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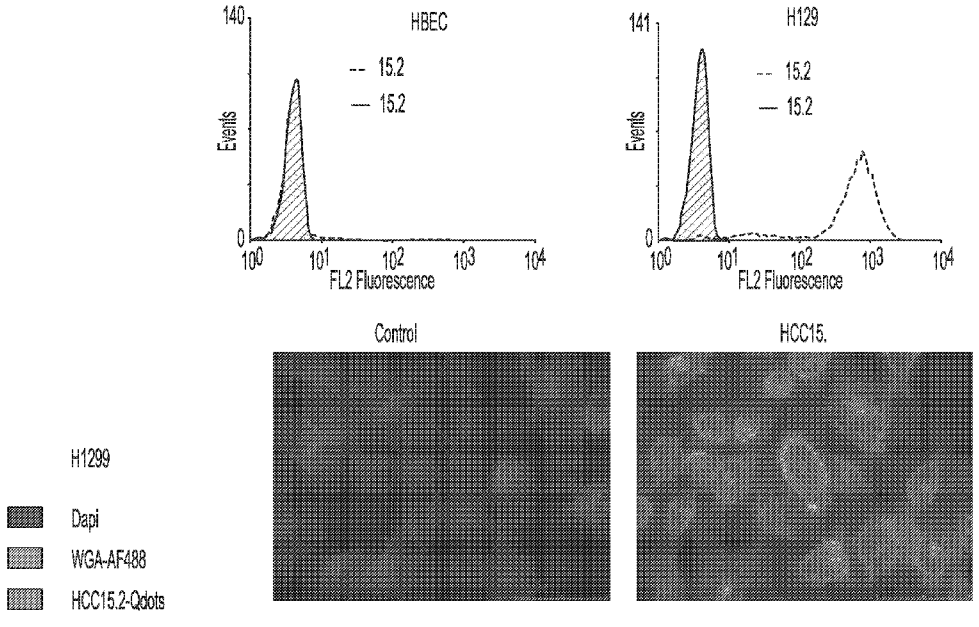

FIG. 1

(57) Abstract: Disclosed herein, are compositions comprising one or more a molecular guidance system (MGS) peptides and a cytotoxic agent. Also described herein, are methods of administering the compositions to patients with cancer.

[Continued on next page]

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A PEPTIDE SAPORIN CONJUGATE FOR THE TREATMENT OF CANCER

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

[0001] This invention was made with government support under Award Number, 7R01CA164447-04 awarded by the Department of Health and Human Services of the National Cancer Institute. The government has certain rights in the invention.

BACKGROUND

[0002] American Cancer Society estimates that more than 1.6 million new cancer cases will be diagnosed in the Unites States this year with almost 600,000 deaths due to cancer. The costs to society are immense. The NIH estimates that cancer costs $216.6 billion yearly, of which $89 billion is direct medical costs. First line treatment for most cancers still relies on cytotoxic therapies. These therapies require actively dividing cells resulting in untoward side effects. Treatments are typically given at the maximum tolerated dose not the maximum effective dose. Additionally, these treatments are ineffective in quiescent cancer cells, stems cells or poorly differentiated cancer cells because they are not actively dividing. This leads to reoccurrence. Most targeted therapies that are more specific for tumor biomarkers are cytostatic not cytotoxic. Hence, they may slow the growth of the tumor but not cure it. Finally, resistance to both targeted and untargeted therapies is a clinical reality. Thus, new specific cytotoxic therapies with minimal side effects are needed.

SUMMARY

[0003] Saporin is a ribosome inactivating protein (RIP, 34 KD) with no cell entry domain. Given an entry mechanism into the cell, it catalytically removes a single adenine from ribosomal RNA of the large subunit of the ribosome, completely inactivating it. As such, if it can be delivered inside the cell, it is a potent toxin that rapidly kills the cell. Described herein are targeted cancer therapies comprising one or more MGS peptides and the plant toxin saporin. The compositions described herein can, in some aspects, bind to and mediate internalization of the compositions into cancer cells resulting in rapid cell death. Because of the cancer specificity of the MGS peptides, the treatment has minimal effect on normal tissues.

[0004] The compositions described herein can be created as a fusion protein or by chemical conjugation. The conjugate can be delivered and the MGS directs the saporin to the tumor and mediates its uptake in the tumor cells where it can bind its target. This compound

has several advantages over current therapies. For example, in some aspects, the disclosed compositions (1) can allow for a highly potent cytotoxic to be delivered intracellularly where it is functional; (2) the MGS can be cancer specific minimizing uptake of the toxin in other tissues; (3) because all cells, even non dividing cells, are dependent on protein synthesis, the MGS peptide-saporin conjugate can be effective against cells that are refractory to common cytotoxic agents; (4) resistance is unlikely due to the mechanism of action as a protein, multiple drug resistance pumps are unlikely to negate activity; and (5) the peptidic targeting agent is small, relatively inexpensive compare to antibody targeting agents, and can be conjugated to saporin.

[0005] Disclosed herein, are compositions comprising one or more a molecular guidance system (MGS) peptides and a cytotoxic agent.

[0006] Disclosed herein, are membrane-permeable conjugates for transport across a lipid membrane comprising: one or more a molecular guidance system (MGS) peptides and a cytotoxic agent.

[0007] Disclosed herein, are methods of targeting an intracellular target, the method comprising administering one or more MGS peptides conjugated to a cytotoxic agent, wherein the cytotoxic agent targets an intracellular target.

[0008] Other features and advantages of the present compositions and methods are illustrated in the description below, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed method and compositions and together with the description, serve to explain the principles of the disclosed method and compositions.

[0010] FIG. 1 shows that HCC15.2 is specific for and internalizes in cancer cells.

[0011] FIG. 2 shows that HCC15.2 binds to all subtypes of NSCLC.

[0012] FIG. 3 shows that peptide internalization is receptor mediated.

[0013] FIG. 4 shows that tetramerization of SEQ ID NO: 1 does not significantly improve internalization compared to the monomer of SEQ ID NO: 1.

[0014] FIG. 5 shows that truncations from N- and C-Terminal ends reveal minimal binding sequence (FHAVPQSFYTAP, SEQ ID NO: 1; FHAVPQSFYTA, SEQ ID NO: 2; and FHAVPQSFYT, SEQ ID NO: 3).

[0015] FIG. 6 shows that acetylation improves binding of the truncated peptide (SEQ ID

NO: 3, FHAVPQSFYTAP, SEQ ID NO: 1).

[0016] FIG. 7 shows that peptide co-localizes with the lysosome.

[0017] FIG. 8 shows that HCC15.2 accumulates in the lysosome over time.

[0018] FIG. 9 shows that in vitro drug data of HCC15.2 conjugated to saporin.

[0019] FIG. 10 shows that the HCC15.2-saporin conjugate reduces tumor growth in a human NSCLC xenograft model.

[0020] FIG. 11 depicts a histogram and a quantification of a histogram from flow cytometry experiments.

[0021] FIG. 12 is a bar graph showing that HCC15.2 binds to other cancer cell lines.

[0022] FIG. 13 is a line graph showing the flow cytometry results to measure KD.

[0023] FIG. 14 shows that tetramerization does not significantly improve half maximal binding.

[0024] FIG. 15 shows that truncations from N- and C-Termini reveal minimal binding sequence. Top panel refers to the following sequences from top to bottom: SEQ ID NOs: 1, 2, 3, 78, and 79. Bottom panel refers to the following sequences from top to bottom: SEQ ID NOs: 1, 2, 3, 78, 79, and 80.

[0025] FIG. 16 shows that HCC15.2 accumulates in the lysosome over time.

[0026] FIG. 17 shows that HCC15.2-targeted NIR dye accumulates into tumors *in vivo.*

[0027] FIG. 18 shows that HCC 15.2-targerted dye is retained in the tumor over time.

[0028] FIG. 19 demonstrates that ex vivo tumors show clear accumulation in HCC 15.2-targeted dye.

[0029] FIG. 20 shows the results of ex vivo imaging of organs.

[0030] FIG. 21 shows that HCC15.2 targets saporin to cancer cells *in vitro.*

[0031] FIG. 22 is an example of the cancer cell killing assay showing that HCC15.2 targets saporin to cancer cells *in vitro.*

[0032] FIG. 23 shows that HCC15.2 targeting of saporin markedly slowed tumor growth.

[0033] FIG. 24 is a table listing various MGSs, indication, cell type targets, cellular location and payloads delivered.

[0034] FIG. 25 shows sequences (in order from top to bottom: SEQ ID NOs: 22, 39, 40, 23, 47, 25, 26, 27, 28, and 29) cellular location, valency, cancer specificity and binding profile information for select MGS peptides.

[0035] FIG. 26 is a table showing further characterization of select MGS peptides. Starting sequence from top to bottom: SEQ ID NOs: 7, 5, 36, 32, and 1. Current sequence from top to bottom: SEQ ID NOs: 81, 82, 83, 84 and 80.

[0036] FIG. 27 shows co-localization of H1299.3 15-mer with autophagosomes (top panel) and results after administration *in vivo*.

[0037] FIG. 28 is a bar graph showing 1299.3 Ac-15mer cancer cell binding compared to normal cell binding.

[0038] FIG. 29 is a table showing saporin delivery *in vitro* with other peptides (parental peptide not optimized except for HCC15.2).

[0039] FIG. 30 is an example of a multimeric MGS peptide and experimental results using the multimeric MGS peptide (SEQ ID NO: 22).

DETAILED DESCRIPTION

[0040] The disclosed method and compositions may be understood more readily by reference to the following detailed description of particular embodiments and the Examples included therein and to the Figures and their previous and following description.

[0041] Before the present compositions and methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[0042] Moreover, it is to be understood that unless otherwise expressly stated, it is in no way intended that any method set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not actually recite an order to be followed by its steps or it is not otherwise specifically stated in the claims or descriptions that the steps are to be limited to a specific order, it is in no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, and the number or type of aspects described in the specification.

[0043] All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Further, the dates of publication provided herein can be different from the actual publication dates, which can require independent confirmation.

DEFINITIONS

[0044] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0045] The word "or" as used herein means any one member of a particular list and also includes any combination of members of that list. Thus, for example, reference to "a MGS peptide" includes a plurality of such MGS peptides, reference to "the MGS peptide" is a reference to one or more MGS peptides and equivalents thereof known to those skilled in the art, and so forth.

[0046] Throughout the description and claims of this specification, the word “comprise” and variations of the word, such as “comprising” and “comprises,” means “including but not limited to,” and is not intended to exclude, for example, other additives, components, integers or steps. In particular, in methods stated as comprising one or more steps or operations it is specifically contemplated that each step comprises what is listed (unless that step includes a limiting term such as “consisting of”), meaning that each step is not intended to exclude, for example, other additives, components, integers or steps that are not listed in the step.

[0047] Ranges can be expressed herein as from "about" or "approximately" one particular value, and/or to "about" or "approximately" another particular value. When such a range is expressed, a further aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," or "approximately," it will be understood that the particular value forms a further aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint and independently of the other endpoint. It is also understood that there are a number of values disclosed herein and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that each unit between two particular units is also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0048] As used herein, the terms "optional" or "optionally" mean that the subsequently described event or circumstance may or may not occur and that the description includes instances where said event or circumstance occurs and instances where it does not.

[0049] As used herein, the term "subject" refers to the target of administration, e.g., a human. Thus the subject of the disclosed methods can be a vertebrate, such as a mammal, a

fish, a bird, a reptile, or an amphibian. The term "subject" also includes domesticated animals (e.g., cats, dogs, etc.), livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (e.g., mouse, rabbit, rat, guinea pig, fruit fly, etc.). In one aspect, a subject is a mammal. In another aspect, a subject is a human. The term does not denote a particular age or sex. Thus, adult, child, adolescent and newborn subjects, as well as fetuses, whether male or female, are intended to be covered.

[0050] As used herein, the term "patient" refers to a subject afflicted with a disease or disorder. The term "patient" includes human and veterinary subjects. In some aspects of the disclosed methods, the "patient" has been diagnosed with a need for treatment for an autoimmune disorder, such as, for example, prior to the administering step.

[0051] As used herein, the term "amino acid sequence" refers to a list of abbreviations, letters, characters or words representing amino acid residues. The amino acid abbreviations used herein are conventional one letter codes for the amino acids and are expressed as follows: A, alanine; C, cysteine; D aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H histidine; I isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine..

[0052] "Polypeptide" as used herein refers to any peptide, oligopeptide, polypeptide, gene product, expression product, or protein. A polypeptide is comprised of consecutive amino acids. The term "polypeptide" encompasses naturally occurring or synthetic molecules.

[0053] In addition, as used herein, the term "polypeptide" refers to amino acids joined to each other by peptide bonds or modified peptide bonds, e.g., peptide isosteres, etc. and may contain modified amino acids other than the 20 gene-encoded amino acids. The polypeptides can be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. The same type of modification can be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide can have many types of modifications. Modifications include, without limitation, acetylation, acylation, ADP-ribosylation, amidation, covalent cross-linking or cyclization, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphytidylinositol, disulfide bond formation, demethylation, formation of

cysteine or pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation, pergylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-RNA mediated addition of amino acids to protein such as arginylation. (*See Proteins – Structure and Molecular Properties* 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993); *Posttranslational Covalent Modification of Proteins*, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983)).

[0054] The phrase "nucleic acid" as used herein refers to a naturally occurring or synthetic oligonucleotide or polynucleotide, whether DNA or RNA or DNA-RNA hybrid, single-stranded or double-stranded, sense or antisense, which is capable of hybridization to a complementary nucleic acid by Watson-Crick base-pairing. Nucleic acids of the invention can also include nucleotide analogs (e.g., BrdU), and non-phosphodiester internucleoside linkages (*e.g.*, peptide nucleic acid (PNA) or thiodiester linkages). In particular, nucleic acids can include, without limitation, DNA, RNA, cDNA, gDNA, ssDNA, dsDNA or any combination thereof.

[0055] As used herein, "sample" is meant to mean an animal; a tissue or organ from an animal; a cell (either within a subject, taken directly from a subject, or a cell maintained in culture or from a cultured cell line); a cell lysate (or lysate fraction) or cell extract; or a solution containing one or more molecules derived from a cell or cellular material (e.g. a polypeptide or nucleic acid), which is assayed as described herein. A sample may also be any body fluid or excretion (for example, but not limited to, blood, urine, stool, saliva, tears, bile) that contains cells or cell components.

[0056] As used herein, "modulate" is meant to mean to alter, by increasing or decreasing.

[0057] As used herein, "effective amount" of a compound is meant to mean a sufficient amount of the compound to provide the desired effect. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of disease (or underlying genetic defect) that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact "effective amount." However, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

[0058] As used herein, "isolated polypeptide" or "purified polypeptide" is meant to mean a polypeptide (or a fragment thereof) that is substantially free from the materials with which the polypeptide is normally associated in nature. The polypeptides of the invention, or fragments thereof, can be obtained, for example, by extraction from a natural source (for

example, a mammalian cell), by expression of a recombinant nucleic acid encoding the polypeptide (for example, in a cell or in a cell-free translation system), or by chemically synthesizing the polypeptide. In addition, polypeptide fragments may be obtained by any of these methods, or by cleaving full length proteins and/or polypeptides.

[0059] As used herein, "isolated nucleic acid" or "purified nucleic acid" is meant to mean DNA that is free of the genes that, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, such as an autonomously replicating plasmid or virus; or incorporated into the genomic DNA of a prokaryote or eukaryote (e.g., a transgene); or which exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR, restriction endonuclease digestion, or chemical or in vitro synthesis). It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. The term "isolated nucleic acid" also refers to RNA, e.g., an mRNA molecule that is encoded by an isolated DNA molecule, or that is chemically synthesized, or that is separated or substantially free from at least some cellular components, for example, other types of RNA molecules or polypeptide molecules.

[0060] As used herein, "treat" is meant to mean administer a compound or molecule of the invention to a subject, such as a human or other mammal (for example, an animal model), that has a cancer, in order to prevent or delay a worsening of the effects of the disease or condition, or to partially or fully reverse the effects of the disease.

[0061] As used herein, "prevent" is meant to mean minimize the chance that a subject who has an increased susceptibility for developing cancer will develop cancer.

[0062] As used herein, "specifically binds" is meant that an antibody recognizes and physically interacts with its cognate antigen or target (for example, the disclosed synthetic MGS sequences) and does not significantly recognize and interact with other antigens or targets; such an antibody may be a polyclonal antibody or a monoclonal antibody, which are generated by techniques that are well known in the art.

[0063] As used herein, "probe," "primer," or "oligonucleotide" is meant to mean a single-stranded DNA or RNA molecule of defined sequence that can base-pair to a second DNA or RNA molecule that contains a complementary sequence (the "target"). The stability of the resulting hybrid depends upon the extent of the base-pairing that occurs. The extent of base-pairing is affected by parameters such as the degree of complementarity between the probe and target molecules and the degree of stringency of the hybridization conditions. The degree of hybridization stringency is affected by parameters such as temperature, salt

concentration, and the concentration of organic molecules such as formamide, and is determined by methods known to one skilled in the art. Probes or primers specific for nucleic acids capable of encoding the disclosed MGS sequences (for example, genes and/or mRNAs) have at least 80%-90% sequence complementarity, preferably at least 91%-95% sequence complementarity, more preferably at least 96%-99% sequence complementarity, and most preferably 100% sequence complementarity to the region of the nucleic acid capable of encoding the disclosed MGS sequences to which they hybridize. Probes, primers, and oligonucleotides may be detectably-labeled, either radioactively, or non-radioactively, by methods well-known to those skilled in the art. Probes, primers, and oligonucleotides are used for methods involving nucleic acid hybridization, such as: nucleic acid sequencing, reverse transcription and/or nucleic acid amplification by the polymerase chain reaction, single stranded conformational polymorphism (SSCP) analysis, restriction fragment polymorphism (RFLP) analysis, Southern hybridization, Northern hybridization, in situ hybridization, electrophoretic mobility shift assay (EMSA).

[0064] As used herein, "specifically hybridizes" is meant to mean that a probe, primer, or oligonucleotide recognizes and physically interacts (that is, base-pairs) with a substantially complementary nucleic acid (for example, a nucleic acid capable of encoding the disclosed MGS sequence) under high stringency conditions, and does not substantially base pair with other nucleic acids.

[0065] As used herein, "high stringency conditions" is meant to mean conditions that allow hybridization comparable with that resulting from the use of a DNA probe of at least 40 nucleotides in length, in a buffer containing 0.5 M $NaHPO_4$, pH 7.2, 7% SDS, 1 mM EDTA, and 1% BSA (Fraction V), at a temperature of 65°C, or a buffer containing 48% formamide, 4.8X SSC, 0.2 M Tris-Cl, pH 7.6, 1X Denhardt's solution, 10% dextran sulfate, and 0.1% SDS, at a temperature of 42°C. Other conditions for high stringency hybridization, such as for PCR, Northern, Southern, or in situ hybridization, DNA sequencing, etc., are well-known by those skilled in the art of molecular biology. (See, for example, F. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1998).

[0066] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed method and compositions belong. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present method and compositions, the particularly useful methods, devices, and materials are as described. Publications cited herein and the material for which they are cited are hereby specifically

incorporated by reference. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such disclosure by virtue of prior invention. No admission is made that any reference constitutes prior art. The discussion of references states what their authors assert, and applicants reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of publications are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0067] Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

[0068] Also disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein.

[0069] Disclosed herein is a targeted cancer therapy comprising a peptide conjugate comprising the peptide sequence FHAVPQSFYT (SEQ ID NO: 3) conjugated to the plant toxin saporin. Saporin is a ribosome inactivating protein (RIP, 34 KD) with no cell entry domain. Given an entry mechanism into the cell, it catalytically removes a single adenine

from ribosomal RNA of the large subunit of the ribosome, inactivating it. As such, if it can be delivered inside the cell, it is a potent toxin that rapidly kills the cell. As described herein, this problem can be overcome by conjugating saporin to a peptide that is selective for cancer cells and binds to and mediates internalization of the toxin into cancer cells, thus resulting in rapid cancer cell death. Because of the cancer specificity of the MGS peptide conjugate disclosed herein, the treatment has minimal effect on normal tissues.

[0070] Disclosed herein is an anti-cancer therapy with applications in lung, breast, colorectal, ovarian, and pancreatic cancers. The compound, composition, or peptide conjugate disclosed herein represents a targeted cytotoxic therapy that may have wide applicability in the treatment of carcinomas.

[0071] The composition or conjugate (e.g., the MGS peptide-saporin conjugate) disclosed herein can be created as a fusion protein or by chemical conjugation. The conjugate can be delivered and the MGS peptide can direct the toxin, (e.g., saporin) to the tumor or cancer cell where it mediates the uptake of the saporin into the tumor cells where the toxin can bind its target.

[0072] The delivery of the compositions disclosed herein, for example, when one or more molecular guidance system (MGS) peptides when coupled or conjugated to saporin can target and deliver saparoin specifically to cancer cells while avoiding or minimizing the uptake of saporin in normal cells. The MGS peptides disclosed herein have a low nanomolar affinity for a subset of epithelial-derived cancers. Because these MGS peptides are small, they can be easily conjugated to saporin or expressed as a fusion protein. With about 1.6 million new diagnoses and almost 600,000 deaths per year in the U.S. resulting in annual health care costs of approximately $89 billion, the conjugates disclosed herein provide a new therapy for carcinoma that may overcome the limitations of current cancer therapies.

[0073] The peptide HCC15.2 can be truncated by removing removal of –one or more amino acids. Further a PEG linker can be added to the monomeric peptide, and the amino terminus can be acetylated.

COMPOSITIONS

[0074] *MGS peptides.* Disclosed herein are molecular system guidance peptides (MGS) or tumor targeting peptides. These peptides can bind selectively to tumors including malignant tumors. Examples of MGS peptides that can be used or modified in the disclosed compositions can include, but are not limited to, one or more of the MGS peptides disclosed in McGuire et al., Sci Rep. 2014 Mar 27; 4:4480. Examples of MGS peptides that can also be

used in the disclosed compositions and methods, include, but are not limited to the MGS sequences shown in Table 1 and FIGS. 24 and 25.

Table 1. Peptide sequences.

Peptide Name	Peptide Sequence	SEQ ID NO:
HCC15.2	FHAVPQSFYTAP	1
	FHAVPQSFYTA	2
	FHAVPQSFYT	3
	FHAVPQSFY	78
	HAVPQSFYT	79
	CH_3CO-FHAVPQSFYT	80
H1299.1	VSQTMRQTAVPLLWFWTGSL	4
H1299.2	YAAWPASGAWTGTAPCSAGT	5
	YAAWPASGAWT	6
	CH_3CO-YAAWPASGAWT	82
H2009.1	RGDLATLRQLAQEDGVVGVR	7
	D-Leu-RGDLATLRQL	8
	CH_3CO- D-Leu-RGDLATLRQL	81
H460.1	EAMNSAEQSAAVVQWEKRRI	9
HCC15.1	ATEPRKQYATPRVFWTDAPG	10
A549.1	MTVCNASQRQAHAQATAVSL	11
HCC95.1	MRGQTGKLPTEHFTDTGVAF	12
H1155.1	MTGKAAAPHQEDRHANGLEQ	13
H661.1	TNSCRGDWLCDAVPEKARV	14
	EHPWFNMWSWATQVQE	15
H2009.2	YPGSPTQYPSSMHEYHSSSE	16
H2009.3	AHTIDDEWASYHMQQWNSPP	17
	FEEFYSRQSNTIPYPQQYKG	18
	THGNKHQSWTYPSEINHKNY	19
	NLADTWTQTQQHDFHVLRGTR	20
	GYSWWQPNWPSSTWDT	21
H1299.4	EHPWFNMWSWATQVQEKKK	22
H2009.4	NLADTWTQTQQHDFHVLRGT	23

H1993.1	SVEYWGERMYYDVMESLGFS	24
H1993.2	FAAKRAEWWDPGQLWDAVWN	25
H1993.3	QEALEEWFWKMMPWSGPSGQ	26
H1993.4	TWTDFGQWPWPFGAEGTRAF	27
H1993.5	MDGATWWTQLDPLLVWEGET	28
H1993.5	SADWFQGPAEWLLEGWMGPL	29
H1299.3	LQWRRDDNVHNFGVWARYRL	30
	LQWRRNFGVWARYRL	31
HCC15.1	ATEPRKQYATPRVFWTDAPG	32
	KQYATPRVFWT	33
	CH_3CO- KQYATPRVFWT	84
MGS_H1299.1	VSQTMRQTAVPLLWFWTGSL	34
MGS_1299.2	YAAWPASGAWTGTAPCSAGT	35
MGS_1299.3	LQWRRDDNVHNFGVWARYRL	36
	CH_3CO- LQWRRDDNVHNFGVWARYRL	83
MGS_H2009.1	RGDLATLRQLAQEDGVVGVR	37
MGS_H2009.2	EHPWFNMWSWATQVQE	38
MGS_H2009.3	YPGSPTQYPSSMHEYHSSSE	39
MGS_H2009.4	AHTIDDEWASYHMQQWNSPP	40
MGS_H2009.5	FEEFYSRQSNTIPYPQQYKG	41
MGS_HCC15.1	ATEPRKQYATPRVFWTDAPG	42
MGS_HCC15.2	FHAVPQSFYTAP	43
MGS_H460.1	EAMNSAEQSAAVVQWEKRRI	44
MGS_A549.1	MTVCNASQRQAHAQATAVSL	45
MGS_MCF7.1	LTVHGRGPEYNPSWNRRAFP	46
MGS_H1993.1	SVEYWGERMYYDVMESLGFS	47
MGS_H1993.2	FAAKRAEWWDPGQLWDAVWN	48
MGS_H1993.3	QEALEEWFWKMMPWSGPSGQ	49
MGS_H1993.4	TWTDFGQWPWPFGAEGTRAF	50
MGS_H1993.5	MDGATWWTQLDPLLVWEGET	51
MGS_H1993.6	SADWFQGPAEWLLEGWMGPL	52
MGS_HCC95.1	MRGQTGKLPTEHFTDTGVAF	53

MGS_H1155.1	MTGKAAAPHQEDRHANGLEQ	54
MGS_H1155.2	MEKLPLSKTGRTVSEGVSPP	55
MGSH666.1	TNSCRGDWLCDAVPEKARV	56
MGS_A20.1	SAKTAVSQRVWLPSHRGGEP	57
MGS_A20.2	KSREHVNNSACPSKRITAAL	58
MGS_PCM.1	WLSEAGPVVTVRALRGTGSW	59
MGS_C2C12.1	TGGETSGIKKAPYASTTRNR	60
MGS_C2C12.2	SHHGVAGVDLGGGADFKSIA	61
MGS_C2C12.3	SNSPLGLKDEATQRLVLEQAKWLA	62
MGS_XS52.1	GPEDTSRAPENQQKTFHRRW	63
MGS_XS52.2	SGETGSNLVGHELDFRPGSPSP	64
MGS_XS106.1	RYSPAATAEGRSVSKELLRV	65
MGS_717US.1	GQELGAWTRSKGPEVQTSVL	66
MGS_717S.1	ASTWRGTSAGGNRLEKMEVT	67
MGS_RIP.1	LSGTPERSGQAVKVKLKAIP	68
MGS_RIP.2	GAWEAVRDRIAEWGSWGIPS	69
MGS_MArg.1_Bacterial	AMDMYSIEDRYFGGYAPEVG	70
MGS_1299.2 V4	CH_3CO-YAAWPASGAWT-PEG_{11}-C-NH_2	71
MGS_1299.3 V2	CH_3CO-LQWRRNFGVWARYRL-PEG_{11}-C-NH_2	72
MGS_2009.1 V4	CH_3CO-RGDLATLRQL-PEG_{11}-YC-NH_2	73
MGS_H2009.1 V5	CH_3CO-d(Leu)-RGDLATLRQL-PEG_{11}-YC-NH_2	74
MGS_HCC15.1 V4	CH_3CO-LQWRRNFGVWARYRL-PEG_{11}-C-NH_2	75
MGS_HCC15.2 V8	CH_3CO-FHAVPQSFYT-PEG_{11}-C-NH_2	76
MGS_HCC15.2 V9	CH_3CO-FHAVPQSFYT-PEG_{11}-C-NH_2	77

[0075] In an aspect, the compositions comprise one or more a molecular guidance system (MGS) peptides and a cytotoxic agent. In an aspect, the membrane-permeable conjugates for transport across a lipid membrane can comprise one or more a molecular guidance system (MGS) peptides and a cytotoxic agent.

[0076] In an aspect, the one or more MGS peptides can be any of the MGS peptides disclosed herein. In an aspect, the one or more MGS peptides comprise SEQ ID NO: 1, 2, 3, 34, 35, 36, 37, 38, 39, 40, 41, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 5, 54, 55, 56, 57,

58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, or 84 or a combination thereof. In an aspect, the one or more MGS peptides comprise SEQ ID NOs: 1, 2 or 3. In an aspect, the cytotoxic agent can be saporin or a biologically active variant thereof. In an aspect, the one or more MGS peptides can be SEQ ID NO: 3 and the cytotoxic agent can be saporin. In an aspect, the compositions can comprise one or more MGS peptides, for example, in some aspects, the composition can comprise, one, two, three, four or five MGS peptides. In an aspect, the one or more MGS peptides can form a tetrameric scaffold protein. In an aspect, the one or more MGS peptides disclosed herein can be truncated. In an aspect, the one or more MGS peptides can be modified. In an aspect, the one or more MGS peptides can acetylated on the N-terminus. FIG. 26 provides examples of select MGS peptides that have been further characterized. In an aspect, the one or more MGS peptides can be chemically conjugated to the cytotoxic agent. In an aspect, the chemical conjugate can be polyethylene glycol (PEG). In an aspect, the number of PEG units can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or more. In aspect, the number of PEG units can be of sufficient length to separate the one or more MGS peptides from the cytotoxic agent to prevent any steric interference between the one or more MGS peptides and the cytotoxic agent. For example, disclosed herein are compositions comprising a chemical conjugate, wherein the chemical conjugate is PEG and the PEG comprises eleven PEG units. In an aspect, the one or more MGS peptides comprise SEQ ID NO: 3, wherein SEQ ID NO: 3 can be acetylated on the N-terminus and can be chemically conjugated to PEG; and the cytotoxic agent can be saporin, wherein the saporin can be covalently attached to PEG.

[0077] *Cytotoxic Agents*. A wide variety of toxic (e.g., cytotoxic) agents can be included in the disclosed compositions. The cytotoxic agents can be covalently conjugated or form a fusion protein with one or more of the MGS peptides disclosed herein. The cytotoxic agent can be a protein. In an aspect, the cytotoxic agent can be a bacteria or plant toxin. In some aspects, the cytotoxic agent can be a plant toxin. In an aspect, the cytotoxic agent can be saporin or a biological variant thereof. The cytotoxic agent can be modified. In an aspect, the cytotoxic agent is a fragment of a bacterial or plant toxin.

[0078] The methods of making the conjugates or fusion proteins are known to one of ordinary skill in the art and can be carried out using known techniques. In an aspect, the MGS peptides are conjugated to a cytotoxic agent (e.g., saporin) using polyethylene glycol. It is within the skill of an ordinary person in the art to chemically conjugate the MGS peptides disclosed herein with the optimal PEG units.

[0079] *Labels*. Also described herein are composition comprising one or more molecular

guidance system (MGS) peptides and a label. For example, the compositions disclosed herein can include detectable labels. Such detectable labels can include, but are not limited to, a tag sequence designed for detection (e.g., purification or localization) of an expressed polypeptide or sequence. Tag sequences include, for example, green fluorescent protein, glutathione S-transferase, polyhistidine, c-myc, hemagglutinin, or Flag™ tag, and can be fused with an encoded nucleic acid. Such detectable labels can include, but are not limited to, a fluorescent agent, an enzymatic label, or a radioisotope.

PHARMACEUTICAL COMPOSITIONS

[0080] Disclosed herein are pharmaceutical compositions comprising one or more of the compositions disclosed herein and a pharmaceutical acceptable carrier described above. In some aspects, the MSG peptide can be SEQ ID NO: 3 and the cytotoxic agent can be a saporin or a biological variant thereof and the pharmaceutical composition is formulated for intravenous administration. The compositions of the present disclosure also contain a therapeutically effective amount of the cytotoxic agent as described herein. The compositions can be formulated for administration by any of a variety of routes of administration, and can include one or more physiologically acceptable excipients, which can vary depending on the route of administration. As used herein, the term "excipient" means any compound or substance, including those that can also be referred to as "carriers" or "diluents." Preparing pharmaceutical and physiologically acceptable compositions is considered routine in the art, and thus, one of ordinary skill in the art can consult numerous authorities for guidance if needed.

[0081] The pharmaceutical compositions as disclosed herein can be prepared for oral or parenteral administration. Pharmaceutical compositions prepared for parenteral administration include those prepared for intravenous (or intra-arterial), intramuscular, subcutaneous, intraperitoneal, transmucosal (e.g., intranasal, intravaginal, or rectal), or transdermal (e.g., topical) administration. Aerosol inhalation can also be used to deliver the fusion proteins. Thus, compositions can be prepared for parenteral administration that includes fusion proteins dissolved or suspended in an acceptable carrier, including but not limited to an aqueous carrier, such as water, buffered water, saline, buffered saline (e.g., PBS), and the like. One or more of the excipients included can help approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents, and the like. Where the compositions include a solid component (as they may for oral administration), one or more of the excipients can act as a binder or

filler (e.g., for the formulation of a tablet, a capsule, and the like). Where the compositions are formulated for application to the skin or to a mucosal surface, one or more of the excipients can be a solvent or emulsifier for the formulation of a cream, an ointment, and the like.

[0082] The pharmaceutical compositions can be sterile and sterilized by conventional sterilization techniques or sterile filtered. Aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation, which is encompassed by the present disclosure, can be combined with a sterile aqueous carrier prior to administration. The pH of the pharmaceutical compositions typically will be between 3 and 11 (e.g., between about 5 and 9) or between 6 and 8 (e.g., between about 7 and 8). The resulting compositions in solid form can be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. The composition in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

METHODS OF TREATMENT

[0083] Disclosed herein, are methods of treating a subject with cancer, the method comprising: (a) identifying a subject in need of treatment; and (b) administering to the subject a therapeutically effective amount of the pharmaceutical composition comprising one or more a molecular guidance system (MGS) peptides and a cytotoxic agent, and a pharmaceutically acceptable carrier. The MGS peptides can be any of the MGS peptides disclosed herein. The cytotoxic agent can be saporin or a biologically variant thereof.

[0084] Disclosed herein are methods of targeting an intracellular target. The method can comprise administering one or more MGS peptides conjugated to a cytotoxic agent. The cytotoxic agent can target an intracellular target. In an aspect, the intracellular target can be a lysosome, golgi apparatus, endoplasmic reticulum, cytoplasm, or nucleus.

[0085] In an aspect, skilled person can determine an efficacious dose, an efficacious schedule, or an efficacious route of administration for a disclosed composition or a disclosed fusion protein or a disclosed fusion protein so as to induce target an intracellular target such that the intracellular target can be inactivated.

[0086] In an aspect of any of the disclosed methods herein, the composition, conjugate or fusion protein described herein can be combined with one or more additional therapies. In an aspect, the composition, conjugate or fusion protein can be administered alone or in combination with other biologically active agents into compositions suitable for

administration to a subject. In an aspect, methods directed to treating subjects with cancer or at risk for developing cancer, the composition, conjugate or fusion protein disclosed herein can be combined with, for example, therapeutically effective amount of radiation therapy, immunotherapy or chemotherapy or a combination thereof. The combined therapy can be administered as a co-formulation, or separately. When administered separately, the combined therapy can be administered simultaneously or sequentially. The formulations can be made using methods routine in the art.

[0087] The pharmaceutical compositions described above can be formulated to include a therapeutically effective amount of a composition, conjugate or fusion protein as disclosed herein. Therapeutic administration encompasses prophylactic applications. Based on genetic testing and other prognostic methods, a physician in consultation with their patient can choose a prophylactic administration where the patient has a clinically determined predisposition or increased susceptibility (in some cases, a greatly increased susceptibility) to one or more autoimmune diseases or where the patient has a clinically determined predisposition or increased susceptibility (in some cases, a greatly increased susceptibility) to cancer.

[0088] The pharmaceutical compositions described herein can be administered to the subject (e.g., a human subject or human patient) in an amount sufficient to delay, reduce, or preferably prevent the onset of clinical disease. Accordingly, in some aspects, the subject is a human subject. In therapeutic applications, compositions are administered to a subject (e.g., a human subject) already with or diagnosed with an autoimmune disease in an amount sufficient to at least partially improve a sign or symptom or to inhibit the progression of (and preferably arrest) the symptoms of the condition, its complications, and consequences. An amount adequate to accomplish this is defined as a "therapeutically effective amount." A therapeutically effective amount of a pharmaceutical composition can be an amount that achieves a cure, but that outcome is only one among several that can be achieved. As noted, a therapeutically effective amount includes amounts that provide a treatment in which the onset or progression of the cancer is delayed, hindered, or prevented, or the autoimmune disease or a symptom of the autoimmune disease is ameliorated. One or more of the symptoms can be less severe. Recovery can be accelerated in an individual who has been treated.

[0089] In some aspects, the cancer can be a primary, secondary, refractory or relapsing tumor. In an aspect, the cancer can be lung cancer, breast cancer, colorectal cancer, ovarian cancer or pancreatic cancer.

[0090] Amounts effective for this use can depend on the severity of the cancer and the weight and general state and health of the subject. Suitable regimes for initial administration and booster administrations are typified by an initial administration followed by repeated doses at one or more hourly, daily, weekly, or monthly intervals by a subsequent administration.

[0091] The total effective amount of the conjugates or fusion proteins in the pharmaceutical compositions disclosed herein can be administered to a mammal as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol in which multiple doses are administered over a more prolonged period of time (e.g., a dose every 4-6, 8-12, 14-16, or 18-24 hours, or every 2-4 days, 1-2 weeks, or once a month). Alternatively, continuous intravenous infusions sufficient to maintain therapeutically effective concentrations in the blood are also within the scope of the present disclosure.

[0092] The therapeutically effective amount of the toxins (or cytotoxic agents) present within the compositions described herein and used in the methods as disclosed herein applied to mammals (e.g., humans) can be determined by one of ordinary skill in the art with consideration of individual differences in age, weight, and other general conditions (as mentioned above). Because the compositions, conjugates and fusion proteins of the present disclosure can be stable in serum and the bloodstream and in some cases more specific, the dosage of the compositions, conjugates and fusion proteins including any individual component can be lower (or higher) than an effective dose of any of the individual components when unbound. Accordingly, in some aspects, the toxin administered can have an increased efficacy or reduced side effects when administered as part of a conjugate or fusion protein as compared to when the toxin is administered alone or not as part of a conjugate or fusion protein.

Vectors

[0093] Disclosed are vectors comprising the nucleic acid sequence that encodes for one or more of the disclosed compositions. In some aspects, the vector comprises only a nucleic acid sequence capable of encoding one or more of the disclosed MGS peptides.

Kits

[0094] The materials described above as well as other materials can be packaged together in any suitable combination as a kit useful for performing, or aiding in the performance of,

the disclosed method. It is useful if the kit components in a given kit are designed and adapted for use together in the disclosed method. For example disclosed are kits comprising one or more of the disclosed compositions.

[0095] In some aspects the kits comprise a MGS peptide and a cytotoxic agent and instructions for conjugation.

[0096] In some aspects the kits comprise a cell line comprising the nucleic acid sequence that encodes one or more of the MGS peptides.

EXAMPLES

Example 1: HCC15.2 shows specificity to and internalizes in cancer cells

[0097] In order for the peptides to be clinically useful reagents, discriminating between normal and cancer cells can be important. FIG. 1 shows that the MGS peptide, HCC15.2, accumulates in cancer cells and is specific compared to control. HCC15.2 binding was analyzed by flow cytometry analysis (see, FIG. 1). The HCC15.2 does not bind to normal HBEC's but binds to the LC line H1299. The binding was sequence dependent and not specific for a single histopathological class (see, FIG. 2).

[0098] MGS peptides were synthesized using standard solid-phase FMOC techniques on a Prelude synthesizer (Protein Technologies Inc.). MGS peptides were purified via reverse phase HPLC on a C18 prep column to >95% purity, and verified by MALDI-TOF. The optimized HCC15.2 Peptide sequence is Ac-FHAVPQSFYT-PEG11-Biotin and has a molecular weight of 2394 Da, and Ac-FHAVPQSFYT-PEG11-C with a molecular weight of 1940 Da. Tetrameric HCC15.2 was synthesized via previously published protocols. Stock solutions were made in PBS pH 7.4 and concentration determined by absorbance at 280 nM on a Nanodrop 2000 (Thermo Fisher Scientific).

[0099] Flow Cytometry. Biotinylated peptide was conjugated to streptavidin-R-phycoerythrin (1:1) for 30 min at RT. The remaining binding sites on streptavidin were quenched with RPMI 1640 and solution diluted to 25 nM. Tumor cells were grown to 90% confluency in a 12 well plate, then incubated with 500 µl peptide-dye conjugate for 1 hour at 37 C. After 1 hour, peptide was removed and the cells were washed 3x with PBS for 5 min, 2x with acid rinse, and 1x PBS rinse. Added 300 µl trypsin until cells lifted then added 700 µl RPMI +5% FBS to inactivate the trypsin. Cells were transferred to a flow tube, and put on ice in the dark. Flow cytometry was run on BD FACSCelesta and data were analyzed on Flowing software. A region containing < 5% of the cells in the negative control is established and the McGuire score is calculated for each sample by multiplying the % of positive cells by the

fluorescence intensity.

[00100] For the flow cytometry experiments, cells are treated with a peptide (e.g., a MGS peptide) that has a dye on it for one hour. If the peptide enters the cell(s), the dye will also enter the cell(s) (see, FIG. 11). Next, the cells are washed and placed in a tube to run flow cytometry. The flow cytometer can measure the brightness of 10,000 or more individual cells.

[00101] Confocal Microscopy. Plasmids with organelle specific markers labeled with GFP were purchased from Addgene and electroporated into H1299 cells. After G418 selection, GFP-labeled tumor cells were plated on 8 well chamber slides on the day previous to the study. Biotinylated peptide was conjugated to streptavidin-AlexaFluor 555 (1:1) for 30 min at RT and quenched with RPMI, then added to the wells at 50 nM. After 1 hour incubation cells were washed for 5 min 3x with PBS, 2x acid rinses, and 1x PBS rinse. Cells were fixed in 2% formaldehyde for 10 min, washed with PBS, stained with DAPI in mounting media and coverslip was added. Microscopy was acquired on a Zeiss LSM 700 with a Pln Apo 63x/1.4 oil DIC III objective. Compressed images were obtained using ImageJ software maximal intensity projections.

[00102] For the microscopy experiments, cells are treated with a peptide (e.g., a MGS peptide) that has a dye on it for one hour. If the peptide enters the cell(s), the dye will also enter the cell(s) (see, FIG. 1). Next, the cells are washed and fixed to glass plate. Other parts of the cell (e.g., cell membrane, organelles or nucleus) are stained. Fluorescent microscopy is performed to assess the dyes in the cells. Confocal microscopy is used to take a series of sliced images that can be used to build a 3D picture (like an MRI).

Example 2: HCC15.2 Internalization is Receptor Mediated

[00103] Experiments were carried out that show that HCC15.2 can be internalized and this internalization is receptor mediated (see, FIG. 3). More specifically, HCC15.2 binds to a specific subset of NSCLC and other tumor cells (see, FIG. 12). Phage blocking was used as a control. HCC15.2 was internalized by a specific panel of cell lines and not others. The results also show that the internalization mediated by a receptor was sequence specific. Treating with trypsin 2 min before adding HCC15.2 resulted in no intnernalization. Also, HCC15.2 does not internalize at 4° C.

[00104] FIG. 4 shows that the tetramerization of HCC15.2 does not significantly improve receptor mediated internalization. This result is surprising as most peptides studied and identified through the FOX3 molecular guidance system had non-additive increase in

binding/internalization.

Example 3: Truncations of HCC15.2.

[00105] FIG. 5 and FIG. 15 show that the truncations from N- and C-Terminal ends of the HCC15.2 peptide reveal minimal binding sequence.

[00106] FIG. 6 shows that acetylation improves binding of the truncated peptide.

Example 4: Peptide co-localizes with the lysosome.

[00107] FIG. 7 shows that the MGS peptide co-localizes with the lysosome and accumulates in the lysosome over time (see, FIG. 8 and FIG. 16).

Example 5: HCC15.2 conjugated to saporin.

[00108] Additional experiments were conducted to examine HCC15.2 conjugated to saporin in cell viability assays (see, FIG. 9 and Table 2). For this, tumor cell lines were plated on black-walled, clear flat-bottom, 96 well plates. The next day, media was replaced with a concentration gradient of Hcc15.2-Saporin, Saporin alone, or no treatment. After 1 hr incubation, treatment was removed and replaced with culture media. 72 hours later cell viability was measured using Cell Titer Glo ® and luminescence was measured on an Analyst HT from LjL Biosystems. IC50s were calculated on Graphpad Prizm.

[00109] A cancer cell killing assay was performed (see, FIG. 22). Cancer cells were put into a dish. After 24 hours, cells were given different doses of peptide-saporin for 1 hour. Peptide-saporin was washed off and cells were left for 72 more hours. Assay to measure live cells performed. FIG. 21 shows that HCC15.2 targets saporin to cancer cells *in vitro*. These results demonstrate that saporin did not enter the cell by itself, however, saporin did enter the cell when conjugated to a required MGS peptide (e.g., HCC15.2). The results also show that HCC15.2-saporin lowers off target effects and escapes intracellular vesicles.

Table 2. Cell viability in multiple cell lines.

Cell Line	Peptide-Drug	# of Repeats	Average KD
H1299	15.2-Saporin	3	4.97e-09
	Control-Saporin	2	1.05e-07
H2009	15.2-Saporin	2	5.55e-09
	Control-Saporin	2	2.43e-07
HBEC	15.2-Saporin	2	2.58e-07
	Control-Saporin	1	4.18e-07

[00110] FIG. 10 shows that HCC15.2 conjugated to saporin reduces tumor growth in a human NSCLC xenograft model compared to free-saporin.

Example 6: Tetramerization does not significantly improve half maximal binding.

[00111] Flow cytometry is carried out to measure KD. Cells are treated with increasing concentrations of peptide-dye for one hour. MGS peptide(s) that enter or go into the cells, take the dye with it. Cells are washed and placed in a tube to run flow cytometry. The flow cytometer can measure brightness of 10,000 or more individual cells. Each concentration is quantified and plotted (see, FIGS. 13 and 14). The curves are mathematically fitted to data and the halfway point is KD (e.g., a measure of how good the binding is).

Example 7: HCC15.2-targeted NIR dye accumulates into tumors *in vivo*.

[00112] *In Vivo* Imaging. H2009 tumor cells were suspended in 106 cells/100 µl sterile PBS and were injected subcutaneously on the flank of female athymic nude mice (Jackson Labs.) Cysteine labeled peptides were conjugated to maleimide Alexafluor-750 C5 (1:1.1) in sterile PBS pH 7.4 for 1 hour. Peptide-dye conjugate was diluted in sterile PBS to 15 µg dye per 100 µl and 100ul were injected intravenously via lateral tail vein into 4 mice/group. Mice were anesthetized with Isothesia and whole animal images were collected on an IVIS at 12, 24, 48, and 72 hours. *Ex vivo* tumors and organs were then weighed and imaged at 72 hours.

[00113] *In vivo* animal imaging studies were carried out. For these experiments, human cancer cells were placed under the skin of mice and allowed to grow a tumor to a certain size. Peptide with dye was injected by IV when tumors became a predetermined size. Animals were anesthetized and imaged at 12, 24, 48, and 72 hours. Organs and tumors were imaged at 72 hours. Yellow shows most peptide, maroon/red shows less peptide, no color is very

little/no peptide. The HCC15.2-targeted NIR dye accumulates into tumors (see, FIG. 17) and is retained in the tumor over time (see, FIG. 18). FIGS. 19 and 20 show the results of *ex vivo* imaging.

[00114] Subcutaneous Tumor Growth Assay. H2009 tumors were established subcutaneously on the flank of athymic nude mice (Jackson Labs). Saporin conjugated to Streptavidin (SAZAP) was purchased from Advanced Targeting Systems and conjugated 1:1 with optimized, biotinylated Hcc15.2 and control peptides. When the tumors reached ~1003 mM in size, mice were injected with nontargeted saporin (no MGS peptide), 15.2-saporin conjugate, or nothing via lateral tail vein IV. Targeted and nontargeted saporin toxin was administered 2x/week for 2.5 weeks at ~7 ug/dose. Tumors were blindly measured every other day with calipers and tumor volumes were calculated using the equation $\pi/6*(length*width)^{3/2}$.

[00115] For the next set of experiments, human cancer cells were placed under the skin of mice and allowed to grow a tumor to a certain and predetermined size. The peptide conjugated or linked to saporin was injected by IV when tumors reached the predetermined size. Animals received 5 doses over 2.5 weeks of 7 or 7.5 μg/dose. Tumors were measured every other day. FIG. 23 shows that HCC15.2 targeting of saporin markedly slowed tumor growth.

Example 8: Further characterization of the H1299.3 peptide.

[00116] A modified MGS peptide (SEQ ID NO: 31) that fuses amino acids 1-5 and 11-20 of the original MGS peptide: LQWRRNFGVWARYRL (SEQ ID NO: 31) was assessed. This MGS peptide maintains its cancer specificity and increases its ability to traffic to the autophagosome. Acetylation protects degradation *in vivo* and improves solubility. This MGS peptide hones to a NSCLC tumor in an animal (see, FIG. 27). The data show that no tumor targeting was observed for the nonacetylated version, This MGS peptide (e.g., the modified 15-mer) has >2-fold reduction in heart, lung and kidney tumors (see, FIG. 27). 1299.3 Ac-15mer cell binding results are shown in FIG. 28.

[00117] Cell Culture. Human NSCLC lines were provided. Cells were cultured in RPMI 1640 with L-glutamine and 5% FBS at 37° C and 5% CO2.

Example 9: *In vitro* delivery of saporin with other peptides.

[00118] FIG. 29 shows the IC50 in various cell lines of the administration of saporin conjugated or linked to select MGS peptides.

[00119] It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other aspects of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

[00120] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the method and compositions described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising one or more molecular guidance system (MGS) peptides and a cytotoxic agent.
2. The composition of claim 1, wherein the one or more MGS peptides comprise SEQ ID NO: 1, 2, 3, 34, 35, 36, 37, 38, 39, 40, 41, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 5, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or a combination thereof.
3. The composition of any of claims 1-2, wherein the one or more MGS peptides comprise SEQ ID NOs: 1, 2, 3, 34, 35, 36, 37, 38, 39, 40, 41, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 5, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83 or 84.
4. The composition of any of claims 1-3, wherein the cytotoxic agent is saporin or a biologically active variant thereof.
5. The composition of claim 1, wherein the one or more MGS peptides is SEQ ID NO: 3 and the cytotoxic agent is saporin.
6. The composition of claim 1, wherein the one or more are four MGS peptides.
7. The composition of claim 6, wherein the one or more MGS peptides form a tetrameric scaffold protein.
8. The composition of claim 1, wherein the one or more MGS peptides are acetylated on the N-terminus.
9. The composition of any of claims 1-8, wherein the one or more MGS peptides are chemically conjugated to the cytotoxic agent.

10. The composition of claim 9, wherein chemical conjugate is polyethylene glycol (PEG).
11. The composition of claim 10, wherein the PEG is 11 units in length.
12. The composition of claim 1, wherein the one or more MGS proteins comprises SEQ ID NO: 3, wherein SEQ ID NO: 3 is acetylated on the N-terminus and is chemically conjugated to PEG; and the cytotoxic agent is saporin, wherein the saporin is covalently attached to PEG.
13. A membrane-permeable conjugate for transport across a lipid membrane comprising: one or more a molecular guidance system (MGS) peptides and a cytotoxic agent.
14. The conjugate of claim 13, wherein the one or more MGS peptides comprises SEQ ID NOs: 1, 2 3, 34, 35, 36, 37, 38, 39, 40, 41, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 5, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or a combination thereof.
15. The conjugate of any of claims 13-14, wherein the one or more MGS peptides comprises SEQ ID NOs: 1, 2, 3, 34, 35, 36, 37, 38, 39, 40, 41, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 5, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, or 84.
16. The composition of any of claims 13-15, wherein the cytotoxic agent is saporin or a biologically active variant thereof.
17. The composition of claim 13, wherein the one or more MGS peptides is SEQ ID NO: 3 and the cytotoxic agent is saporin.
18. The composition of claim 13, wherein the one or more MGS peptides are four MGS peptides.
19. The composition of claim 13, wherein the one or more MGS peptides form a tetrameric scaffold protein.

20. The composition of claim 13, wherein the one or more MGS peptides are acetylated on the N-terminus.
21. The composition of any of claims 13-20, wherein the one or more MGS peptide are chemically conjugated to the cytotoxic agent.
22. The composition of claim 21, wherein chemical conjugate is polyethylene glycol (PEG).
23. The composition of claim 22, wherein the PEG is 11 units in length.
24. The composition of claim 13, wherein the one or more MGS proteins comprises SEQ ID NO: 3, wherein SEQ ID NO: 3 is acetylated on the N-terminus and is chemically conjugated to PEG; and the cytotoxic agent is saporin, wherein the saporin is covalently attached to PEG.
25. A pharmaceutical composition comprising the composition of claim 1 and pharmaceutically acceptable carrier.
26. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is formulated for intravenous administration.
27. A method of treating a cancer, the method comprising: (a) identifying a patient in need of treatment; (b) administering to the patient a therapeutically effective amount of the composition of any of claims 1 - 27; and (c) a pharmaceutically acceptable carrier.
28. The method of claim 27, wherein the patient is a human patient.
29. The method of claim 27, wherein the cancer is a primary, secondary, refractory or relapsing tumor.
30. The method of claim 27, wherein the cancer is lung cancer, breast cancer, colorectal cancer, ovarian cancer or pancreatic cancer.

31. The method of claim 27, further comprising administering to the patient a therapeutically effective amount of radiation therapy, immunotherapy or chemotherapy or a combination thereof.
32. A method of targeting an intracellular target, the method comprising administering one or more MGS peptides conjugated to a cytotoxic agent, wherein the cytotoxic agent targets an intracellular target.
33. The method of claim 32, wherein the intracellular target is the lysosome.
34. A composition comprising one or more molecular guidance system (MGS) peptides and a cytotoxic agent, wherein the one or more MGS peptides are selected from group listed in Table 1.
35. An MGS peptide as disclosed herein.
36. A nucleic acid sequence capable of encoding an MGS peptide disclosed herein.
37. A vector comprising the nucleic acid sequence of claim 36.
38. A cell line comprising the MGS peptide of claim 34, the nucleic acid sequence of claim 36 or the vector of claim 37.
39. An MGS peptide comprising the sequence of SEQ ID NO: 1, 2, 3, 34, 35, 36, 37, 38, 39, 40, 41, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 5, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83 or 84.
40. A nucleic acid sequence capable of encoding an MGS peptide of claim 39.
41. A vector comprising the nucleic acid sequence of claim 40.
42. A cell line comprising the MGS peptide of claim 39, the nucleic acid sequence of claim 40 or the vector of claim 41.

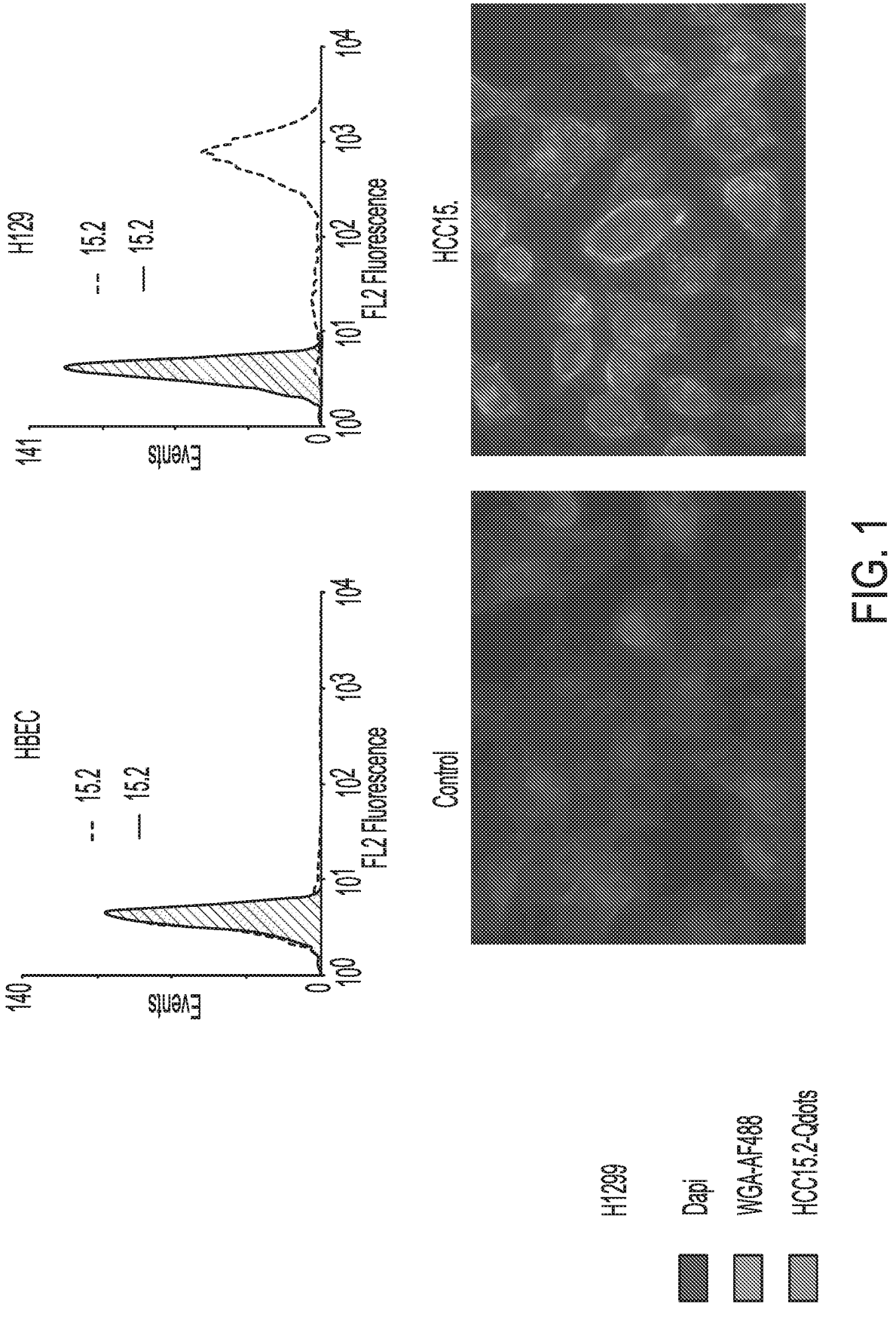

H1299
Dapi
WGA-AF488
HCC15.2-Qdots
Control
HCC15.
HBEC
H1299
-- 15.2
— 15.2
Events
FL2 Fluorescence
0
100
101
102
103
104
140
141

FIG. 1

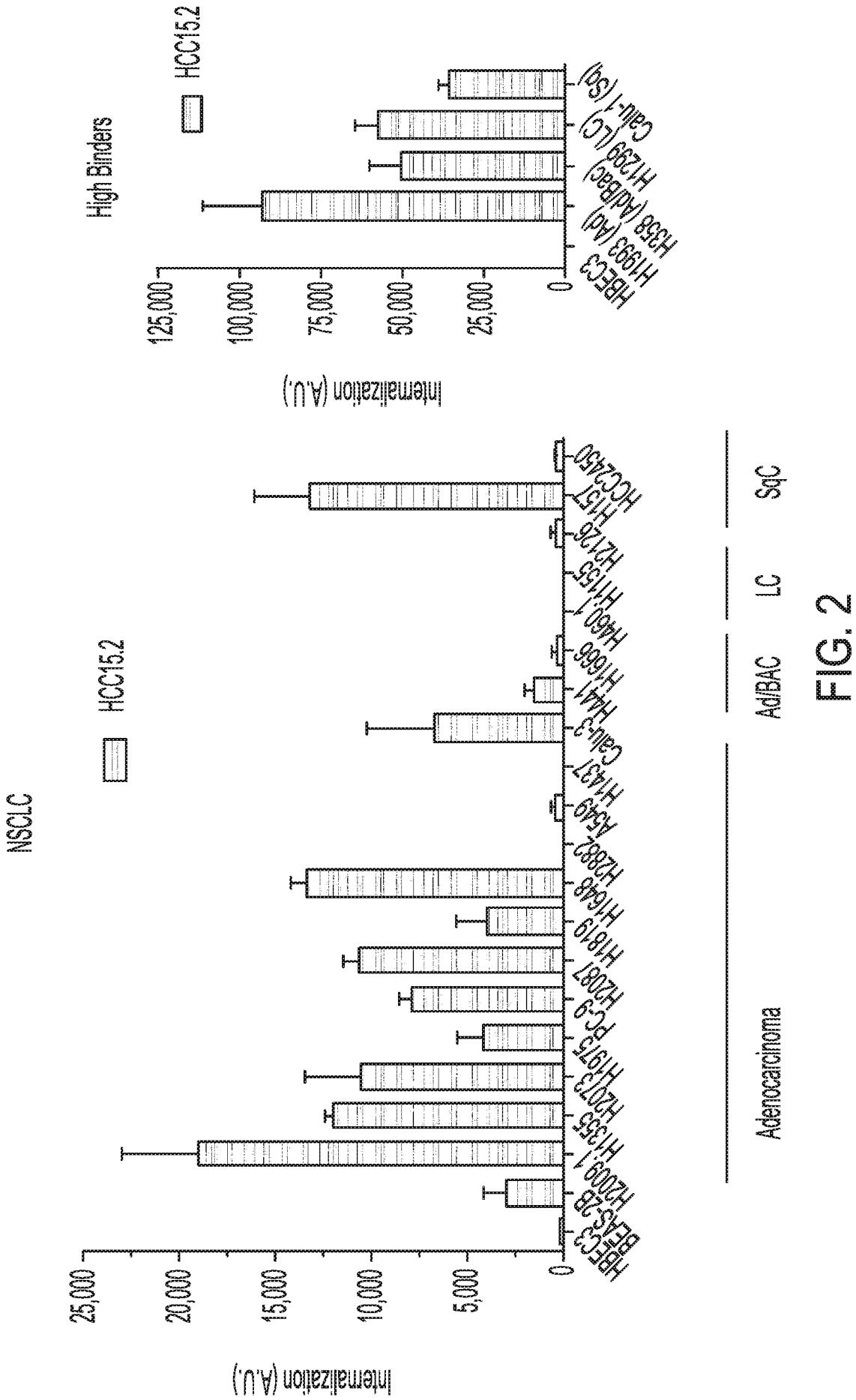
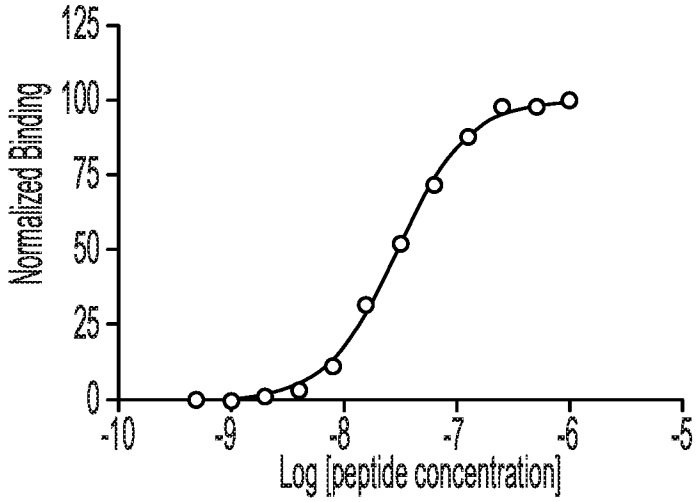

High Binders
HCC15.2
Internalization (A.U.)
125,000
100,000
75,000
50,000
25,000
0
HBEC3
H1993 (Ad)
H358 (Ad/Bac)
H1299 (LC)
Calu-1 (Sq)
NSCLC
HCC15.2
Internalization (A.U.)
25,000
20,000
15,000
10,000
5,000
0
HBEC3
BEAS-2B
H2009
H1355
H2073
H1975
PC-9
H2087
H819
H1648
H2882
A549
H1437
Calu-3
H441
H1666
H460
H1155
H2126
H157
HCC2450
Adenocarcinoma
Ad/BAC
LC
SqC

FIG. 2



Half Maximal Internalization

26 ± 4.5 nM

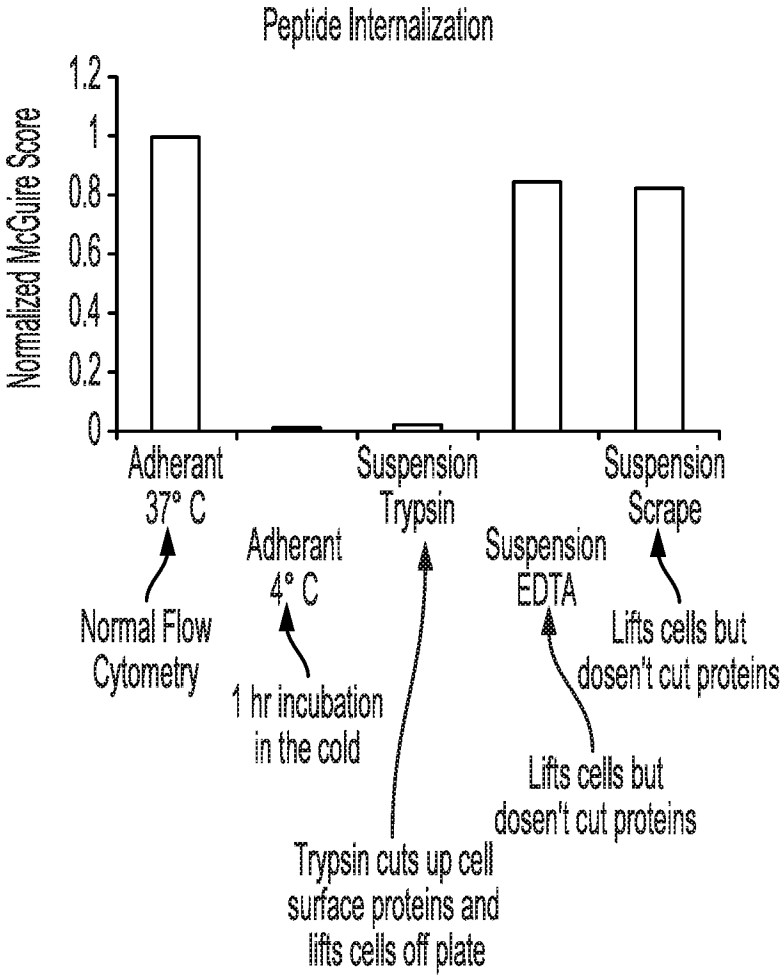
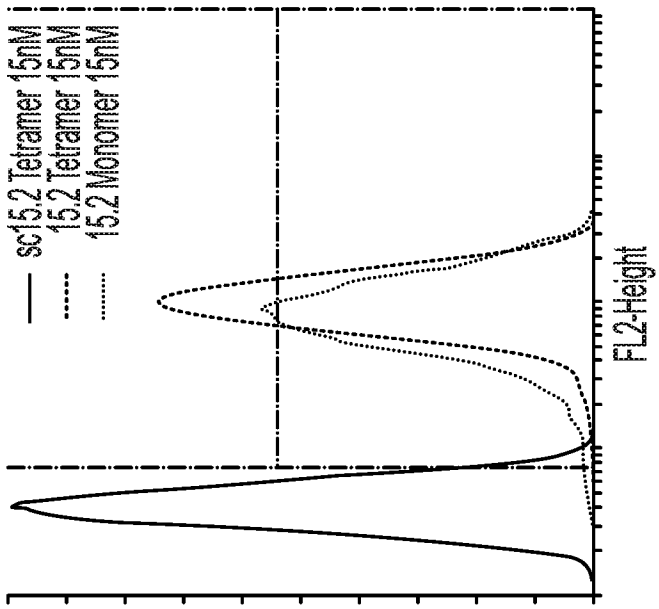


FIG. 3


sc15.2 Tetramer 15nM
15.2 Tetramer 15nM
15.2 Monomer 15nM
FL2-Height

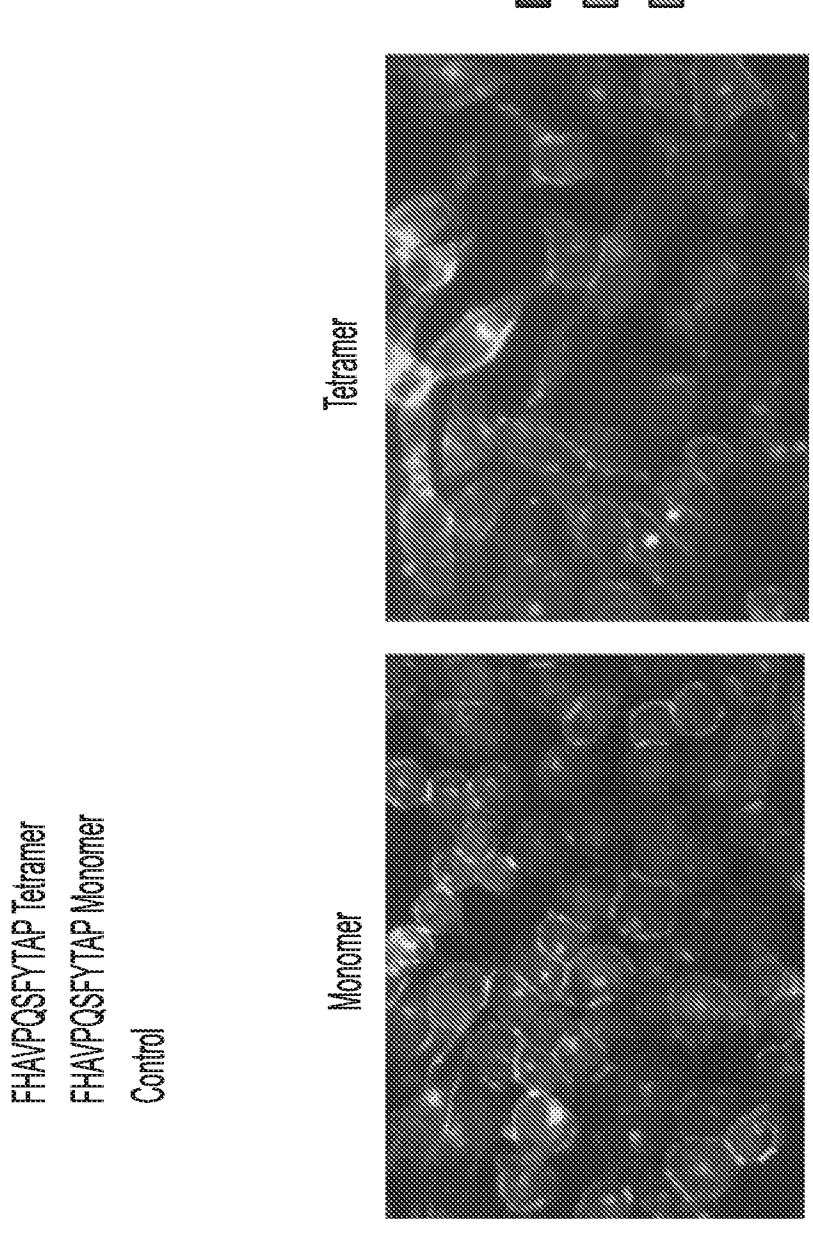

Dapi
WGA-AF488
15.2-AF555
Tetramer
Monomer
FHAVPQSFYTAP Tetramer
FHAVPQSFYTAP Monomer
Control

FIG. 4

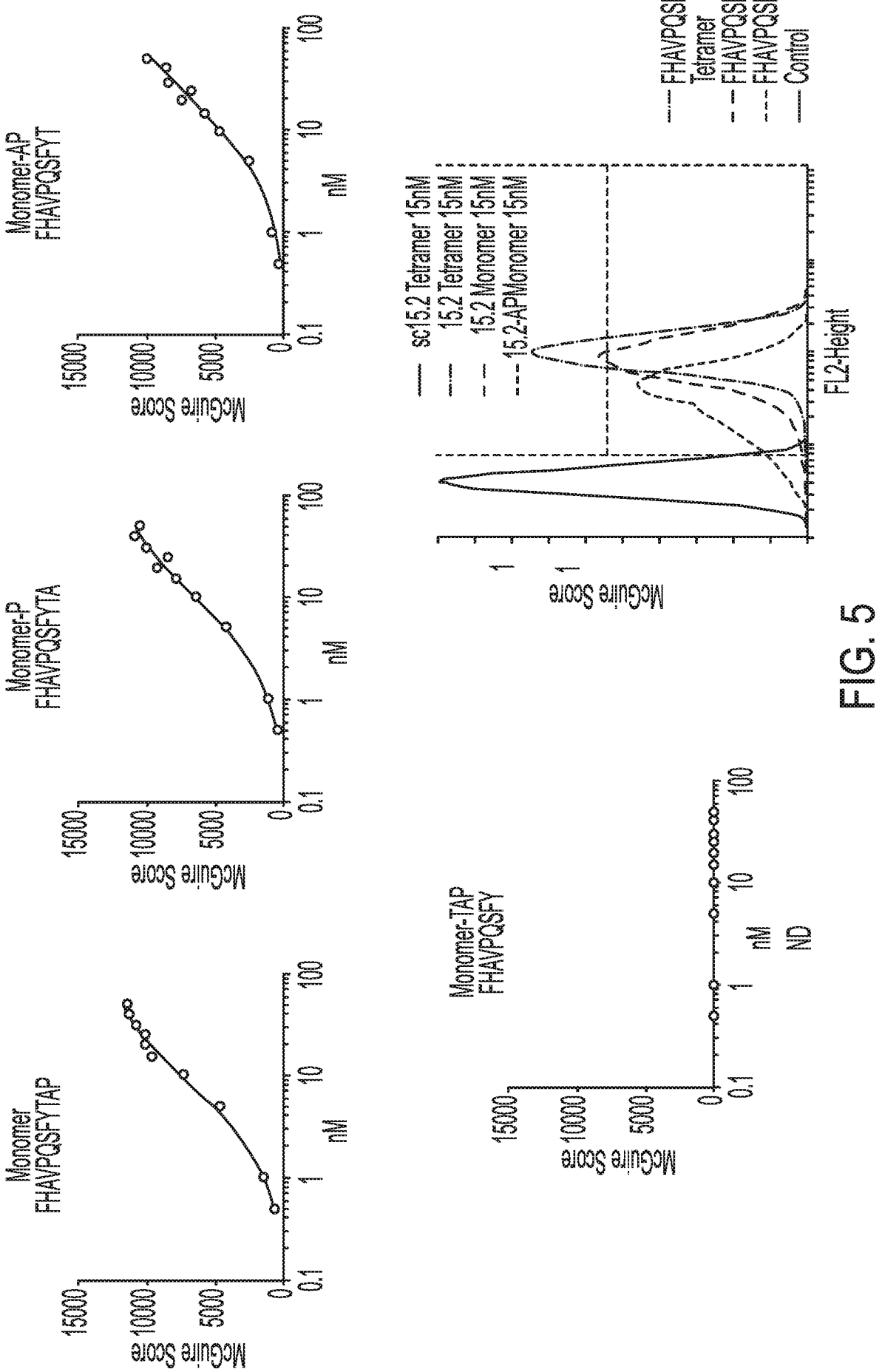
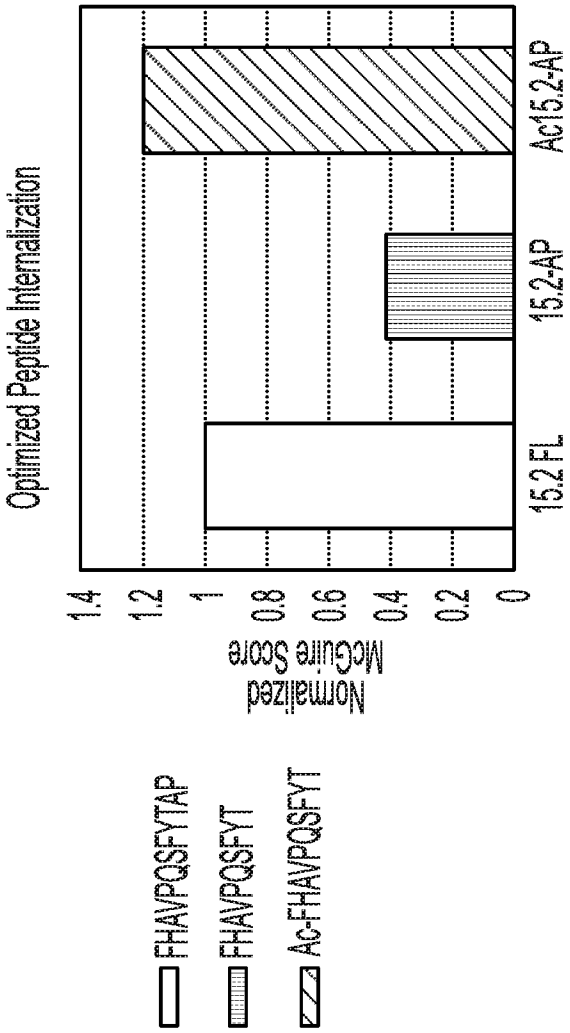
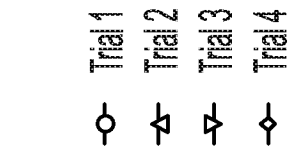

Monomer-AP
FHAVPQSFYT
Monomer-P
FHAVPQSFYTA
Monomer
FHAVPQSFYTAP
Monomer-TAP
FHAVPQSFY
McGuire Score
nM
ND
sc15.2 Tetramer 15nM
15.2 Tetramer 15nM
15.2 Monomer 15nM
15.2-APMonomer 15nM
FHAVPQSFYTAP Tetramer
FHAVPQSFYTAP
FHAVPQSFYT
Control
FL2-Height

FIG. 5



KD
Trial 1	2.93e-9
Trial 2	3.10e-9
Trial 3	1.69e-9
Trial 4	2.93e-9
Average	2.66e-9 ±0.57e-9



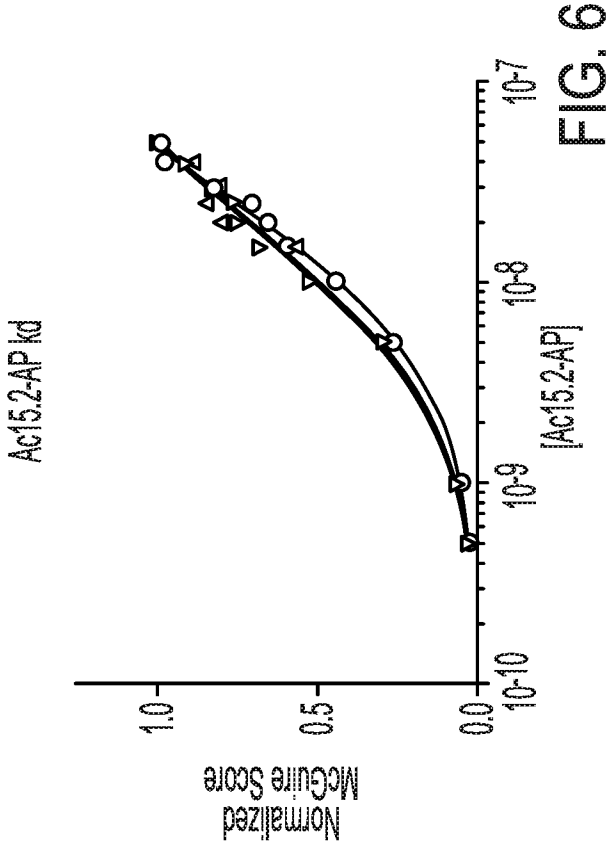


FIG. 6

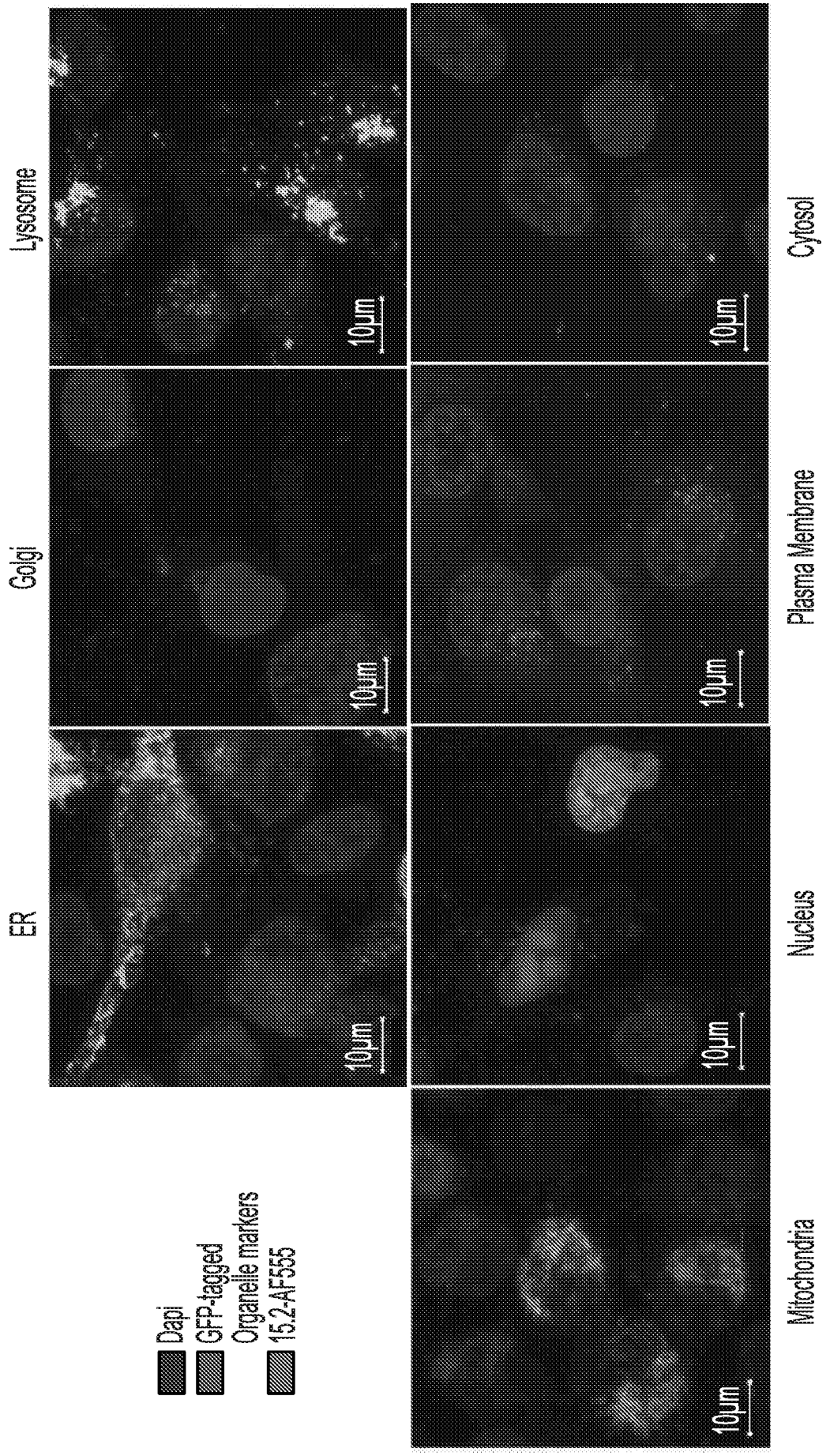

Lysosome
Golgi
ER
Mitochondria
Nucleus
Plasma Membrane
Cytosol
10μm
Dapi
GFP-tagged Organelle markers
15.2-AF555

FIG. 7

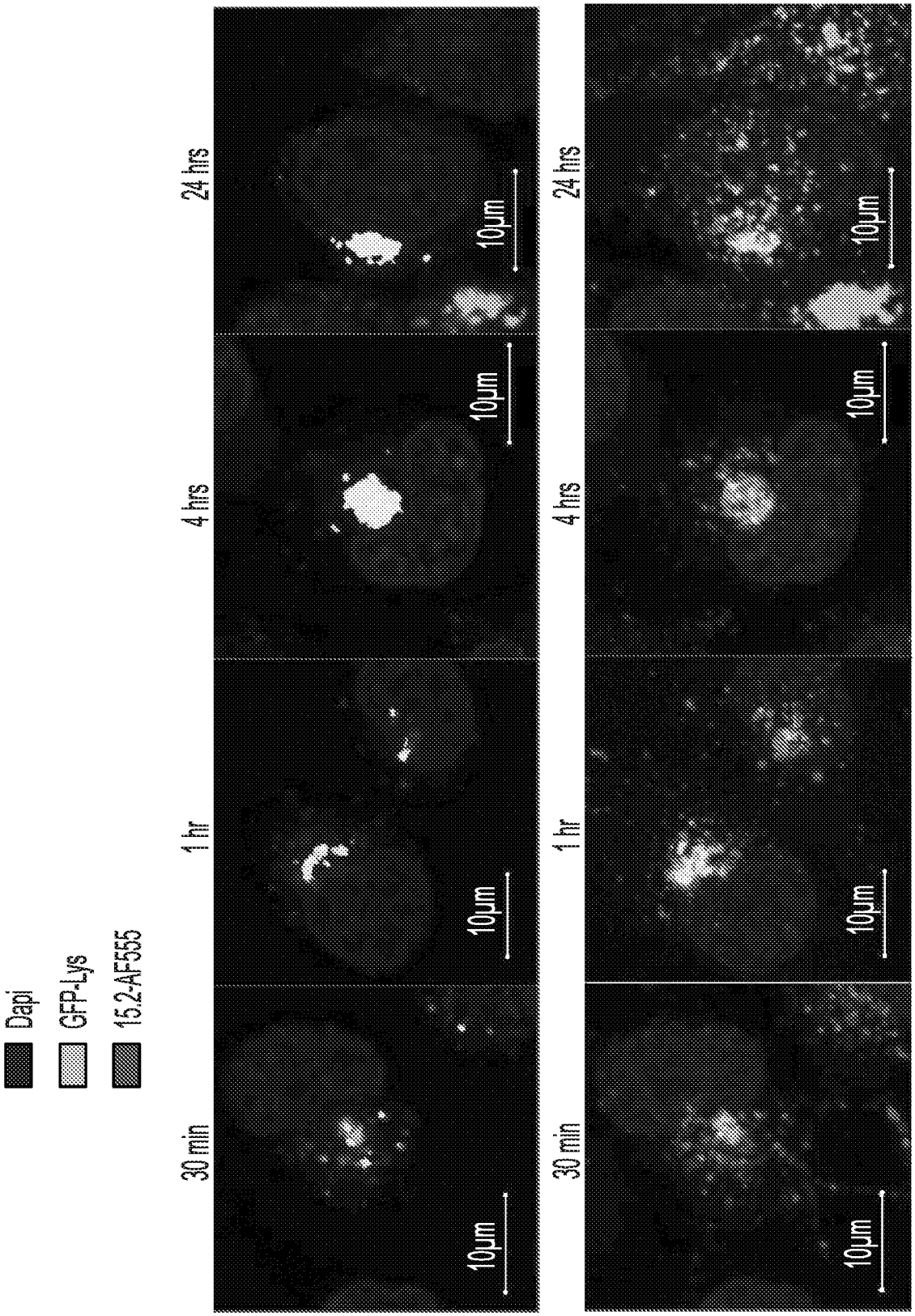


FIG. 8

	IC50
Trial 1	4.04e-9
Trial 2	3.97e-9
Trial 3	8.28e-9
Average	5.43e-9 ±2.47e-9

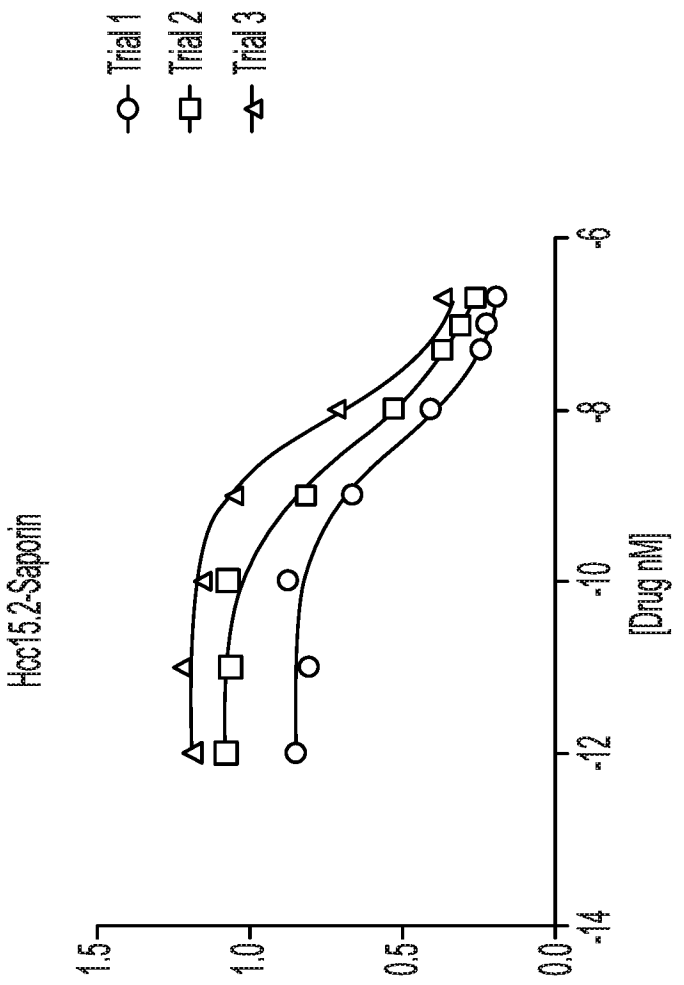


FIG. 9

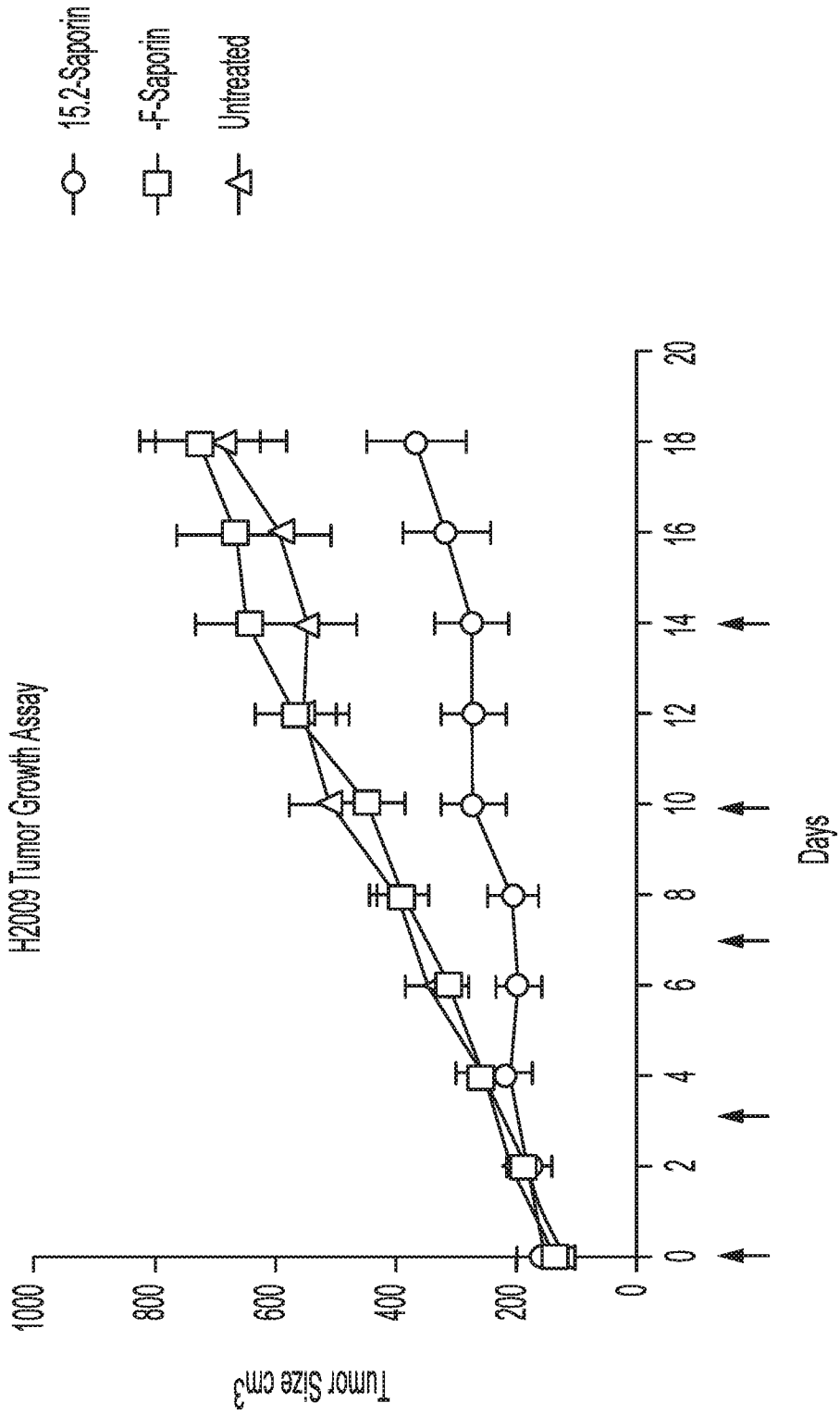

15.2-Saporin
-F-Saporin
Untreated
H2009 Tumor Growth Assay
Tumor Size cm3
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
Days

FIG. 10

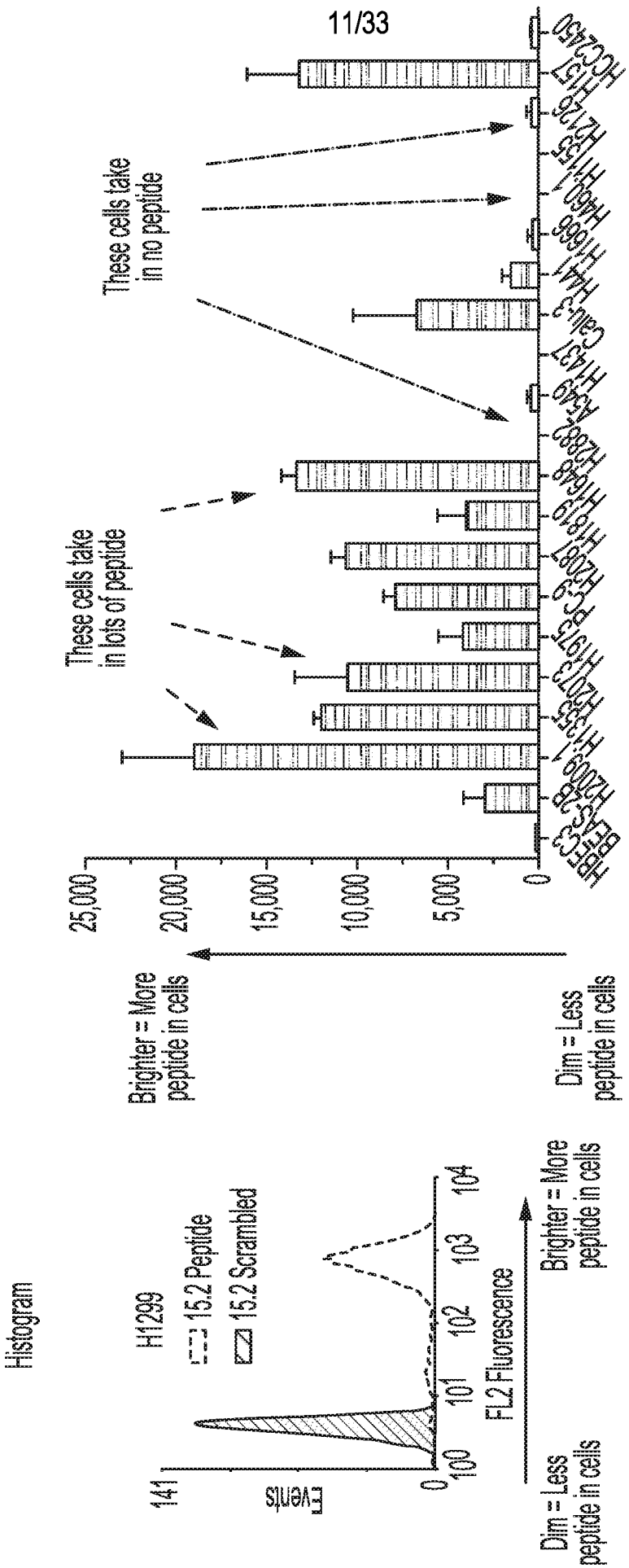

Quantification of Histogram
These cells take in no peptide
These cells take in lots of peptide
25,000
20,000
15,000
10,000
5,000
0
HBEC3
BEAS-2B
H2009.1
H1355
H2073
H1975
PC9
H2087
H1819
H1648
H2882
A549
H1437
Calu-3
H441
H1666
H460
H1355
H2126
H157
HCC2450
Brighter = More peptide in cells
Dim = Less peptide in cells
Histogram
H1299
15.2 Peptide
15.2 Scrambled
141
0
100
101
102
103
104
FL2 Fluorescence
Dim = Less peptide in cells
Brighter = More peptide in cells

FIG. 11

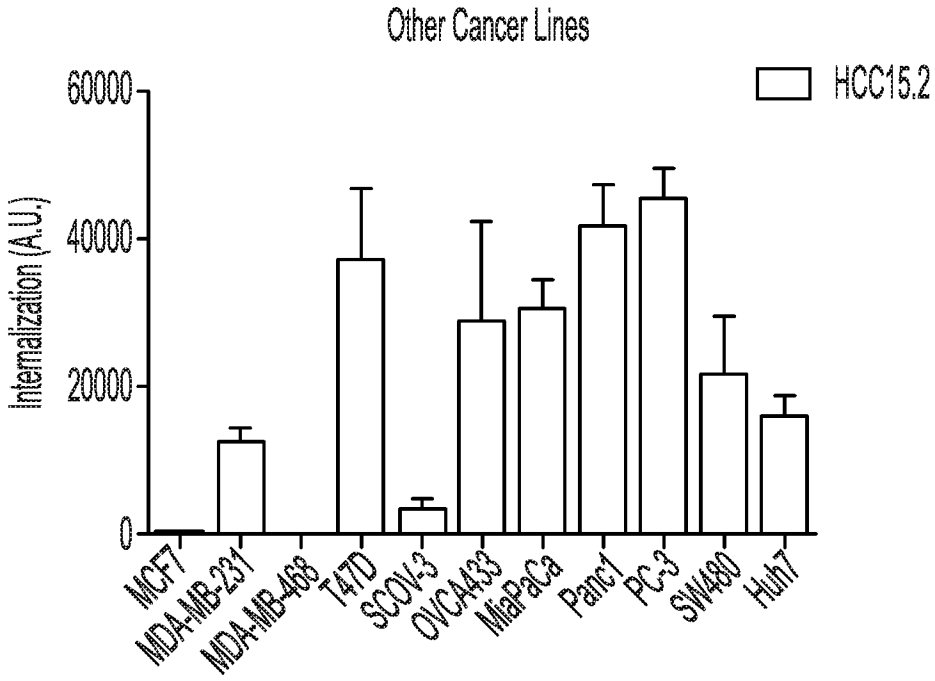

Other Cancer Lines
HCC15.2
Internalization (A.U.)
60000
40000
20000
0
MCF7
MDA-MB-231
MDA-MB-468
T47D
SCOV-3
OVCA433
MiaPaCa
Panc1
PC-3
SW480
Huh7

FIG. 12

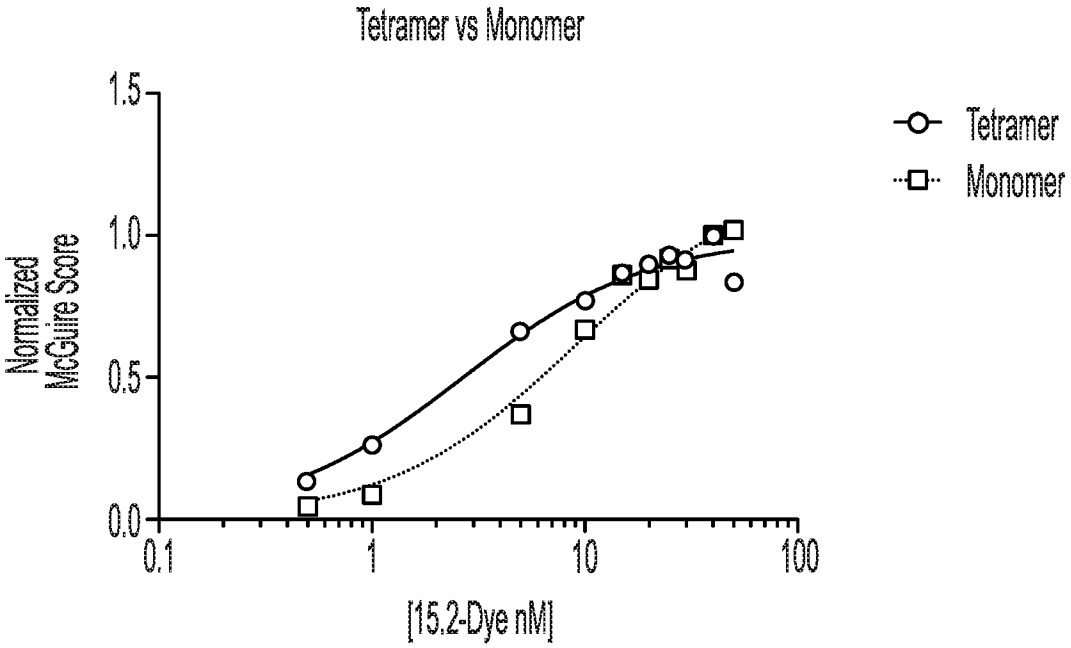

Tetramer vs Monomer
Tetramer
Monomer
Normalized McGuire Score
1.5
1.0
0.5
0.0
0.1
1
10
100
[15.2-Dye nM]

FIG. 13

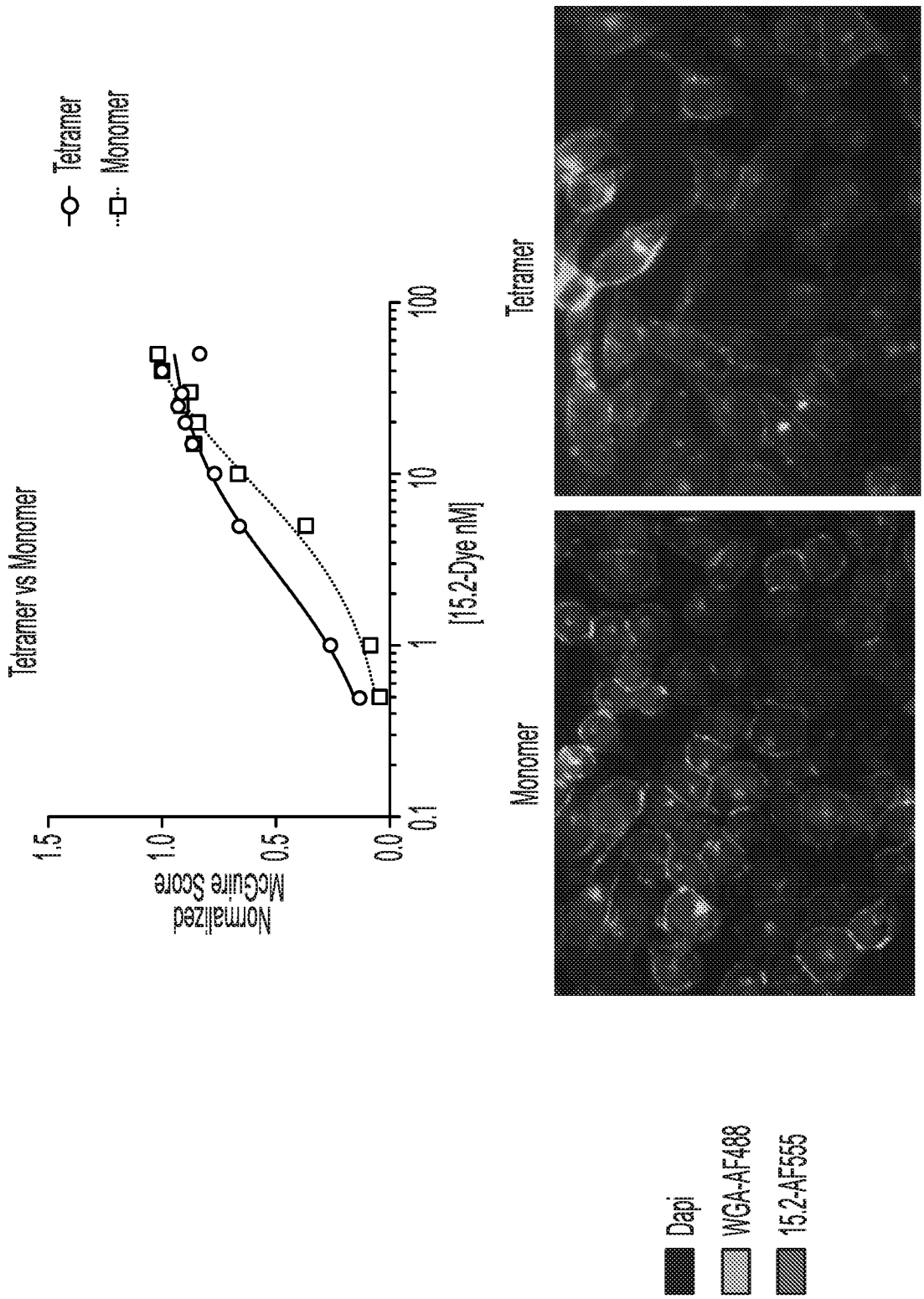
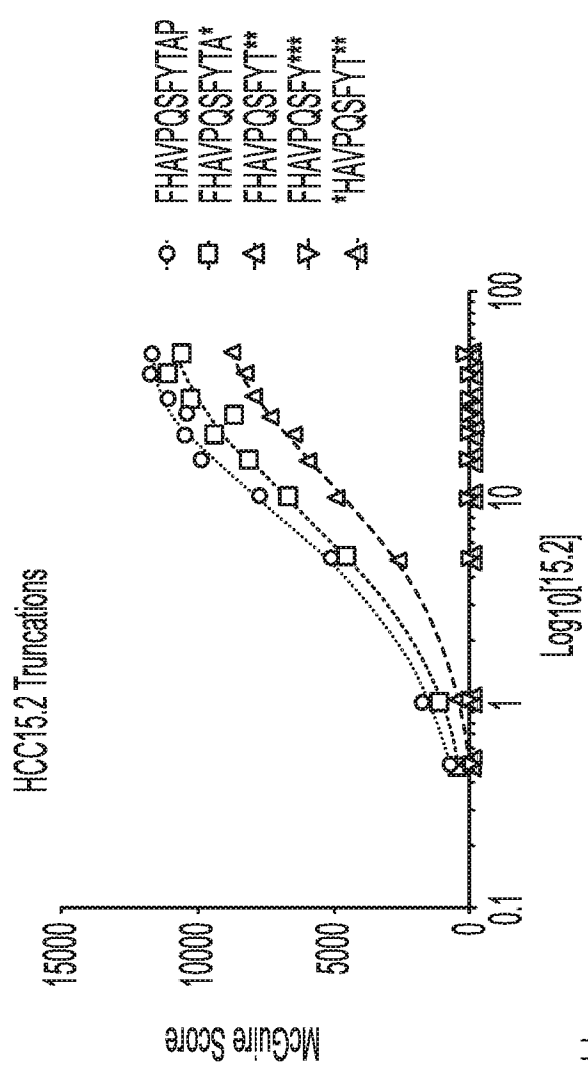

Tetramer vs Monomer
Tetramer
Monomer
Normalized McGuire Score
1.5
1.0
0.5
0.0
0.1
1
10
100
[15.2-Dye nM]
Tetramer
Monomer
Dapi
WGA-AF488
15.2-AF555

FIG. 14



Peptide	Valency	KD
FHAVPQSFYTAP	Tetramer	3.73 ± 0.57 nM
FHAVPQSFYTAP	Monomer	6.38 ± 1.88 nM
FHAVPQSFYTA*	Monomer	12.97 ± 2.07 nM
FHAVPQSFYT**	Monomer	20.32 ± 2.00 nM
FHAVPQSFY***	Monomer	ND
*HAVPQSFYT**	Monomer	ND
(Ac) FHAVPQSFYT**	Monomer	2.57 ± 0.38 nM

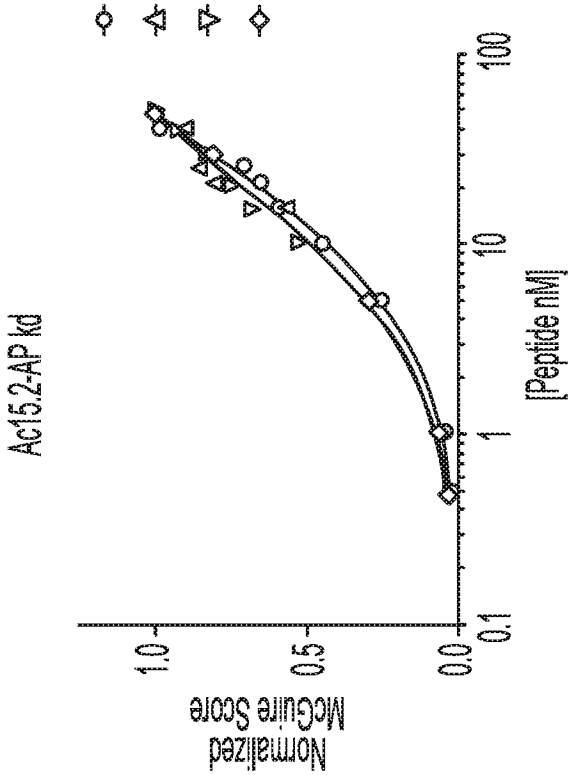


FIG. 15

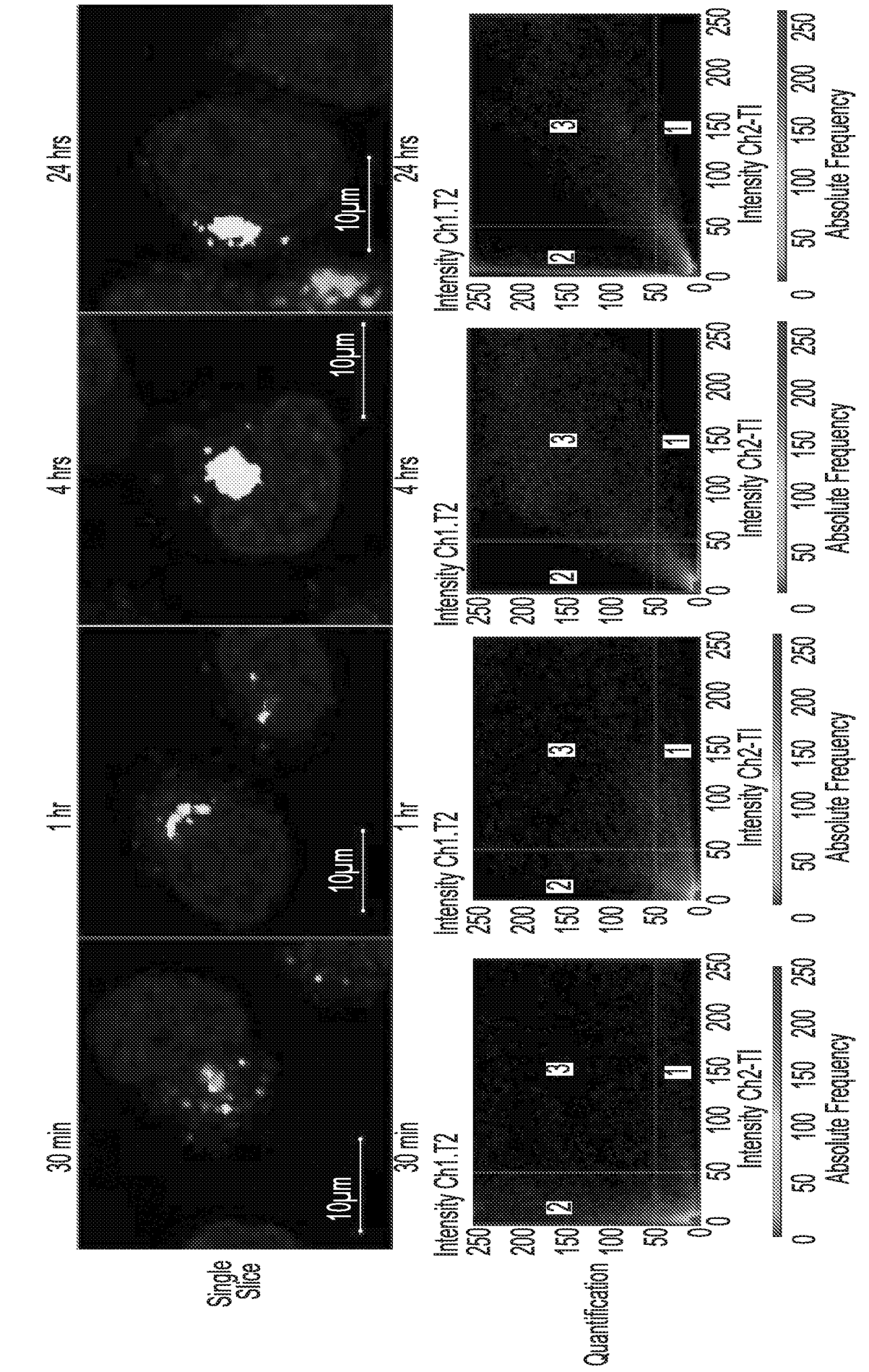

30 min
1 hr
4 hrs
24 hrs
Single Slice
Quantification
10µm
Intensity Ch1.T2
Intensity Ch2-TI
Absolute Frequency
1
2
3

FIG. 16

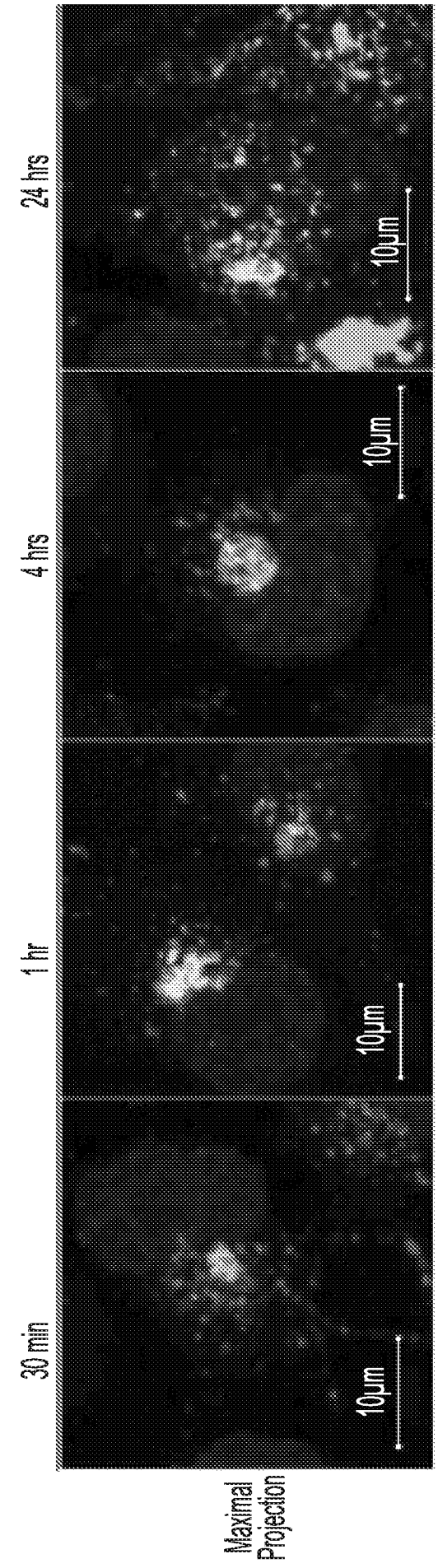


FIG. 16
CONTINUED

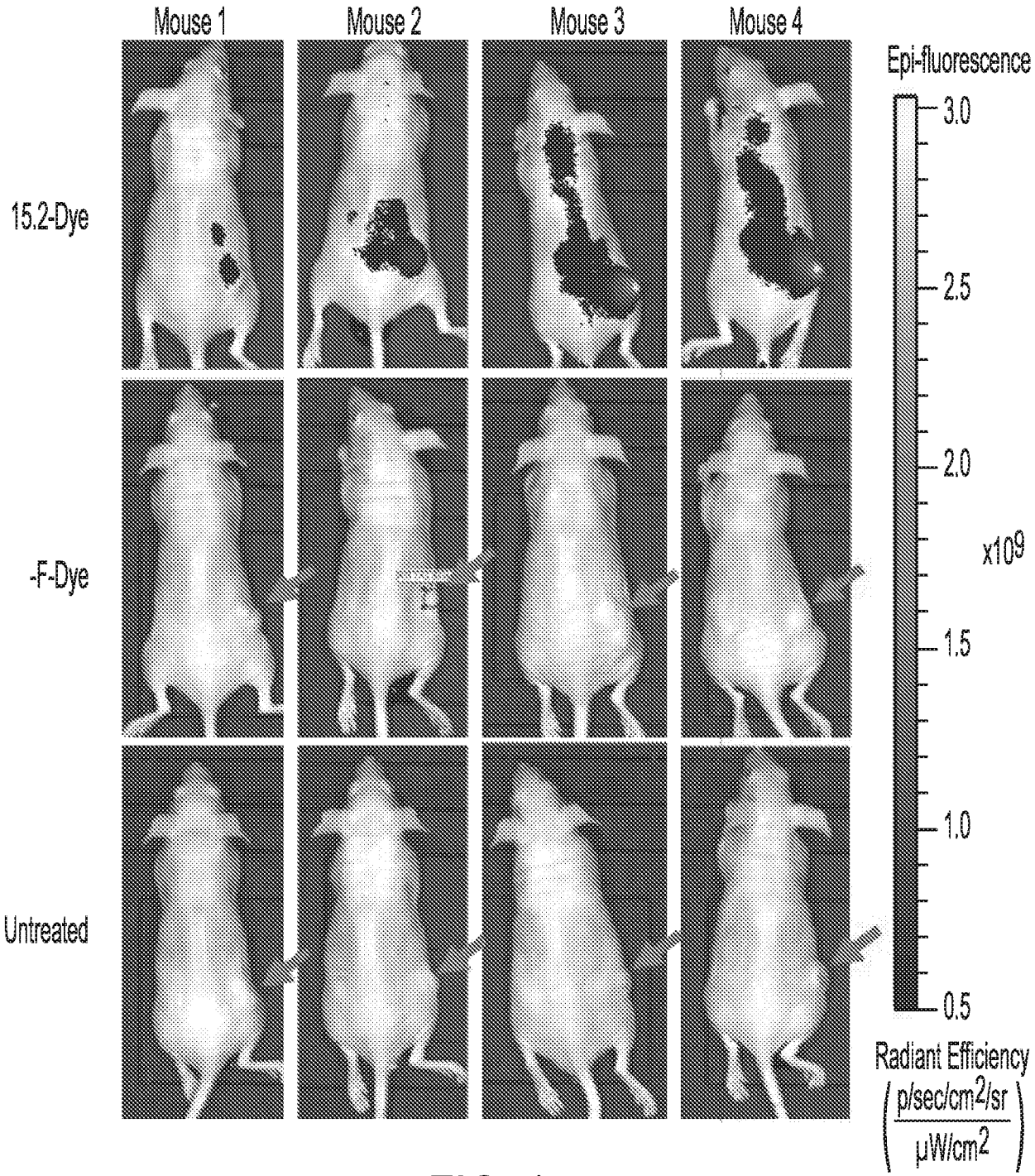
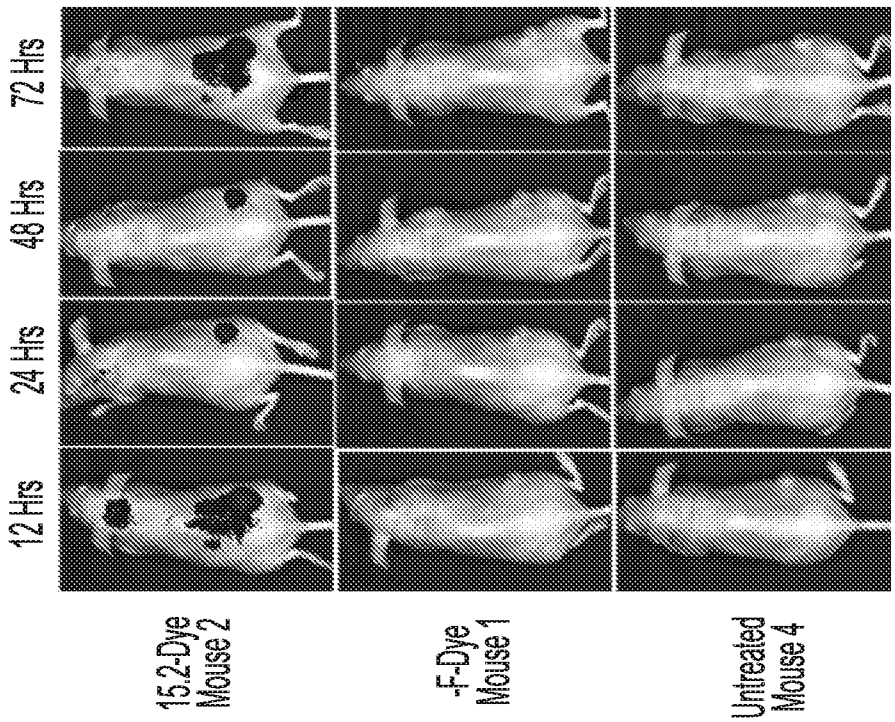

Mouse 1
Mouse 2
Mouse 3
Mouse 4
15.2-Dye
-F-Dye
Untreated
Epi-fluorescence
3.0
2.5
2.0
x10^9
1.5
1.0
0.5
Radiant Efficiency
p/sec/cm2/sr
µW/cm2

FIG. 17


12 Hrs
24 Hrs
48 Hrs
72 Hrs
15.2-Dye Mouse 2
-F-Dye Mouse 1
Untreated Mouse 4

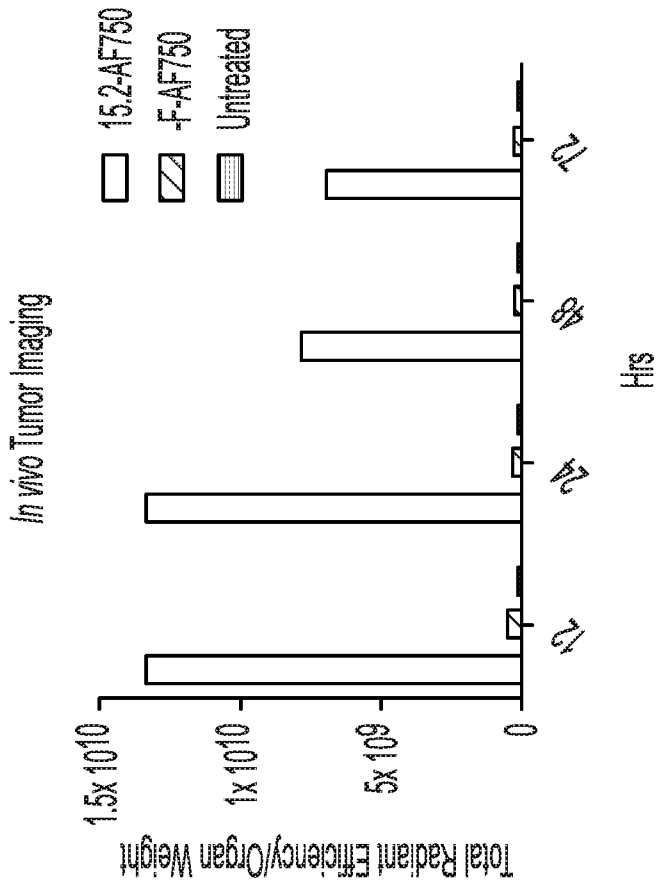
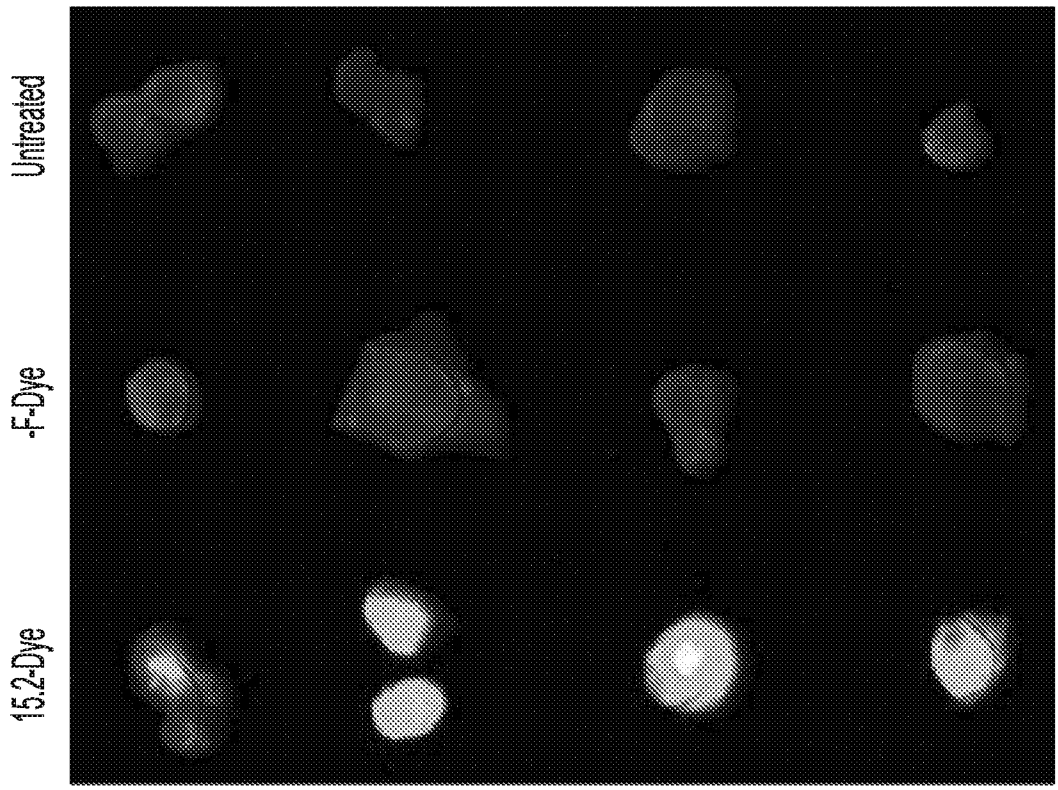

In vivo Tumor Imaging
15.2-AF750
-F-AF750
Untreated
Total Radiant Efficiency/Organ Weight
1.5x10^10
1x10^10
5x10^9
0
12
24
48
72
Hrs

FIG. 18


Untreated
-F-Dye
15.2-Dye

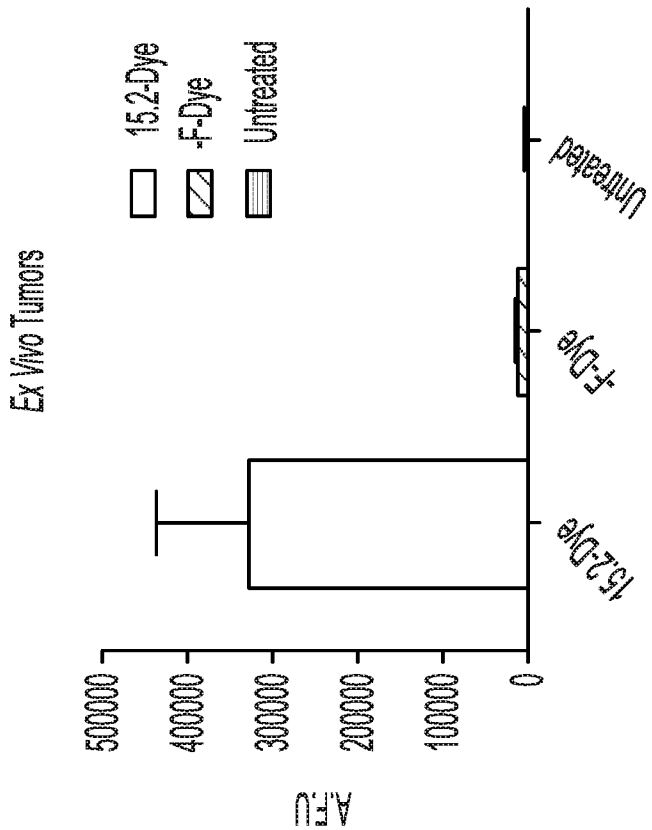

Ex Vivo Tumors
15.2-Dye
-F-Dye
Untreated
500000
400000
300000
200000
100000
0
15.2-Dye
-F-Dye
Untreated

FIG. 19

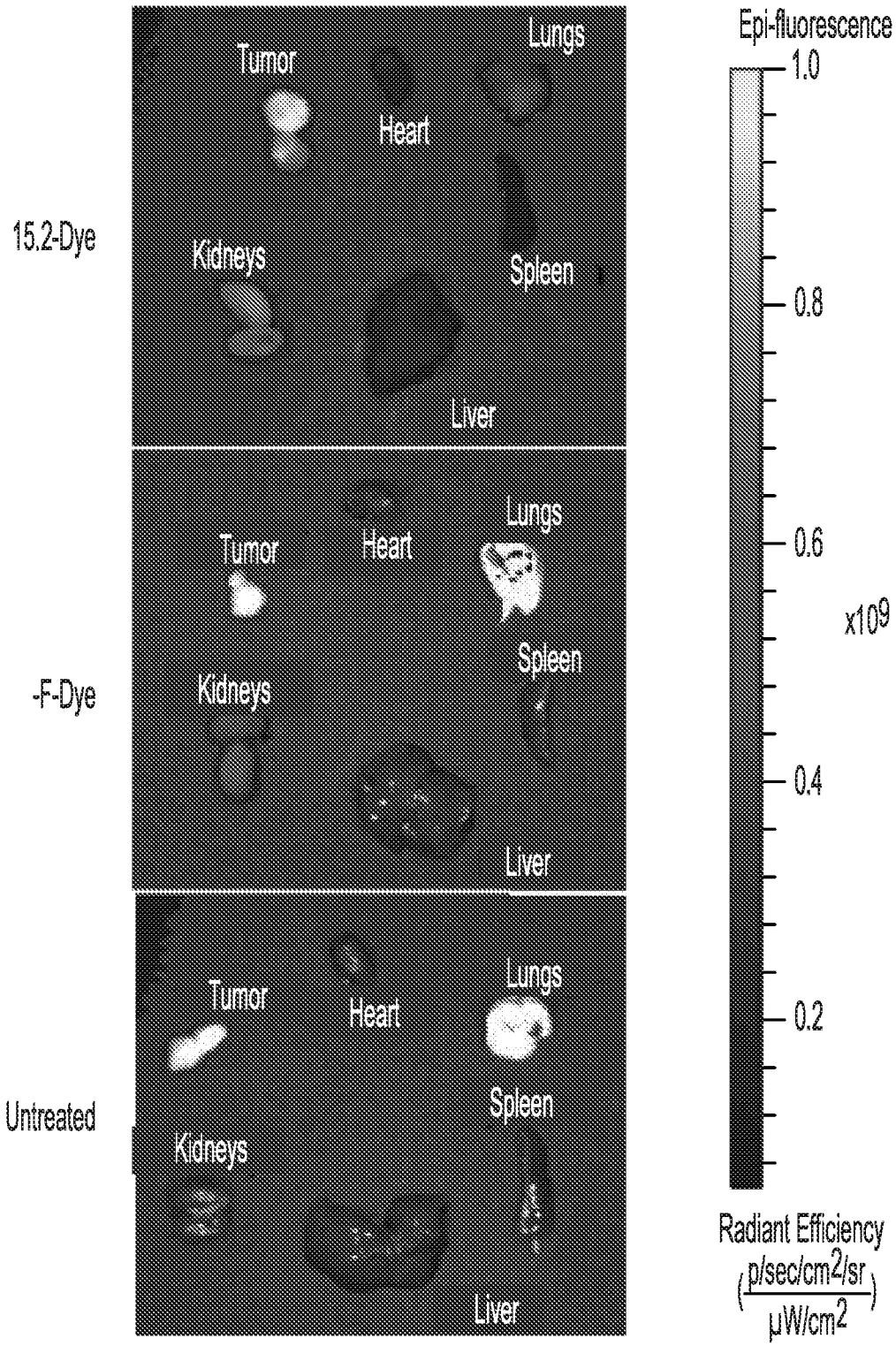

15.2-Dye
Tumor
Heart
Lungs
Kidneys
Spleen
Liver
-F-Dye
Tumor
Heart
Lungs
Kidneys
Spleen
Liver
Untreated
Tumor
Heart
Lungs
Kidneys
Spleen
Liver
Epi-fluorescence
1.0
0.8
0.6
0.4
0.2
x10^9
Radiant Efficiency
(p/sec/cm2/sr / µW/cm2)

FIG. 20

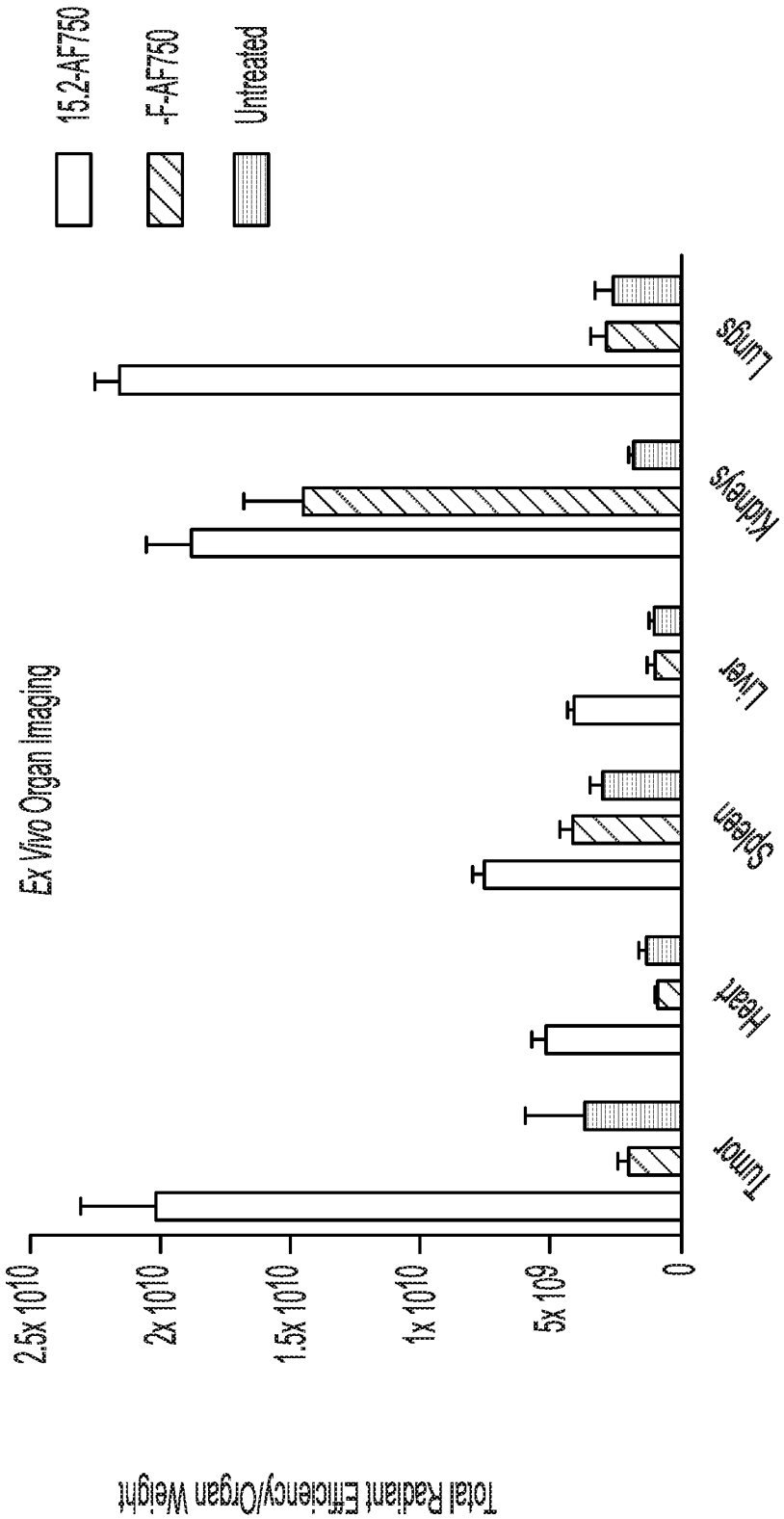


FIG. 20
CONTINUED

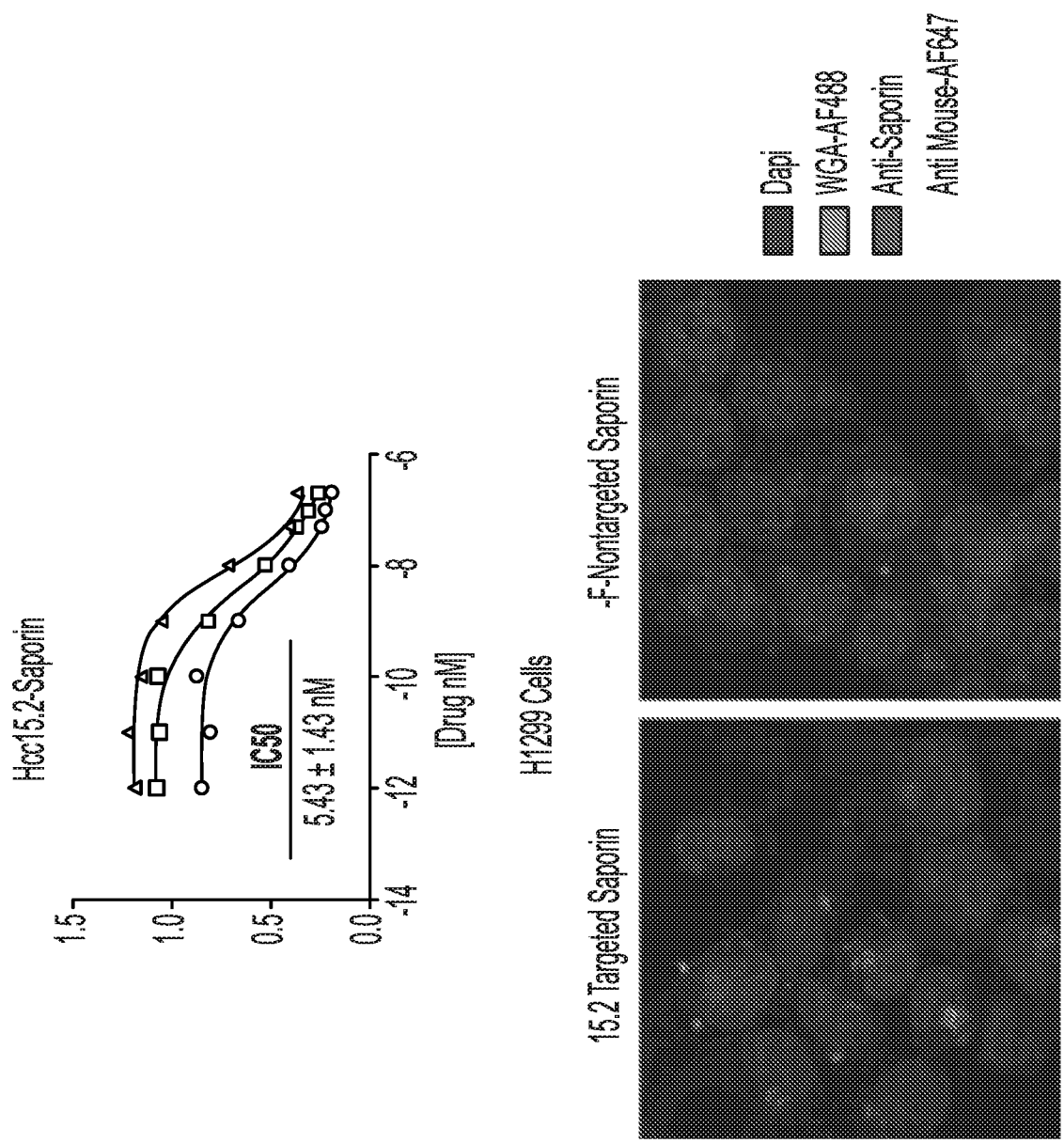

Hcc15.2-Saporin
1.5
1.0
0.5
0.0
-14
-12
-10
-8
-6
[Drug nM]
IC50
5.43 ± 1.43 nM
H1299 Cells
15.2 Targeted Saporin
-F-Nontargeted Saporin
Dapi
WGA-AF488
Anti-Saporin
Anti Mouse-AF647

FIG. 21

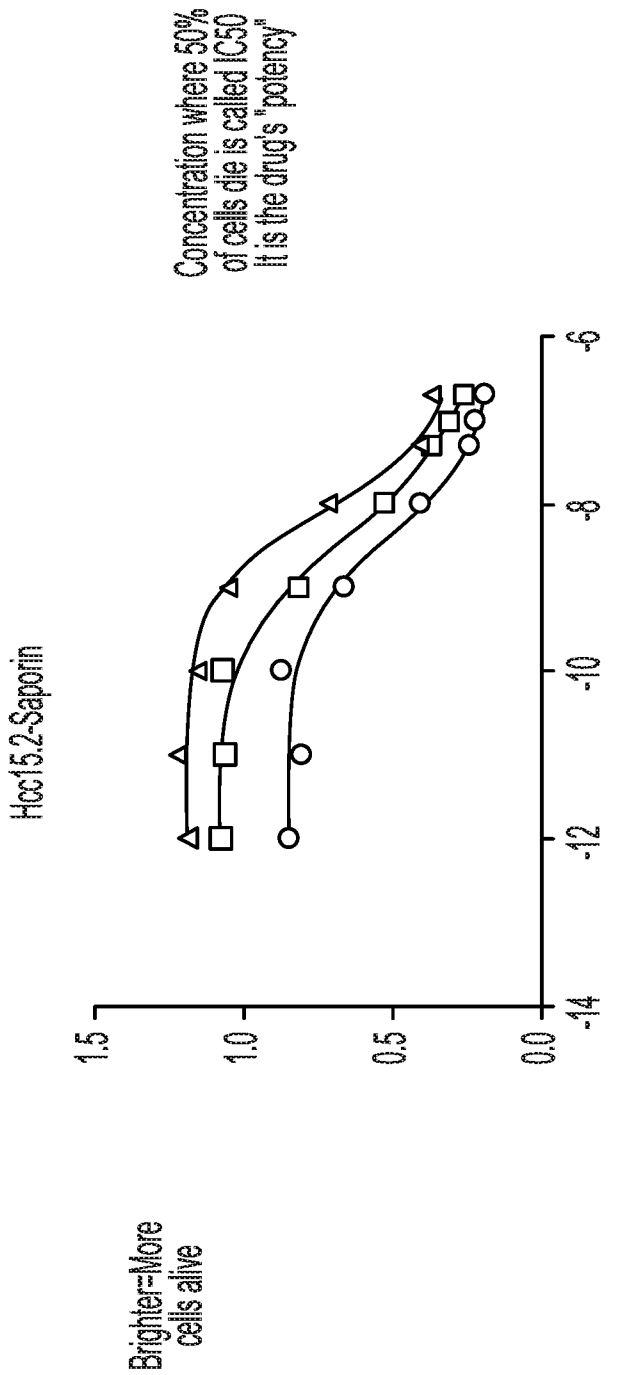

Concentration where 50% of cells die is called IC50 It is the drug's "potency"
Hoc15.2-Saporin
Brighter=More cells alive
1.5
1.0
0.5
0.0
-14
-12
-10
-8
-6
[Drug nM]

FIG. 22

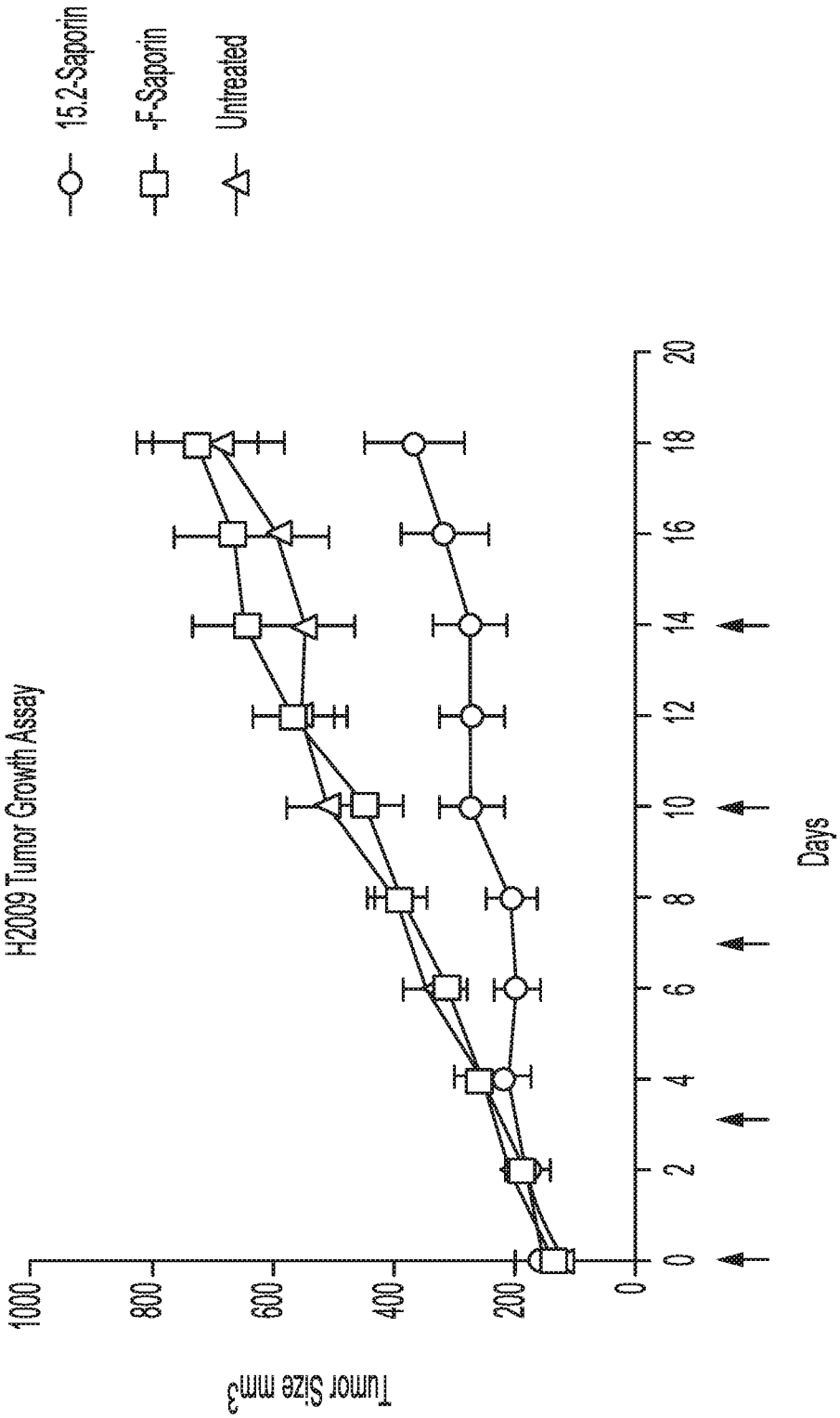

H2009 Tumor Growth Assay
15.2-Saporin
-F-Saporin
Untreated
Tumor Size mm3
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
Days

FIG. 23

MGSs identified

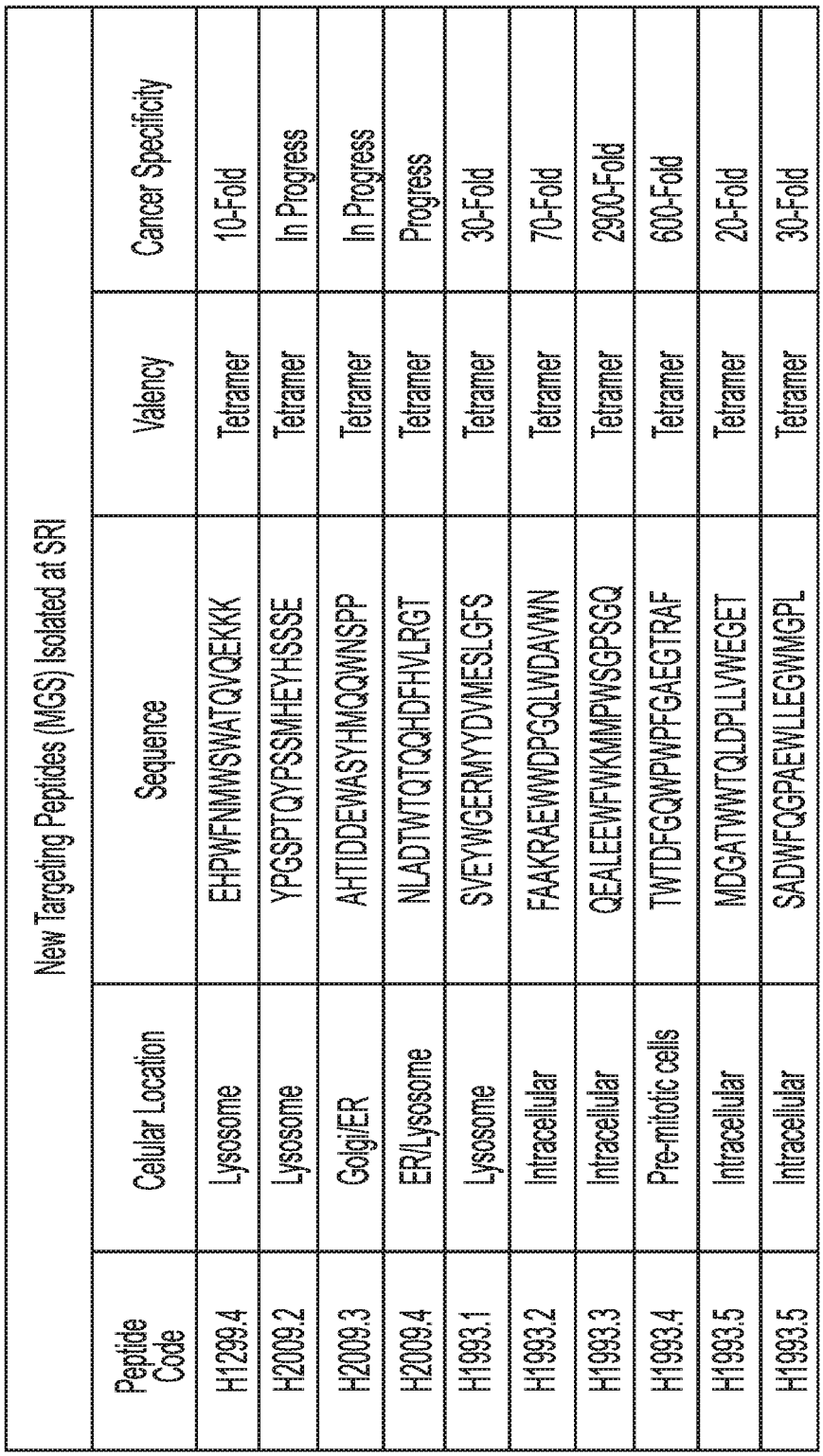
Indication	Cell Type Targeted	MSG Code and Cellular Location	Payloads Delivered
Carcinomas and Solid Tumors	Human Non-Small Cell Lung Cancer Cells	MGS_H1299.1_Intracellular **MGS_H1299.2_Intracellular** **MGS_H1299.3_Autophagosomes** **MGS_H2009.1_Golgi** MGS_H2009.2_Lysosome MGS_H2009.3_Lysosome MGS_H2009.4_Golgi/ER MGS_H2009.5_ER/Lysosome MGS_H1993.1_Lysosome MGS_H1993.2_Intracellular MGS_H1993.3_Intracellular MGS_H1993.4_Pre-mitotic cells only MGS_H1993.5_Intracellular MGS_H1993.6_Intracellular **MGS_H460.1_Plasmallembrane** **MGS_HCC15.1_Intracellular** **MGS_HCC15.2_Lysosomal** MGS_A549.1_Intracellular MGS_HCC95.1 MGS_H1155.1 MGS_H1155.2 MGS_H666.1	• Monoclonal Antibodies • Small molecule therapeutics (doxorubicin, paclitaxel, DM1, amanitin, auristatin and duocarmycin) • Fluorophores • Nonoparticles (liposomes, micelles, quantum dots, SPIO) • Imaging agents (PET, NIR, MR) • Peptide and proteins • Proteinaceous toxins • Antigenic peptides • Bead-based capture

FIG. 24

Lymphoma and Leukemia	Lymphoma Cells	MGS_A20.1_Plasma Membrane MGS_A20.2_Plasma Membrane **MGS_PCM.1_Plasma Membrane**	• Proteins • Fluorophores • Bead-based capture
Vaccine Development	Dendritic Cells	**MGS_XS2.1_Intracellular** **MGS_XS2.3_Intracellular**	• Liposomes • DNA • Protein antigens • Fluorophores
Diabetes	β-Cells of the Islets of Langerhans	**MGS_RII.1** MGS_RII.2	• Proteins • Radionuclides
Pathogen Infected Cells	Mycoplasma Arginine Infected Cells	MGS_MArg.1_Bacterial	• Fluorescent dyes • Bead-based capture
Cardiovascular Disease	Cardiomyocytes	**MGS_PCM.1_Intracellular**	• DNA • Fluorophores

1. Intracellular indicates that cellular uptake has been confirmed but the exact intracellular compartment has not been determined.
2. MGS with no location information indicated that cellular binding has been confirmed but cellular location not determined.
3. Peptides highlighted in **blue** have been shown to home to their target cell in an animal model.
4. The peptides that target NSCLC also bind to cancers derived from other organ sites such as pancreatic, colorectal, breast, and ovarian cancers.

FIG. 24
CONTINUED



New Targeting Peptides (MGS) Isolated at SRI

Peptide Code	Cellular Location	Sequence	Valency	Cancer Specificity
H1299.4	Lysosome	EHPWFNMWSWATQVQEKKK	Tetramer	10-Fold
H2009.2	Lysosome	YPGSPTQYPSSMHEYHSSSE	Tetramer	In Progress
H2009.3	Golgi/ER	AHTIDDEWASYHMQQWNSPP	Tetramer	In Progress
H2009.4	ER/Lysosome	NLADTWTQTQQHDFHVLRGT	Tetramer	Progress
H1993.1	Lysosome	SVEYWGERMYYDVMESLGFS	Tetramer	30-Fold
H1993.2	Intracellular	FAAKRAEWWDPGQLWDAVWN	Tetramer	70-Fold
H1993.3	Intracellular	QEALEEWFWKMMPWSGPSGQ	Tetramer	2900-Fold
H1993.4	Pre-mitotic cells	TWTDFGQWPWPFGAEGTRAF	Tetramer	600-Fold
H1993.5	Intracellular	MDGATWWTQLDPLLVWEGET	Tetramer	20-Fold
H1993.5	Intracellular	SADWFQGPAEWLLEGWMGPL	Tetramer	30-Fold

FIG. 25

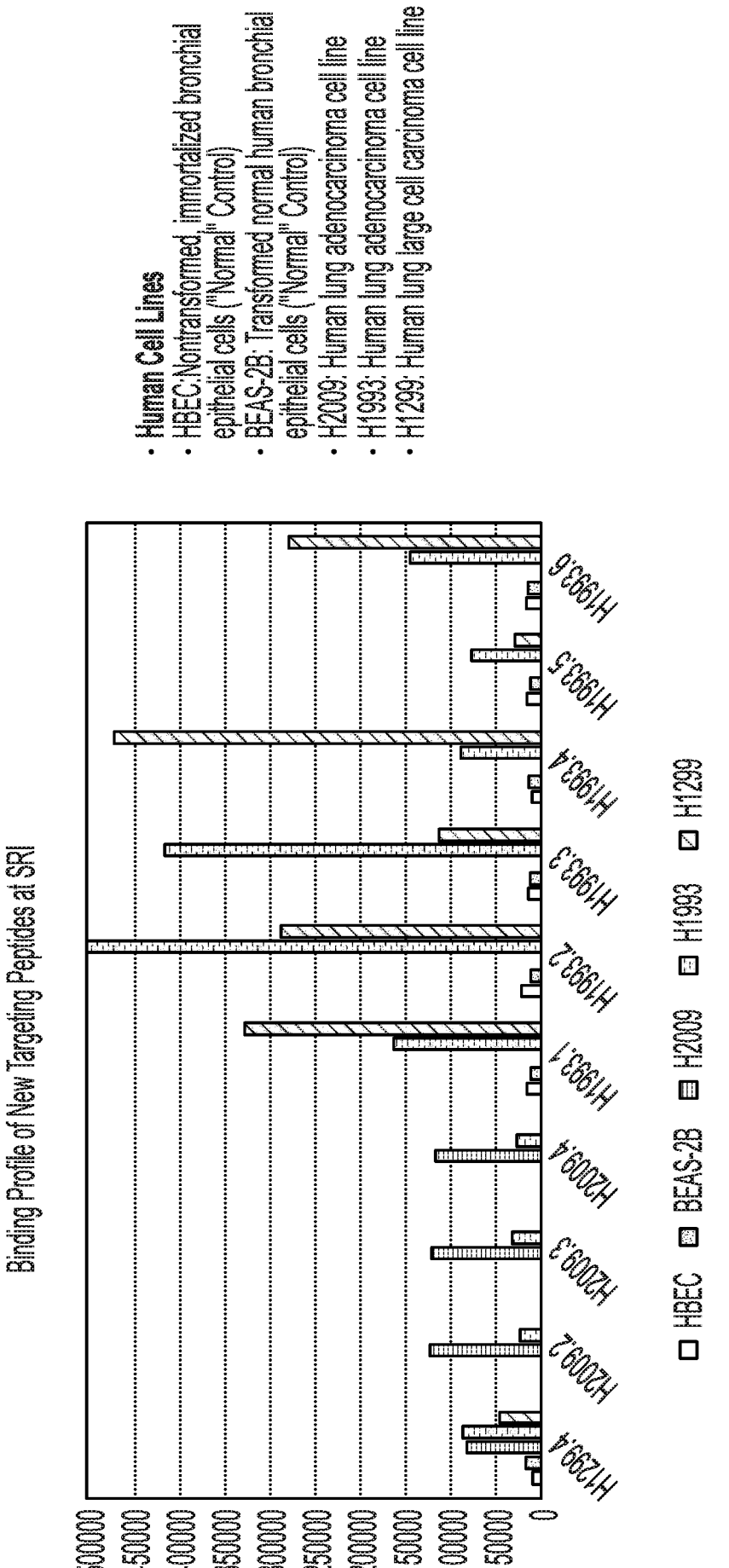


FIG. 25
CONTINUED

	Starting Sequence	Current Sequence	Valency	Half-Maximal Binding for target cell	Serum Stability	bCancer Specificity	Subcellular Location	Validated in Vivo
[a]H2009.1	RGDLATLRQLAQED GVVGVR	Ac-D-Leu-RGDLATLRQL	dimer	17 nM	>99%intact at 12H		Golgi	Yes
H1299.2	YAAWPASGAWTGT APCSAGT	Ac-YAAWPASGAWT	dimer	3.4 nM			Internalized	
[c]H1299.3	LQWRRDDNVHNFG VWARYRL	Ac-LQWRRNFGVWARYRL	dimer			>250 fold	autophagasome	Yes
HCC15.1	ATEPRKQYATPRVF WTDAPG	Ac-KQYATPRVFWT	dimer	5.6 nM			Internalized	
HCC15.2	FHAVPQSFYTAP	Ac-FHAVPQSFYT	monomer	2.7 nM			Lysosome	Yes

a. We should discuss this peptide further

b. Relative to normal control cell. HBEC. We know the optimized peptides still show specificity but I don't have the numbers relative to the HBEC cell line. These are in progress

c. Related to SRI PCT/US15/66519 filed 12/17/2015

FIG. 26

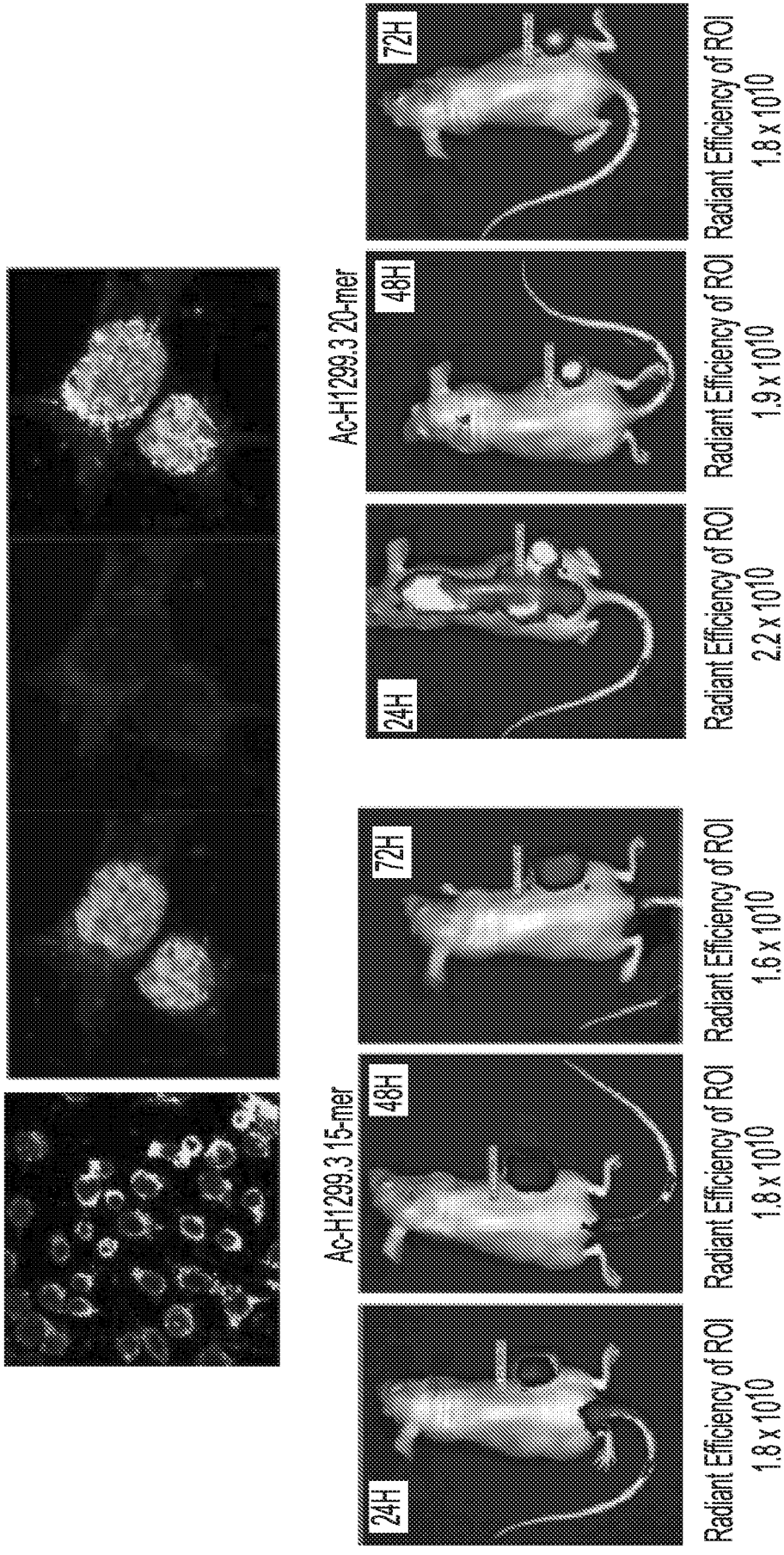

Ac-H1299.3 15-mer
Ac-H1299.3 20-mer
24H
48H
72H
Radiant Efficiency of ROI
1.8 x 10^10
1.8 x 10^10
1.6 x 10^10
2.2 x 10^10
1.9 x 10^10
1.8 x 10^10

FIG. 27

Saporin Delivery		
MGS	Cell Line	IC50
H2009.1	H2009	1.8 nM
H1299.2	H1299	2.3 nM
H1299.3	H1299	840 nM
HCC15.1	H1299	2.5 nM
HCC15.2	H1299	4.6 nM
H1299.4	H2009	84 nM

FIG. 29

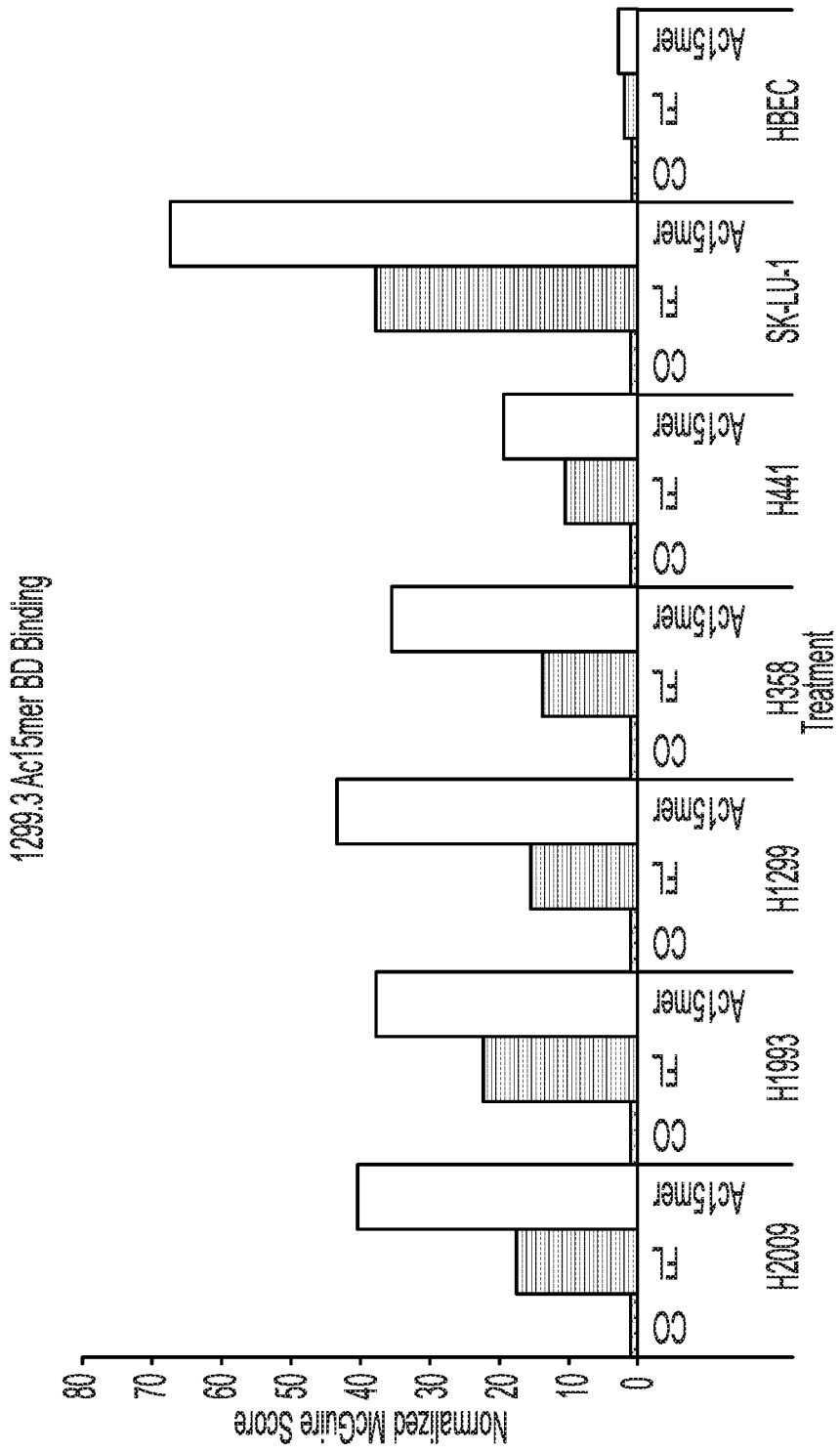


FIG. 28

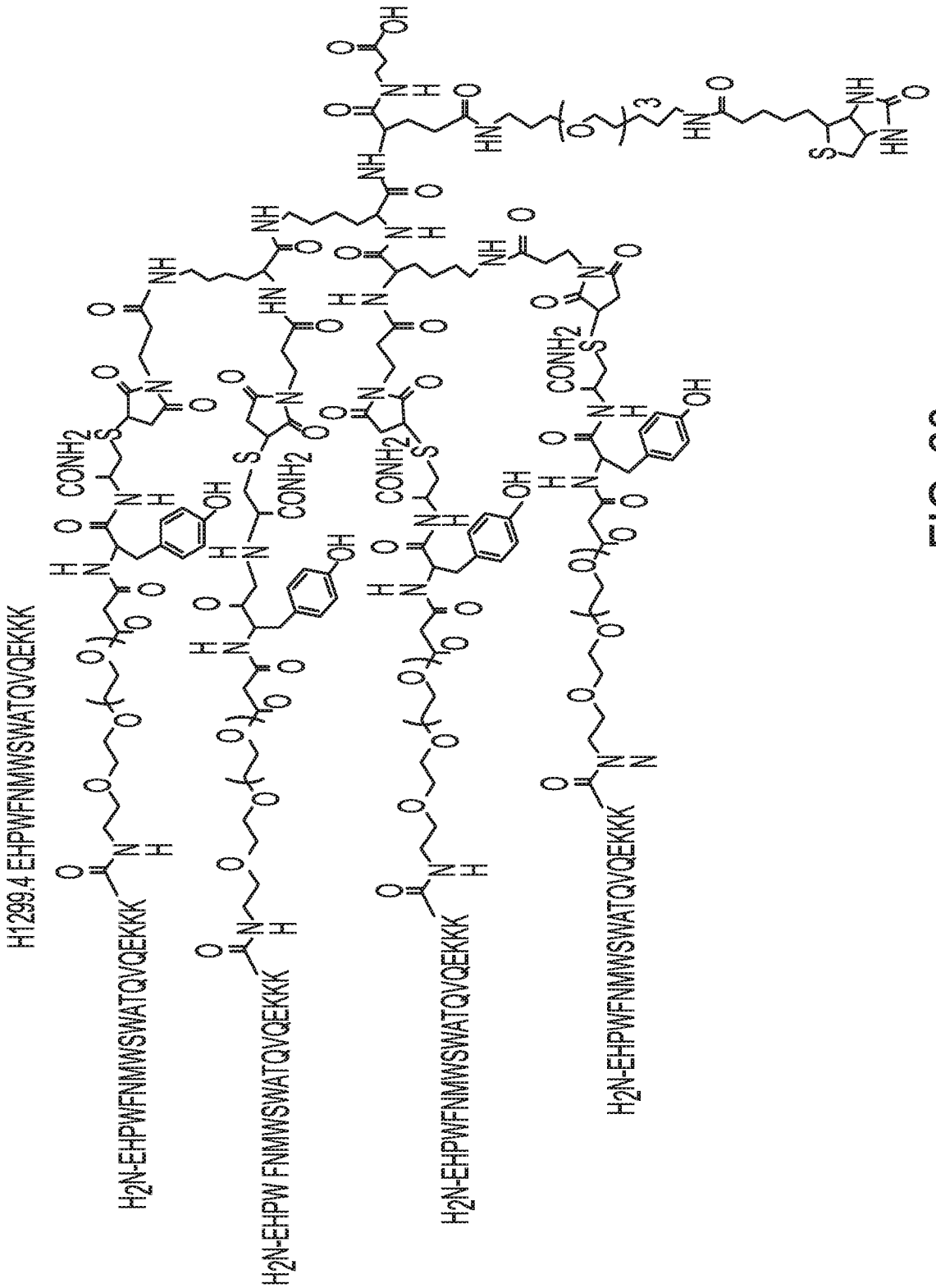
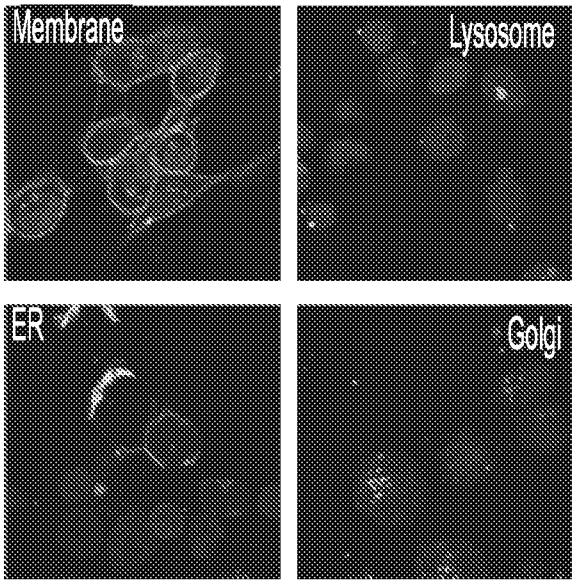

H1299.4 EHPWFNMWSWATQVQEKKK
H2N-EHPWFNMWSWATQVQEKKK
H2N-EHPW FNMWSWATQVQEKKK
H2N-EHPWFNMWSWATQVQEKKK
H2N-EHPWFNMWSWATQVQEKKK

FIG. 30



Cell Line	McGuire Score	Cancer Specificity
H2009	80,700	13
H1299	80,900	13
H1993	44800	7.2
H460	10,500	1.7
HBEC9	6,200	—
BEAS-2B	12,700	—

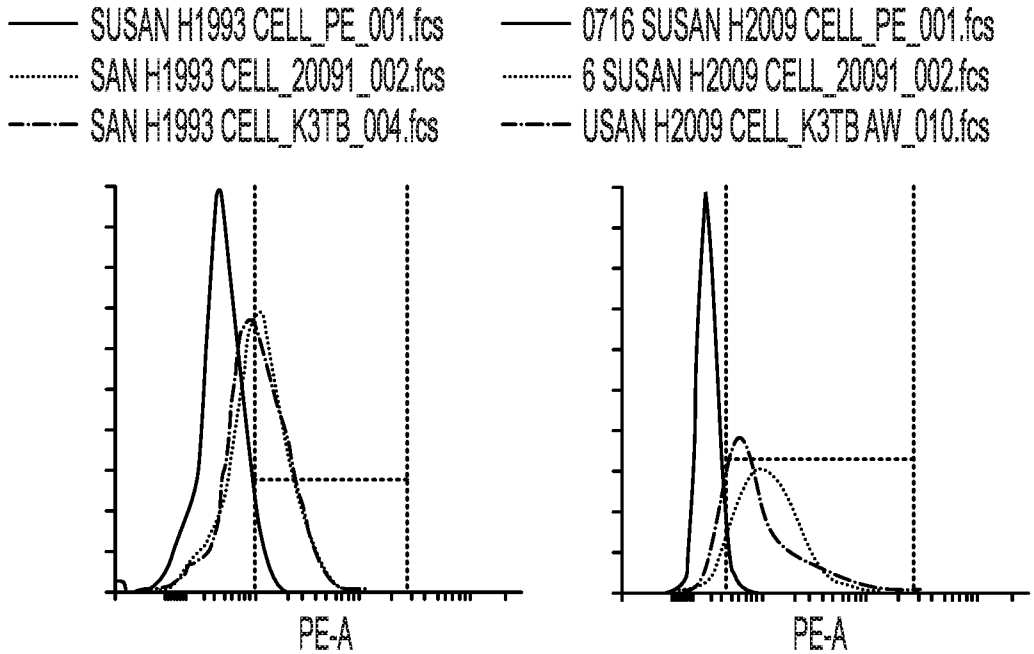


FIG. 30
CONTINUED

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/41412

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 7/06, C07K 14/00, C07K 7/08 (2018.01)
CPC - C07K 7/06, A61K 47/64, C07K 14/001, C07K 7/08, C07K 2319/00

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0094404 A1 (VILLAVERDE CORRALES et al.) 3 April 2014 (03.04.2014) Abstract; Claim 19; para [0018]; para [0032]; para [0049] ; para [0128]; para [0173]; para [0175]	1, 8, 13, 20, 25, 26 --- 2, 3, 14, 15, 19, 34
X --- Y	MCGUIRE te al., Identification and Characterization of a Suite of Tumor Targeting Peptides for Non-Small Cell Lung Cancer. Scientific Reports, 27 March 2014, Vol. 4, page 4480. Abstract; p2, Table 1	39-42 --- 2, 3, 14, 15, 34
Y	US 2008/0206136 A1 (GREENE et al.) 28 August 2008 (28.08.2008) Abstract; Claim 2	19

☐ 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	Date of mailing of the international search report
20 September 2018	04 DEC 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/41412

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 35-38
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claim 35-38 are an unsearchable omnibus claim under PCT Rule 6.2(a). The claims include omnibus reference to "as disclosed herein" and thus does not clearly set forth a specific invention for which protection is being sought.

3. ☒ Claims Nos.: 4, 9-11, 16, 21-23, 27-31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

----Please see continuation in first extra sheet ----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 8, 13-15, 19, 20, 25, 26, 34, 39-42, limited to SEQ ID NO: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/41412

Continuation of Box No. III. Observations where unity of invention is lacking.

Group I+: Claims 1-3, 5-8, 12-15, 17-20, 24-26, 34, 39-42, drawn to a composition comprising one or more molecular guidance system (MGS) peptides and a cytotoxic agent. The composition will be searched to the extent that the MGS peptide encompasses SEQ ID NO: 1. It is believed that claims 1-3, 8, 13-15, 19, 20, 25, 26, 34, 39-42, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1. Additional MGS peptide will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected MGS peptide(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a MGS peptide encompassing SEQ ID NO: 3 (Claims 1-3, 5, 8, 12-15, 17, 19, 20, 24-26, 34, 39-42).

Group II: claims 32-33, drawn to a method of targeting an intracellular target.

The inventions listed as Groups I+ and do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I+ include the special technical feature of a composition comprising one or more molecular guidance system (MGS) peptides and a cytotoxic agent, not required by Group II.
Group II includes the special technical feature of a method of targeting an intracellular target, not required by Groups I+.

No technical features are shared between the amino acid sequences of MGS peptide of Groups I+ and, accordingly, these groups lack unity a priori. Additionally, even if Groups I+ were considered to share the technical features of including: Claims 1, 13, and 34, these shared technical features are previously disclosed by US 2014/0094404 A1 to Villaverde Corrales et al., (hereinafter 'Villaverde Corrales').

Villaverde Corrales teaches (instant claims 1 and 34) a composition comprising one or more molecular guidance system (MGS) peptides and a cytotoxic agent (Abstract - 'The invention relates to conjugates comprising a targeting moiety specific for the CXCR4 based on the polyphemusin-derived peptide and a therapeutic or imaging agent. The invention relates as well to the application of said conjugates for the therapy and diagnostics which require the specific targeting to CXCR4+ cells.'; Claim 19 - 'A conjugate comprising (i) a targeting peptide comprising the sequence RRCYRKCYKGYCYRKCR (SEQ ID NO: 5) or a functionally equivalent variant thereof, and (ii) a therapeutic agent wherein the targeting peptide is capable of specifically binding to CXCR4 and promoting internalization of the therapeutic agent in a cell expressing CXCR4.'; para [0128] - 'Examples of proteinaceous cell toxins useful for incorporation into the conjugates according to the invention include, but are not limited to, type one and type two ribosome inactivating proteins (RIP). Useful type one plant RIPs include, but are not limited to... saporins').

Villaverde Corrales teaches (instant claim 13) membrane-permeable conjugate for transport across a lipid membrane comprising: one or more a molecular guidance system (MGS) peptides and a cytotoxic agent (Claim 19 - 'A conjugate comprising: (i) a targeting peptide comprising the sequence RRCYRKCYKGYCYRKCR (SEQ ID NO: 5) or a functionally equivalent variant thereof, and (ii) a therapeutic agent wherein the targeting peptide is capable of specifically binding to CXCR4 and promoting internalization of the therapeutic agent in a cell expressing CXCR4.'; para [0128] - 'Examples of proteinaceous cell toxins useful for incorporation into the conjugates according to the invention include, but are not limited to, type one and type two ribosome inactivating proteins (RIP). Useful type one plant RIPs include, but are not limited to... saporins'; para [0018] - 'FIG. 2. Confocal analysis of T22, vCCL2, V1 and CXCL12-GFP-H6 upon internalization in HeLa cells.'; para [0049] - 'As used herein, "internalization" refers to a process by which a molecule or a construct comprising a molecule binds to a target element on the outer surface of the cell membrane and the resulting complex is internalized by the cell.').

Villaverde Corrales further teaches a pharmaceutical composition (para [0173] - 'the invention relates to a pharmaceutical composition comprising a conjugate according to the invention and a pharmaceutically acceptable carrier.').

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of Item 4:
Claims 4, 9-11, 16, 21-23, 27-31 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Claim 35-38 are an unsearchable omnibus claim under PCT Rule 6.2(a). The claims include omnibus reference to "as disclosed herein" and thus does not clearly set forth a specific invention for which protection is being sought.

Note, Claim 27 which depends from "any of claim 1-27", is objected to, as a claim cannot depend from itself. Claim 27 is reconstrued to depend from "any of claim 1-26".