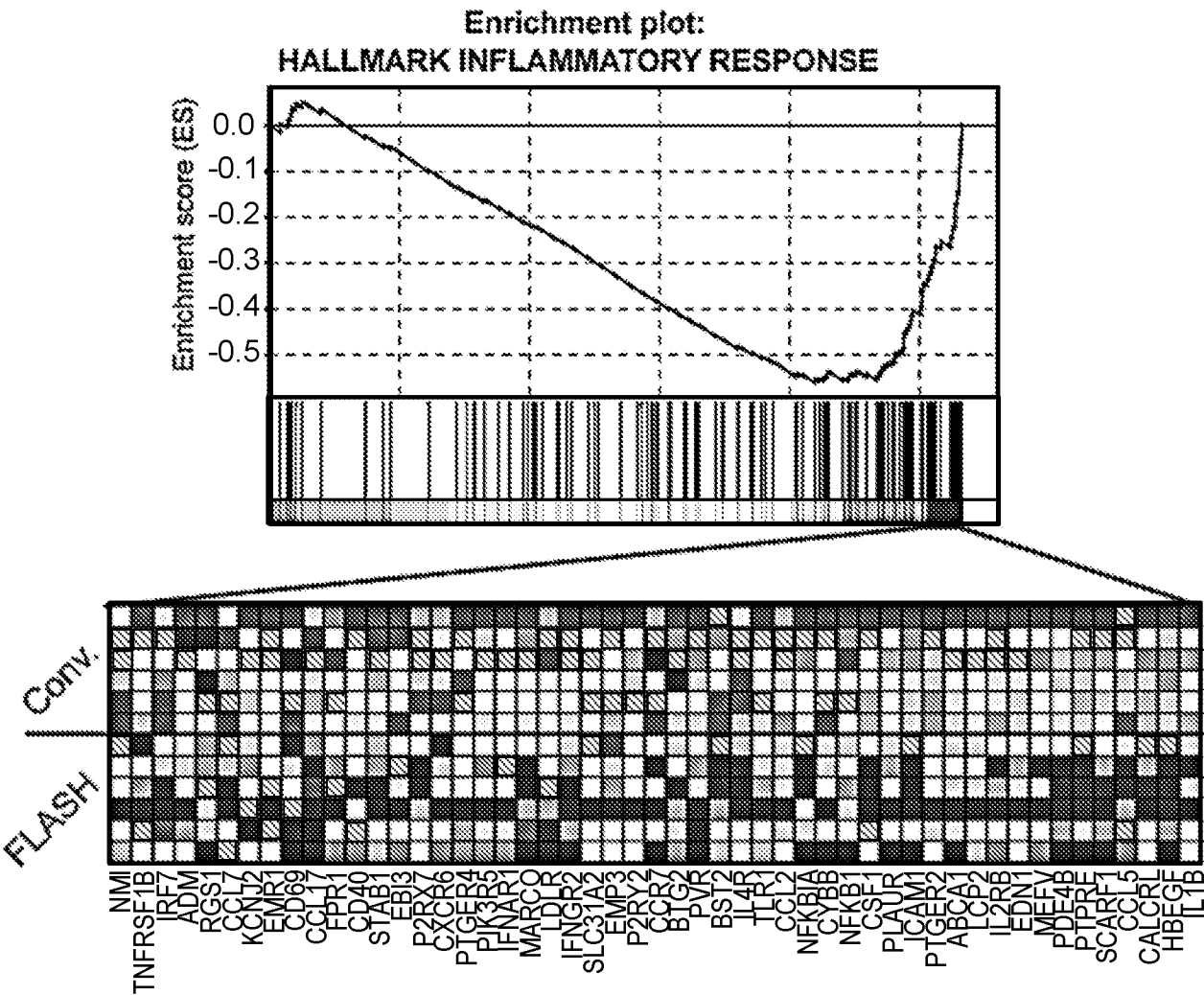


FIG. 25C

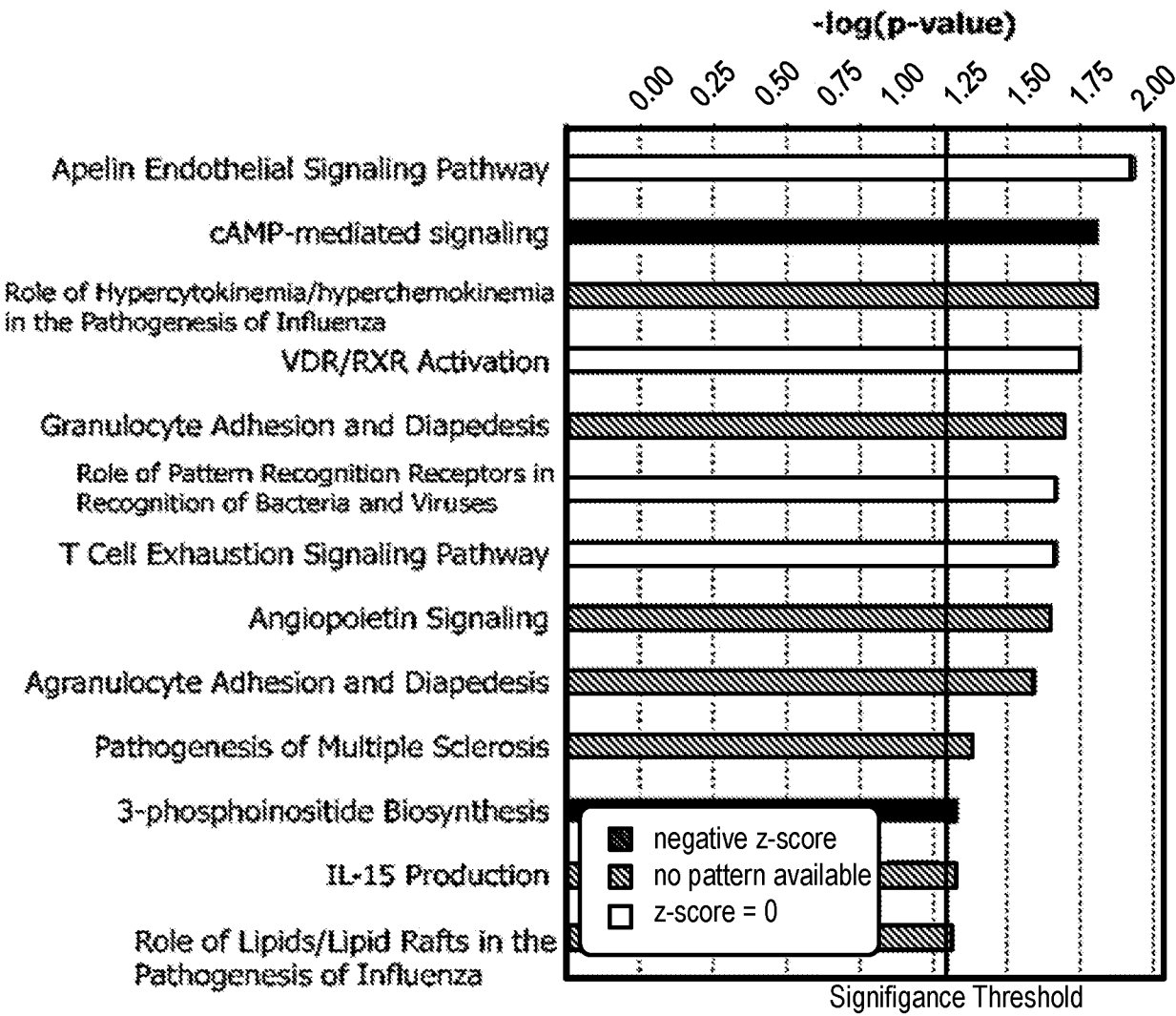


FIG. 25D

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/042603

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/436 A61N5/10 A61K39/395 A61K31/352 A61K31/366
A61K31/404 A61K31/4439 A61P35/00 G01N33/5005 G01N33/50

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61N A61P G01N

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/011817 A1 (FENG YUXIN [US]) 8 January 2015 (2015-01-08) claims 1-12 paragraphs [0001] - [0009] claim 7 -----	1-20, 40-60
X	SPRATT D ET AL: "Long-term Survival and Toxicity of Patients Treated With Ultra-high-dose IMRT for Localized Prostate Cancer", INTERNATIONAL JOURNAL OF RADIATION: ONCOLOGY BIOLOGY PHYSICS, vol. 84, no. 3, 2012, XP028954087, ISSN: 0360-3016, DOI: 10.1016/J.IJROBP.2012.07.041 the whole document ----- -/--	40-60



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 October 2019

Date of mailing of the international search report

30/10/2019

Name and mailing address of the ISA/

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

Authorized officer

Albayrak, Timur

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/042603

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TECKIE S ET AL: "Ultra-high-dose Stereotactic Body Radiation Therapy (SBRT) is Feasible for Tumors in the Head and Neck (HN)", INTERNATIONAL JOURNAL OF RADIATION: ONCOLOGY BIOLOGY PHYSICS, vol. 84, no. 3, 2012, XP028954869, ISSN: 0360-3016, DOI: 10.1016/J.IJROBP.2012.07.1372 the whole document	40-54, 58,59
X	STOKES W A ET AL: "Organ Preservation for Muscle-Invasive Squamous Cell Carcinoma of the Urinary Bladder in the United States", INTERNATIONAL JOURNAL OF RADIATION: ONCOLOGY BIOLOGY PHYSICS, vol. 99, no. 2, 2017, XP085192883, ISSN: 0360-3016, DOI: 10.1016/J.IJROBP.2017.06.1238 the whole document	1-20, 40-54
X	"Induction chemotherapy plus high-dose radiotherapy versus radiotherapy alone in locally advanced unresectable non-small-cell lung cancer", LUNG CANCER, ELSEVIER, AMSTERDAM, NL, vol. 11, no. 3-4, 1 September 1994 (1994-09-01), page 343, XP026296088, ISSN: 0169-5002, DOI: 10.1016/0169-5002(94)90654-8 [retrieved on 1994-09-01] the whole document	1-20, 40-54
Y		22-34
X	WO 2014/144804 A1 (VARIAN MED SYS INC [US]) 18 September 2014 (2014-09-18) column 28	1-20, 35-54
X	WO 2017/044562 A1 (VARIAN MED SYS INC [US]; VARIAN MEDICAL SYSTEMS INT AG [CH]) 16 March 2017 (2017-03-16) claim 1	22-54
Y		22-54
Y	WO 2014/205128 A1 (UNIV DUKE [US]) 24 December 2014 (2014-12-24) claim 28 paragraph [0053]	22-54
X	WO 2006/039569 A1 (UNIV CHICAGO [US]; WEICHSSELBAUM RALPH R [US] ET AL.) 13 April 2006 (2006-04-13) claim 24	21

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/042603

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed:
 - ☐ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
 - ☒ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/042603

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2015011817 A1	08-01-2015	NONE	
WO 2014144804 A1	18-09-2014	BR 112015023493 A2 CN 105209070 A EP 2968542 A1 JP 2016521141 A JP 2019103838 A US 2016024594 A1 US 2018305769 A1 WO 2014144804 A1	10-10-2017 30-12-2015 20-01-2016 21-07-2016 27-06-2019 28-01-2016 25-10-2018 18-09-2014
WO 2017044562 A1	16-03-2017	CN 108348771 A EP 3347093 A1 US 2017072220 A1 US 2019015684 A1 WO 2017044562 A1	31-07-2018 18-07-2018 16-03-2017 17-01-2019 16-03-2017
WO 2014205128 A1	24-12-2014	CN 105358217 A EP 3010585 A1 EP 3384959 A1 US 2016129282 A1 WO 2014205128 A1	24-02-2016 27-04-2016 10-10-2018 12-05-2016 24-12-2014
WO 2006039569 A1	13-04-2006	JP 2008514726 A US 2007281040 A1 US 2011046211 A1 WO 2006039569 A1	08-05-2008 06-12-2007 24-02-2011 13-04-2006