(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 December 2016 (22.12.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/205515 A1

(51) International Patent Classification:
A61K 47/48 (2006.01) *A61K 9/127* (2006.01)
A61K 49/00 (2006.01) *A61K 38/00* (2006.01)
A61K 51/06 (2006.01)

(21) International Application Number:
PCT/US2016/037874

(22) International Filing Date:
16 June 2016 (16.06.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/180,857 17 June 2015 (17.06.2015) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: GOLD NANOROD BASED DIAGNOSTIC AGENT FOR DETECTION AND QUANTIFICATION OF c-MET (HGF) RECEPTORS

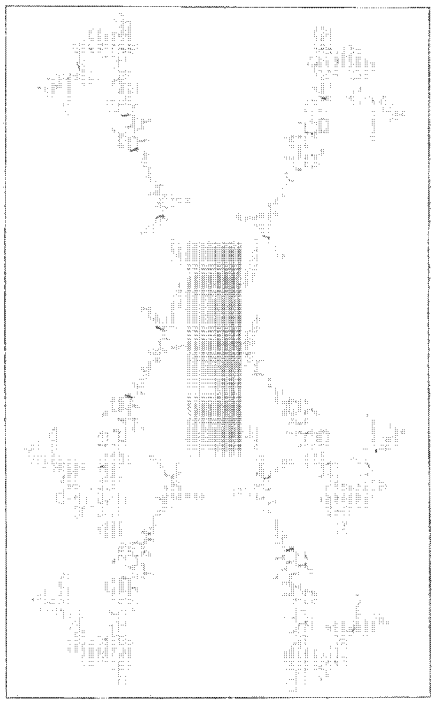


Figure 1

(57) Abstract: Provided is a nanoparticle conjugate comprising a nanoparticle linked via a linker to a target molecule with the specific affinity for c-MET receptor. Methods for diagnosing and treating patients are provided as well.

WO 2016/205515 A1

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

GOLD NANOROD BASED DIAGNOSTIC AGENT FOR DETECTION AND QUANTIFICATION OF c-MET (HGF) RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims a benefit of priority from US provisional patent application 62/180,857, filed June 17, 2015, the entire disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention relates to compositions comprising a gold nanorod conjugated with a peptide which selectively binds c-MET (HGF) receptor and cells expressing the receptor. Also provided are methods in which the compositions are used to diagnose and treat patients.

BACKGROUND

[0003] In the last several years, it has become clear that due to the heterogeneous nature of cancers, focus must shift towards treating each cancer based on its genetic makeup and expression abnormalities. In order to properly tailor treatment to each individual, evaluation of potential biomarker targets must be carefully conducted. One such subclass of biomarkers is comprised of cell-surface receptors, which are commonly overexpressed in cancerous cells.

[0004] The most widely practiced method of cell-surface receptor detection is through immunohistochemistry (IHC), which uses antibody-antigen interactions for detection. Common IHC detection is through an indirect method, where a primary antibody against the desired target antigen is then labeled with a secondary antibody directed at the lgG of the primary antibody species. This secondary antibody is usually tagged with an enzymatic reporter such as alkaline phosphatase (AP) or horseradish peroxidase (HRP). Once the secondary antibody is bound, a substrate, usually a chromagen, is applied to the tissue to yield a colored product from the reporter that is identifiable through light microscopy. There are many commercially available kits for IHC testing of human tumor tissues, such as the FDA-approved DAKO EGFR PharmDx kit, approved for diagnosis of EGFR in colorectal tumors.

[0005] Though IHC is the preferred method of diagnosis, recent studies have shown problems with the IHC analysis of membrane protein expression due to

problems inherent to the indirect IHC design such as background staining, variation in staining intensity, antibody cross-reactivity, enzyme activity, and the qualitative nature of the diagnosis method.

[0006] The rise of personalized medicine has become a promising option for cancer treatment due to the specificity of biomarker-targeted therapies. In order to properly treat a patient with targeted drugs, a testing must be performed first on biopsied samples to establish a profile of suitable biomarker targets. One such biomarker is the hepatocyte growth factor (HGF) receptor also known as c-MET receptor. Normally, c-MET receptor is only expressed in stem and progenitor cells. The c-MET receptor is overexpressed in the cell membrane of many cancers, including non-small cell lung carcinoma (NSCLC), gastric, ovarian, thyroid, breast, and colon cancers, including colorectal adenocarcinoma.

[0007] In cancers, the expression of the MET pathway affects the development of cancer through activation of oncogenic pathways such as RAS, PI3K, and STAT3, and also promotes angiogenesis in tumors. The expression of MET in tumors is also linked to resistance of anti-EGFR therapies, whereby the tumors will circumvent the EGFR pathway by internalizing the receptor and up regulating surface c-MET receptor expression to proliferate. Currently, there are only very few FDA-approved anti-cMET drugs available. These drugs include crizotinib for NSCLC and cabozantinib for thyroid cancers.

[0008] Despite the need to test for c-MET receptor expression in cancers, current methods of evaluating c-MET receptor expression have not been accurate enough to warrant a clinical diagnosis.

SUMMARY

[0009] In one aspect, the present application provides a reagent and sensitive methods using the reagent for accurate detection of c-MET receptor expression on cancer cells and cancer tissues. The reagent comprises a nanoparticle conjugate in which a nanoparticle is linked via a linker to a target molecule with the specific affinity for c-MET receptor.

[0010] In some embodiments, the target molecule in the nanoparticle conjugate is selected from the group consisting of a peptide, ligand, antibody, small molecule and any combination thereof.

[0011] One of the preferred nanoparticle conjugates comprises a peptide consisting essentially of the amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1).

[0012] In some preferred embodiments, the nanoparticle is a nanorod.

[0013] Some suitable reagents comprise a nanoparticle conjugate in which the nanoparticle is a nanorod linked to a peptide consisting essentially of the amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1) by the linker comprising a thiol moiety selected from the group consisting of thioctic acid, monothioctic acid, dithioctic acid, and trithioctic acid.

[0014] In some embodiments, the linker comprises at least one or more moiety selected from the group consisting of a thiol moiety, lysine residue and ethylene glycol.

[0015] Further embodiments provide methods of treatment, including a method of treating a cancer patient, the method comprising reacting the patient's cancer tissue biopsy sample with a reagent comprising a nanoparticle conjugate comprising a nanorod linked to a peptide with specific affinity to human c-MET receptor, and wherein the reaction detects expression of c-MET receptor in the cancer tissue biopsy sample, administering to the patient an anti-cMET drug.

[0016] These methods of treatment may be particularly useful for cancer patients with non-small cell lung carcinoma, gastric cancer, ovarian cancer, thyroid cancer, breast cancer or colon cancer.

[0017] Further embodiments include methods for making the reagent comprising a nanoparticle conjugate. Such methods include a method of forming a nanoparticle conjugate comprising a nanoparticle linked to a target molecule specific for cMET receptor, with the method comprising the following steps: mixing solutions of cetyltrimethylammonium bromide and chlorauric acid, adding a solution of cetyltrimethylammonium bromide, silver nitrate, and ascorbic acid to obtain gold nanorods, purifying the gold nanorods by filtration and centrifugation; conjugating a polyethylene glycol linker modified with thiol to the gold nanorods to obtained gold nanorods with a linker; and conjugating a peptide to the gold nanorods with the linker.

[0018] Various uses for the reagent comprising comprises a nanoparticle conjugate in which a nanoparticle is linked via a linker to a target molecule with the specific affinity for c-MET receptor include any of the following:

- use of the reagent as an agent for targeting cMET receptors on cancerous tissue;
- use of the reagent as an agent for targeting cMET receptors on one or more human cancers from the list comprising non-small cell lung carcinoma, gastric cancer, ovarian cancer, thyroid cancer, breast cancer, and colon cancer;
- use of the reagent as an agent for detecting cMET receptors on cancerous tissue;
- use of the reagent as an agent for detecting cMET receptors on human tissue;
- use of the reagent as a means for detecting and quantifying the number cMET receptors on cancerous tissue by utilizing polarization microscopy to image said nanoparticle conjugates bound to said cMET receptors; and
- use of said nanoparticle conjugate of claim 1 as a therapy agent for one or more human cancers selected from the group consisting of non-small cell lung carcinoma, gastric cancer, ovarian cancer, thyroid cancer, breast cancer, and colon cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a scheme 1, showing a nanorod linked with peptides with specific affinity to cMET receptor.

FIG. 2 is a chemical structure of reagent GNR-1093, the portion comprising the thioctic-acid disulfide bound to the N-terminus of the 1093 peptide through a poly-lysine bridge is shown.

FIG. 3 is an HPLC chromatogram for the 1093 peptide.

FIG. 4 is a mass spectrogram for the 1093 peptide.

FIG. 5A is a UV-visible spectrum and **Fig. 5B** is a Zeta potential of GNR-1093.

Fig. 6 are micrographs showing detection of c-MET receptor on non-small lung cancer cells with GNR-1093.

Fig. 7 is a graph for relative intensities of each sample from Fig. 6.

Figs. 8-25 are scanned images of tumor and normal tissue microarray reacted with GNR-1093. See Table 2 for a microarray panel legend.

DETAILED DESCRIPTION

[0019] The present disclosure provides various embodiments of a reagent suitable for detection of cancer cells which express c-MET receptor. In some embodiments, the reagent can be used for identifying a cancer patient who may benefit from treatment with an anti-cMET drug which target cancer cells expressing c-MET receptor. Such cancer patients include those afflicted with non-small cell lung carcinoma (NSCLC), gastric, ovarian, thyroid, breast, or colon cancers, including colorectal adenocarcinoma.

[0020] The reagent comprises a gold nanorod conjugated through a linker with a peptide which selectively recognizes and binds human c-MET receptor expressed at the cell surface of cancer cells. Gold nanoparticles have been used as imaging labels due to their light scattering properties and ease of surface modification. While nanospheres have attracted much attention, the inventors have unexpectedly discovered that gold nanorods (GNRs) of the same volume have an optical efficiency 20 times that of the spheres. GNR also have a high affinity for thiol groups, which allows them to be stabilized with thiolated polyethylene glycol, and conjugated with a desired biomolecule such as c-MET receptor binding peptide for targeting. Gold nanorods of various length can be obtained. Preferably, the length of a gold nanorod is in the range from about 30 nm to about 60 nm. In some embodiments, the length of a gold nanorod is in the range from 30 nm to 60 nm. Suitable gold nanorods include those with the length/width ratio from 2 to about 100, from 2 to 50, from 2 to 20, from 2 to 10, and from 2 to 6.

[0021] GNRs are an attractive alternative to organic fluorophores, which can suffer from photodecomposition or sensitivity to quenching. GNRs are also biocompatible and are thus also an attractive alternative to cytotoxic quantum dots. Various detection technologies can be used with GNRs conjugated to a peptide which binds to c-MET receptor. These technologies include dark field microscopy, near-infrared (NIR) transmission imaging, photoacoustic tomography (PAT), two-photon excited

luminescence (TPL) imaging, and surface enhanced Raman spectroscopy (SERS) imaging. Additional detection technologies may include polarized imaging, either in the dark field or through differential interference contrast (DIC) microscopy.

[0022] In some embodiments a nanorod-based tissue diagnostic agent specific for the c-MET receptor comprises a gold nanorod (GNR) attached through a linker to a peptide with the specific affinity for c-MET receptor. A person of skill will appreciate that suitable peptides include those obtained by screening a phage display library against human c-MET receptor or any extra-cellular portion of human c-MET receptor.

[0023] In some embodiments, an antibody specific to the c-MET receptor, a ligand specific to the c-MET receptor or a small molecule can be used instead of the peptide, or in combination with the peptide as a chimeric molecule.

[0024] The term "specific affinity" means binding of a peptide or an antibody or a ligand to the c-MET receptor with the equilibrium dissociation constant (K_d) in the range from 10^{-6} to 10^{-12}, and preferably in the range from 10^{-9} to 10^{-12}.

[0025] In one embodiment, a gold nanorod-based c-Met tissue diagnostic agent comprises a gold nanorod conjugated through a linker to a peptide comprising the following amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1). In other embodiment, a gold nanorod-based c-Met tissue diagnostic agent comprises a gold nanorod conjugated through a linker to a peptide consisting essentially of the following amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1). In further embodiment, a gold nanorod-based c-Met tissue diagnostic agent comprises a gold nanorod conjugated through a linker to a peptide consisting of YLFSVHWPPLKA (SEQ ID NO. 1, also referred to as the 1093 peptide).

[0026] GNR-1093 binds the c-Met receptor to give a point-specific image signal, rather than a basic membrane stain which cannot be accurately quantified. This method of c-Met detection improves on current immunohistochemical methods that involve multi-step processes and qualitative data.

[0027] One of the methods in which a gold nanorod-based c-Met tissue diagnostic agent can be used is the polarized light mode which allows to distinctly detect a GNR-peptide conjugate. Polarization microscopy is used for imaging anisotropic (directionally dependent) materials.

[0028] The plasmon peaks seen in the UV absorption spectrum show that GNR has two localized plasmon resonances: the transverse and longitudinal modes. This important fact confirms that GNR is directionally dependent, given its rod shape, and is thus an anisotropic material that can be imaged using polarized tight. Using crossed polarization imaging, which can be achieved by simply adding a polarizer and analyzer to a bright field microscope, GNR can be seen as a bright gold/red color. DIC microscopy mode also uses cross polarized light, with the addition of two Wollaston prisms. These prisms bias the tight in such a way that the image seen looks more three dimensional. Since polarized tight is present, the inventors also have detected some of the GNR on samples imaged with DIC when the prisms are at the correct bias.

[0029] The 1093 peptide is comprised of the 12 amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1 and Figures 1 and 2). The 1093 peptide was identified to bind to c-MET specifically and efficiently by screening a phage display library. Due to solubility issues, the 1093 peptide as such cannot be conjugated to GNR by simply adding a thiol group. The inventors have modified the 1093 peptide to address the solubility issues, while leaving the binding sequence intact. Such modification includes attaching at least one or more moiety selected from the group consisting of ethylene glycol, lysine and any combination thereof to the N-terminus of the peptide.

[0030] As shown in Figs. 1 and 2, several lysine groups are incorporated within the structural motif of the GNR-1093 peptide that is expected to enhance the interaction of receptors with the peptide. The thioctic acid moiety is incorporated at the far end of the binding site. The thioctic acid-disulfide interacts with gold atoms on the GNR to form a stable 6-membered ring for utilization in diagnostic testing.

[0031] A person of skill will appreciate that in some embodiments a linker comprises the thioctic acid-disulate attached to polylysine comprising several lysine residues attached to a peptide specific for c-MET receptor. Thus, the polylysine bridge connects the thioctic acid moiety to the peptide. In addition to the thioctic acid moiety or instead of the thioctic acid moiety, any moiety selected from monothioctic acid, dithioctic acid, and trithioctic acid can be used.

[0032] In some embodiments, a gold nanorod-based c-MET tissue diagnostic agent comprises a gold nanorod conjugated with a peptide with the specific affinity to c-MET receptor through a linker comprising a thiol group. In some embodiments, a gold nanorod-based c-MET tissue diagnostic agent comprises a gold nanorod conjugated with a peptide with the specific affinity to c-MET receptor through a linker comprising a thiol group and ethylene glycol. In some embodiments, the thiol group is selected from at least one moiety from thioctic acid, monothioctic acid, dithioctic acid, and trithioctic acid.

[0033] Various ethylene glycols are suitable, including monoethylene glycol, diethylene glycol, and polyethylene glycol.

[0034] In some embodiments, a gold nanorod-based c-MET tissue diagnostic agent comprises a gold nanorod conjugated with the thioctic-acid disulfide bound to the N-terminus of the 1093 peptide through a poly-lysine bridge comprising several lysine residues. The number of lysine residues may vary. In some embodiments, the number of lysine residues is from 2 to 3.

[0035] In some embodiments, the polylysine bridge comprises at least two lysine residues. In other embodiments, the polylysine bridge comprises at least three lysine residues.

[0036] A peptide with the selective affinity to c-MET receptor can be synthetized and purified using high pressure liquid chromatography (HPLC). Fig. 3 reports an HPLC chromatogram for the 1093 peptide purified by HPLC. Further confirmation of the 1093 peptide purification was obtained from mass spectroscopy (MS) as shown in Fig. 4.

[0037] A further embodiment provides a method of forming a nanoparticle conjugate comprising a nanorod linked to a target molecule that is a peptide with the specific affinity for cMET receptor. In this method, gold nanorods are prepared using a seed-mediated growth method which utilizes mixing solutions of cetyltrimethylammonium bromide and chlorauric acid, followed by addition of a solution of cetyltrimethylammonium bromide, silver nitrate, and ascorbic acid, and the final solution then washed, filtered, and centrifuged to remove excess cetyltrimethylammonium bromide. In the next step, a polyethylene glycol linker is modified with thiol in a solution containing the gold nanorods. Completion of the

conjugation of a peptide to the gold nanorods is then achieved by addition of a solution containing a twelve amino acid sequence YLFSVHWPPLKA peptide (SEQ ID NO. 1) or some other peptide with the selective binding to c-MET receptor to the solution containing the gold nanorods. The nanoparticle conjugates comprising the nanorod attached to the peptide through the linker are then washed. These nanoparticle conjugates retain the Zeta potential is in the range about +10 mV to about +60 mV.

[0038] Further embodiments include a method for making a nanoparticle linked to a target molecule specific for cMET receptor. This method comprises the following steps: preparing nanoparticles; conjugating a linker to the nanoparticles; and conjugating of a target molecule specific for cMET receptor to the nanoparticle. Other methods may be employed for alternative nanoparticle conjugates. At least in some embodiments, a peptide may be modified by attaching several lysine residues to its N-terminus prior being linked with a gold nanorod by a linker comprising at least a thiol moiety.

[0039] Various analytical techniques can be used to confirm conjugation of a peptide to GNR. For example, UV-visible spectrum and Zeta potential of GNR-1093 as quality control can be used as shown in Figs. 5A and 5B, respectively.

[0040] In some embodiments, a nanoparticle conjugate comprising a nanorod linked to a peptide with the affinity specific for cMET receptor is used for treating patients where patients are to be treated with anti-cMET drugs. These drugs may include crizotinib and cabozantinib, as well as drugs currently in clinical trials as shown in Table 1. In these embodiments, a sample of patient's cancer tissue is reacted with the reagent such as for example GNR-1093, and the cancer tissue positive for expression of c-MET receptor is identified. A patient with cancer tissues positive for cMET receptor expression is then treated with at least one anti-cMET drug.

TABLE 1: cMET RECEPTORS TARGETING DRUG MOLECULES

No.	DRUG	COMPANY	STATUS	REASON
1	Onartuzumab (MetMab)	Roche	2014: FAILED Phase III trial	EFFICACY
2	Tivantinib (ARQ197)	ArQule	Phase III trial	
3	INC280	Novartis	Phase I/II	

4	Rilotumumab (AMG102) HGF-AB	Amgen	Phase I/II/III	
5	Ficlatuzumab (AV-299) HGF-AB	Aveo	Phase II	
6	TAK701 HGF-AB	Millennium	Phase I	
7	Cabozantinib (XL184)	Callagen Technology	Phase II	
8	Foretinib (GSK1363089)	GlaxoSmithKline	2014: WITHDRAWN Phase I/II	Product development termination
9	Golvatinib (E7050)	Eisai	Phase I/II	
10	AMG337	Amgen	Phase I	
11	MGCD265	MethylGene	Phase 1/II	
12	Amuvatinib	Astex	Phase II	
13	Crizotinib	Pfizer	Phase I	
14	LY2875358	Eli Lily	Phase II	
15	MSC2156119J	EMD Serono	Phase I	
16	Volitinib	Hutchinson medipharma	Phase I	

[0041] Further embodiments provide diagnostic methods in which a cancer tissue is analyzed for expression of c-MET receptor with a reagent comprising GNR conjugated to a peptide which selectively binds c-MET receptor. Cancer cells which express c-MET receptor are then detected based on identification of cells bound to a nanoparticle conjugate comprising a nanorod linked to a peptide with the specific affinity specific for cMET receptor. Such detection methods may include any of the following: dark field microscopy, near-infrared (NIR) transmission imaging, photoacoustic tomography (PAT), two-photon excited luminescence (TPL) imaging, surface enhanced Raman spectroscopy (SERS) imaging, polarized imaging, either in the dark field or through differential interference contrast (DIC) microscopy.

[0042] The invention will be now described by the way of the following non-limiting examples.

EXAMPLE 1. SYNTHESIS OF A NANOROD LINKED TO A PEPTIDE WITH SPECIFIC AFFINITY TO C-MET RECEPTOR

[0043] To synthesize a combined c-MET-targeted, GNR-based diagnostic and imaging agent GNR-1093, the 1093 peptide (SEQ ID NO. 1) was conjugated to GNRs functionalized with a polyethylene glycol.

[0044] Scheme 1 shown in Fig. 1 is (PEG) surface modification synthesis. In this method, gold nanorods (GNR) of aspect ratio 3.4 were prepared using an established seed-mediated growth method. All solutions were made in fresh de-ionized water (DI H_2O). A seed solution was prepared by making a 10ml solution of 0.1M cetyltrimethylammonium bromide (CTAB). The CTAB solution was lightly heated until the CTAB had dissolved, giving a clear solution. 250 ml of a 0.01M solution of chlorauric acid ($HAuCI_4$) was then added to the CTAB solution while stirring. Immediately after addition of chlorauric acid, 600 ml of ice-cold 0.01M sodium borohydride ($NaBH_4$) was added to the solution, which changed color from gold to light brown.

[0045] The seed solution was then left to stir for 5 minutes. While the seed solution stirred, 500 ml of growth solution was then prepared. The first step was to make 250 ml of a 0.1M CTAB solution, and then heat until the CTAB had dissolved as had been done with the seed solution. 250 ml of 0.001 M chlorauric acid was then added to this CTAB solution and stirred lightly by hand. 10 ml of 0.0043 M silver nitrate ($AgN0_3$) was added to the solution and again stirred gently by hand. 4 ml of 0.1M ascorbic acid was then added, and the solution was stirred very gently until the solution had turned from gold-orange to clear. This last step completed the growth solution. 0.5 ml of the seed solution was then added in to the growth solution. The solution was not touched after this point due to the delicate nature of the synthesis. Minutes later the solution turned from clear to purple, indicating the formation of GNR. The GNR solution was left alone for 24 hours, and then washed of CTAB. The GNR solution was twice filtered through filter paper to remove excess CTAB. To further remove excess CTAB, the solution was then centrifuged at 16,000 RPM for 10 minutes at 25°C. The supernatant was removed and replaced with fresh DI H_2O.

[0046] The centrifuging step was then repeated. The sample of GNR was then characterized using UV-Vis spectroscopy, TEM imaging, and Zeta potential. The GNR synthesized characteristically shows a UV-Vis transverse peak near 540 nm and much larger longitudinal peak near 780 nm. The Zeta potential for GNR-CTAB is highly positive, showing stable values of +20 mV and above. In order to attach the 1093 peptide, a 750 Dalton polyethylene glycol (PEG_{750}) linker modified with thiol was conjugated to the GNR. A solution of PEG750 was added to the GNR solution at

a molar ratio of 1:2 (GNR:Peg 750). This solution was allowed to stir for 24 hours. The washing step as described above was then repeated exactly to remove unbound PEG.

[0047] After washing, GNR-PEG was then characterized through UV-Vis spectroscopy, TEM imaging, and Zeta potential. The UV-Vis spectrum of GNR-PEG showed only a slight shift in absorbance, whereas the Zeta potential became a largely negative and stable value of -15mV and below. A solution of the 1093 peptide was then added to the GNR-PEG solution at a molar ratio of 1:1 GNR-PEG:1093 peptide. This solution was stirred for 24 hours to ensure maximum binding of peptide to PEG. The same washing protocol as with GNR-CTAB was followed after the 24-hour period. GNR-1093 was then characterized using UV-Vis spectroscopy, TEM, and Zeta potential. The UV-Vis spectrum shifted only slightly, but the Zeta potential once again became a highly positive with a value of +20mV and above.

EXAMPLE 2. DETECTION AND QUANTIFICIATION OF c-MET RECEPTORS IN CANCER CELLS

[0048] This example reports specific binding of the GNR-peptide reagent (GNR-1093) to c-MET receptors in MET-overexpressing cells lines and tissues, such as NSCLC. GNR-1093 interacts with c-MET receptors and can be visualized using polarized light as described in previous sections.

[0049] To further confirm that GNR-1093 is targeting c-MET receptors, cell microarrays with varying degrees of c-MET receptors were used. If GNR-1093 is targeting c-MET receptors, then there should be a linear increase in the number of GNR present in the surface of cells with increase in c-MET receptors. As shown in Fig. 6, there was a linear increase in GNR-1093 on the surface of cells with increasing c-MET receptors.

[0050] It is important to note that cellular microarrays used in this study contained three different cell lines that have been verified by Lilly Labs (Indianapolis, IN) as being low, medium, and highly c-MET receptor expressing. For quantification of GNR on cellular microarray, we used MATLAB image processing tools.

[0051] Using MATLAB for image processing, the DAPI (4',6-diamidino-2-phenylindole, dihydrochloride) stained nuclei of the cells were isolated in order to

evaluate the staining on a per cell basis. Each cell was then analyzed for expression of the intense red signals seen through polarized microscopy of gold nanorods.

[0052] The results of each image analysis were graphed on a log scale of the relative intensity of the pixels per cell. The pixel intensity surrounding each of the c-Met 'low' samples did not give much indication of GNR signals, whereas for the medium and highly MET expressing cell lines a much higher value of pixel intensity was detected.

[0053] The relative intensities of each sample were graphed according to the intensity scale, and a linear pattern was established (Figure 7). This study confirms that GNR-1093 is selective in identifying c-MET receptors. Further, it confirms that receptors present on the surface can be quantified using GNR signals.

[0054] The following protocol was followed for imaging: paraffin was removed from the slides by immersing twice in 100% xylene for 5 minutes, and then rehydrated in graded ethanol and Dl H_2O. Once prepared for staining, slides were stained using GNR-1093. For GNR staining, the slides were treated with 2.5% BSA solution for 10 minutes prior to addition of the nanorod solution. Once washed of BSA solution, the samples were treated with 50 ug/ml GNR-1093 solution in a humid chamber for 2 hours. After this time, the slides were washed thoroughly with PBS and Dl H_20. The GNR slides were mounted using fluorescent DAPI nuclear stain and imaged on a Leica DMSSOO using DAPI for nuclear identification, and either polarized or dark field light microscopy was used for gold nanorod detection. Using the scanning capabilities of Leica DMSSOO instrument, a tile-scan was constructed of one whole spot of the GNR-1093 treated NSCLC tissue microarray.

[0055] This capability allows for a bigger picture of expression in a given tissue, and will be further explored for image processing.

EXAMPLE 3. DETECTION AND QUANTIFICIATION OF c-MET RECEPTORS IN CANCER TISSUES

[0056] GNR-1093 was utilized in identifying c-MET receptors present in human tissues. A tissue microarray containing 18 separate cases of non-small cell lung carcinoma (NSCLC) and normal tissue was incubated with the c-MET targeted GNR-

1093 and non-targeted GNR-PEG compounds in order to determine the presence of c-MET receptors in the tissue cases.

[0057] The tissue microarray was tested in the same manner as the cell lines from Lilly Labs, using DAPI as a nuclear counter-stain.

[0058] Pictured are the 18 tissue stained tissue sections with normal tissue, scanned at 20x and stitched using a Leica DMSSOO scanning algorithm. The stitched overview of the tissue cores shows bright areas corresponding to GNR signals in the tissue. The non-targeted GNR-PEG samples show some areas of non-specific gold signals, while the targeted GNR-1093 samples show much brighter and higher intensity gold signals indicative of c-MET expression.

In each sample, the gold signals are localized to the areas where the cell nuclei are present, and the gold expression is confined to the membrane (Figure 8). Scanned images of all tumor and normal tissue sections analyzed are provided in Figs. 8-25. Table 2 below provides description of lung tissue sections of Figs 8-25.

TABLE 2. MICROARRAY PANEL DISPLAY

	1	2	3	4	5	6	7	8	9
A	NT	MT	AT	NT	MT	AT	NT	MT	AT
B	NT	MT	AT	NT	MT	AT	NT	MT	AT
C	NT	MT	AT	NT	MT	AT	NT	MT	AT
D	NT	MT	AT	NT	MT	AT	NT	MT	AT
E	NT	MT	AT	NT	MT	AT	NT	MT	AT
F	NT	MT	AT	NT	MT	AT	NT	MT	AT

AT – Adjacent tissue 1.5 cm away from tumor
MT – Malignant tumor
NT – Normal tissue

[0059] Table 3 below describes the pathology and status of cancer in each patient.

TABLE 3. TISSUE SECTIONS SCANNED WITH GNR-1093 AND THE IMAGES ARE APPENDED ALONG WITH COMMENTS

Number	Position	Sex	Age	Organ	Pathology	Grade	Type
BCO4022	A1	M	55	Lung	Normal lung tissue	-	Normal

BCO4022	A2	M	55	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	A3	M	55	Lung	Bronchus and chronic inflammation of the lung tissue	-	Adjacent
BCO4022	A4	M	46	Lung	Normal lung tissue	-	Normal
BCO4022	A5	M	46	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	A6	M	46	Lung	Congestion and edema	-	Adjacent
BCO4022	A7	M	67	Lung	Normal lung tissue	-	Normal
BCO4022	A8	M	67	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	A9	M	67	Lung	Chronic inflammation of mucous membrane of bronchus	-	Adjacent
BCO4022	B1	M	55	Lung	Chronic pneumonitis	-	Normal
BCO4022	B2	M	55	Lung	Squamous cell carcinoma	II	Malignant
BCO4022	B3	M	55	Lung	Normal lunch tissue	-	Adjacent
BCO4022	B4	M	62	Lung	Chronic congestion and edema	-	Normal
BCO4022	B5	M	62	Lung	Clear cell type squamous cell carcinoma	-	Malignant
BCO4022	B6	M	62	Lung	Chronic congestion	-	Adjacent
BCO4022	B7	M	53	Lung	Normal lunch tissue	-	Normal
BCO4022	B8	M	53	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	B9	M	53	Lung	Congestion	-	Adjacent
BCO4022	C1	M	63	Lung	Edema	-	Normal
BCO4022	C2	M	63	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	C3	M	63	Lung	Edema	-	Adjacent
BCO4022	C4	M	25	Lung	Congestion	-	Normal
BCO4022	C5	M	35	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	C6	M	25	Lung	Congestion and edema	-	Adjacent
BCO40	C7	M	58	Lun	Normal lung tissue	-	Normal

22				g			
BCO4022	C8	M	58	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	C9	M	58	Lung	Chronic pneumonitis and tumoral necrosis	-	Adjacent
BCO4022	D1	M	71	Lung	Normal lung tissue	-	Normal
BCO4022	D2	M	71	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	D3	M	71	Lung	Please read specific diagnoses in note	III	Adjacent
BCO4022	D4	M	40	Lung	Normal lung tissue	-	Normal
BCO4022	D5	M	40	Lung	Squamous cell carcinoma with necrosis	II	Malignant
BCO4022	D6	M	40	Lung	Collapse of lung	-	Adjacent
BCO4022	D7	M	54	Lung	Bronchus and lung tissue	-	Normal
BCO4022	D8	M	54	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	D9	M	54	Lung	Normal lung tissue	-	Adjacent
BCO4022	E1	M	54	Lung	Bronchus and lung tissue	-	Normal
BCO4022	E2	M	54	Lung	Squamous cell carcinoma	II	Malignant
BCO4022	E3	M	54	Lung	Normal lung tissue	-	Adjacent
BCO4022	E4	M	60	Lung	Normal lung tissue	-	Normal
BCO4022	E5	M	60	Lung	Please read specific diagnosis in note	II	Malignant
BCO4022	E6	M	60	Lung	Collapse of lung	-	Adjacent
BCO4022	E7	M	53	Lung	Normal lung tissue	-	Normal
BCO4022	E8	M	53	Lung	Squamous cell carcinoma	II	Malignant
BCO4022	E9	M	53	Lung	Fibrous tissue and blood vessels	-	Adjacent
BCO4022	F1	F	43	Lung	Normal lung tissue	-	Normal
BCO4022	F2	F	43	Lung	Squamous cell carcinoma	III	Malignant
BCO4022	F3	F	43	Lung	Chronic congestion	-	Adjacent

BCO4022	F4	M	62	Lung	Normal lung tissue	-	Normal
BCO4022	F5	M	62	Lung	Squamous cell carcinoma	II	Malignant
BCO4022	F6	M	62	Lung	Mild chronic inflammation of lung tissue and bronchus	-	Adjacent
BCO4022	F7	M	69	Lung	Normal lung tissue	-	Normal
BCO4022	F8	M	69	Lung	Squamous cell carcinoma with necrosis	III	Malignant
BCO4022	F9	M	69	Lung	Bronchus and lung tissue	-	Adjacent

What is claimed is:

1. A nanoparticle conjugate comprising a nanoparticle linked via a linker to a target molecule with the specific affinity for c-MET receptor.

2. The nanoparticle conjugate of claim 1, wherein said target molecule is selected from the group consisting of a peptide, ligand, antibody, small molecule and any combination thereof.

3. The nanoparticle conjugate of claim 1, wherein said target molecule is a peptide consisting essentially of the amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1).

4. The nanoparticle conjugate of claim 1, wherein the nanoparticle is a nanorod.

5. The nanoparticle conjugate of claim 1, wherein the nanoparticle is a nanorod linked to a peptide consisting essentially of the amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1) by the linker comprising a thiol moiety selected from the group consisting of thioctic acid, monothioctic acid, dithioctic acid, and trithioctic acid.

6. The nanoparticle conjugate of claim 1, wherein the linker comprises a thioctic acid moiety and from 2 to 3 lysine residues.

7. The nanoparticle conjugate of claim 1, wherein the linker comprises a thiol moiety and ethylene glycol.

8. The nanoparticle conjugate of claim 1, wherein the linker comprises at least one or more moiety selected from the group consisting of a thiol moiety, lysine residue and ethylene glycol.

9. The nanoparticle conjugate of claim 8, wherein said ethylene glycol is selected from the group consisting of monoethylene glycol, diethylene glycol, and polyethylene glycol.

10. The nanoparticle conjugate of claim 1, wherein the nanoparticle is a nanorod with the length in the range from about 30 nm to about 60 nm.

11. A method of treating a cancer patient, the method comprising:

reacting the patient's cancer tissue biopsy sample with a reagent comprising a nanoparticle conjugate comprising a nanorod linked to a peptide with the specific affinity to human c-MET receptor, and

wherein the reaction detects expression of c-MET receptor in the cancer tissue biopsy sample, administering to the patient an anti-cMET drug.

12. The method of claim 11, wherein the cancer patient is afflicted with a cancer selected from the group consisting of non-small cell lung carcinoma, gastric cancer, ovarian cancer, thyroid cancer, breast cancer, and colon cancer.

13. The method of claim 11, wherein the patient's cancer tissue biopsy sample is reacted with the reagent comprising a nanorod linked to a peptide consisting essentially of the amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1) by the linker comprising a thiol moiety selected from the group consisting of thioctic acid, monothioctic acid, dithioctic acid, and trithioctic acid.

14. The method of claim 11, wherein the detection is performed by a method selected from the group consisting of dark field microscopy, near-infrared (NIR) transmission imaging, photoacoustic tomography (PAT), two-photon excited luminescence (TPL) imaging, surface enhanced Raman spectroscopy (SERS) imaging and polarized imaging.

15. A method of forming a nanoparticle conjugate comprising a nanoparticle linked to a target molecule specific for cMET receptor, with the method comprising the following steps:

mixing solutions of cetyltrimethylammonium bromide and chlorauric acid,

adding a solution of cetyltrimethylammonium bromide, silver nitrate, and ascorbic acid to obtain gold nanorods,

purifying the gold nanorods by filtration and centrifugation;

conjugating a polyethylene glycol linker modified with thiol to the gold nanorods to obtained gold nanorods with a linker; and

conjugating a peptide to the gold nanorods with the linker.

16. The method of claim 15, wherein the peptide consists essentially of the amino acid sequence YLFSVHWPPLKA (SEQ ID NO. 1).

17. The method of claim 16, wherein the peptide is modified with a poly-lysine moiety at the N-terminus prior to be conjugated with a nanorod.

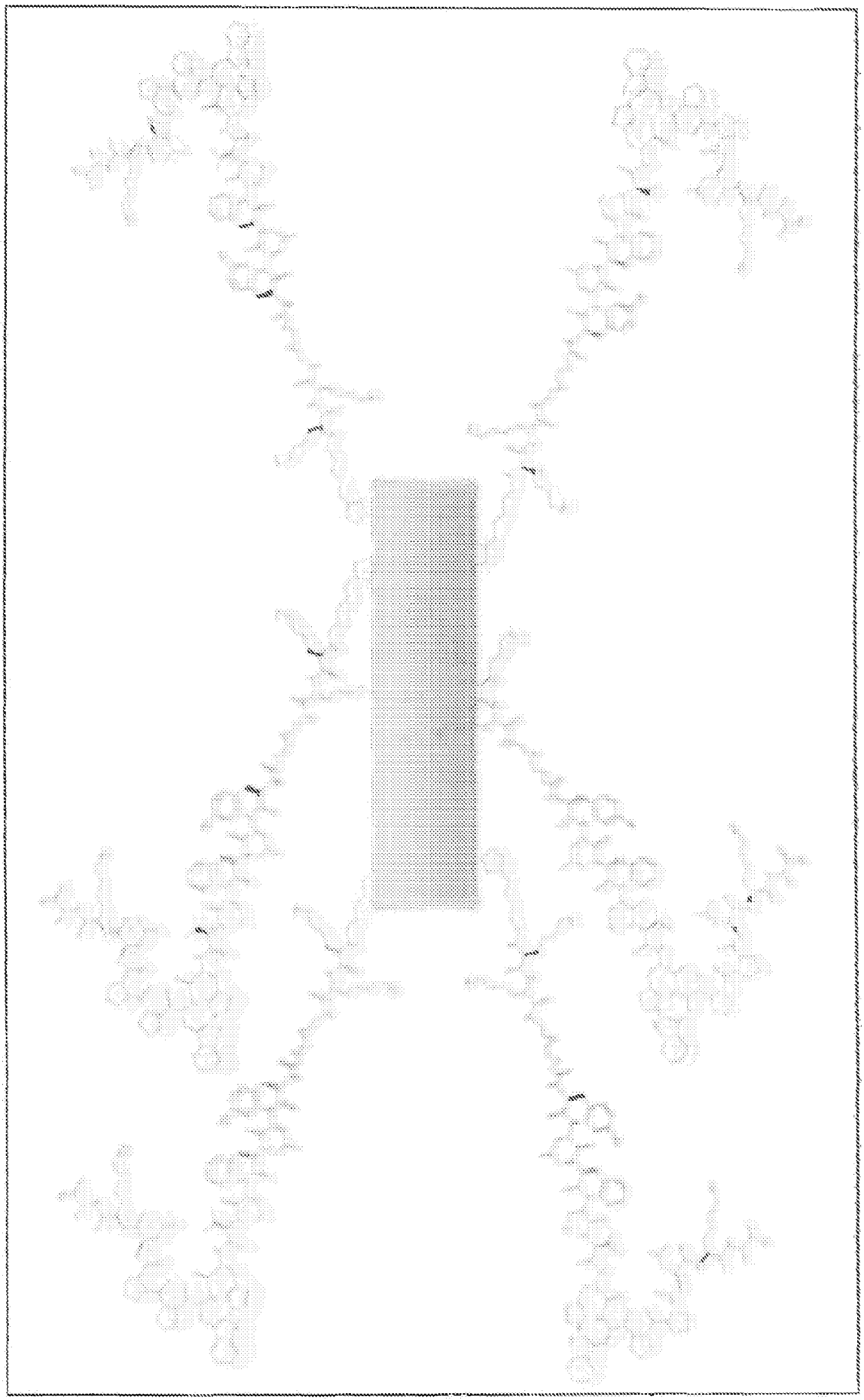


Figure 1

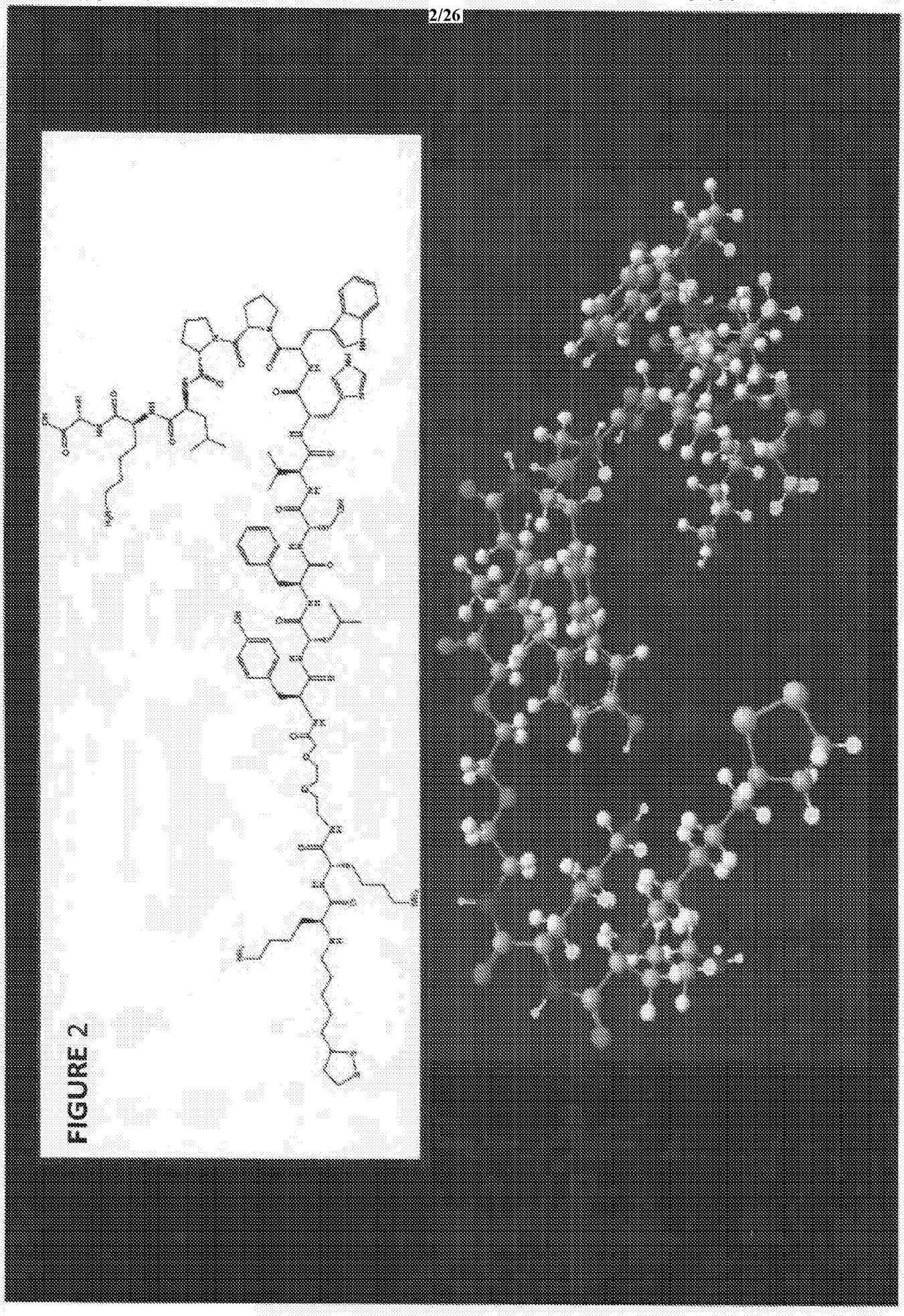


FIGURE 2

FIGURE 3

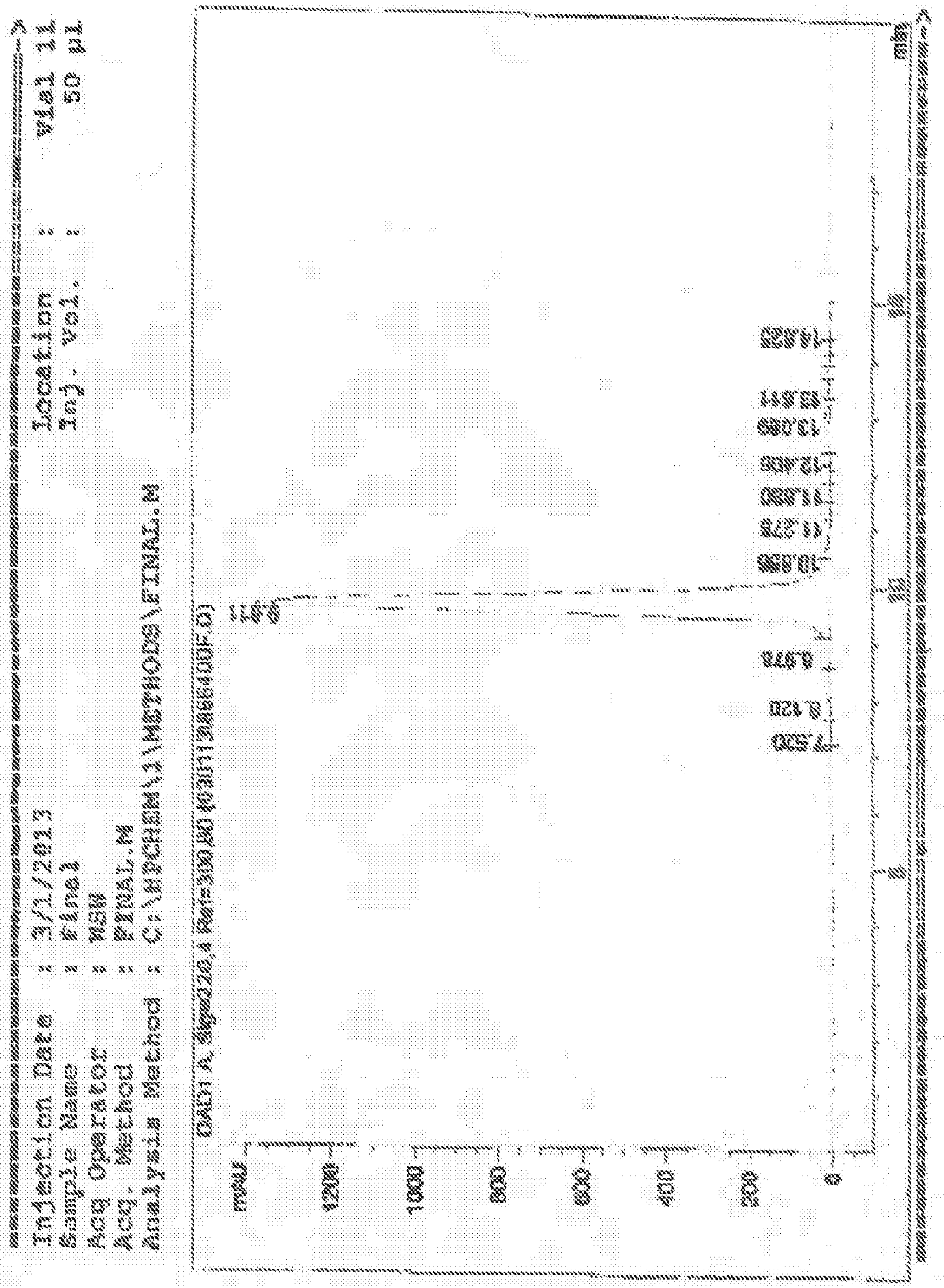
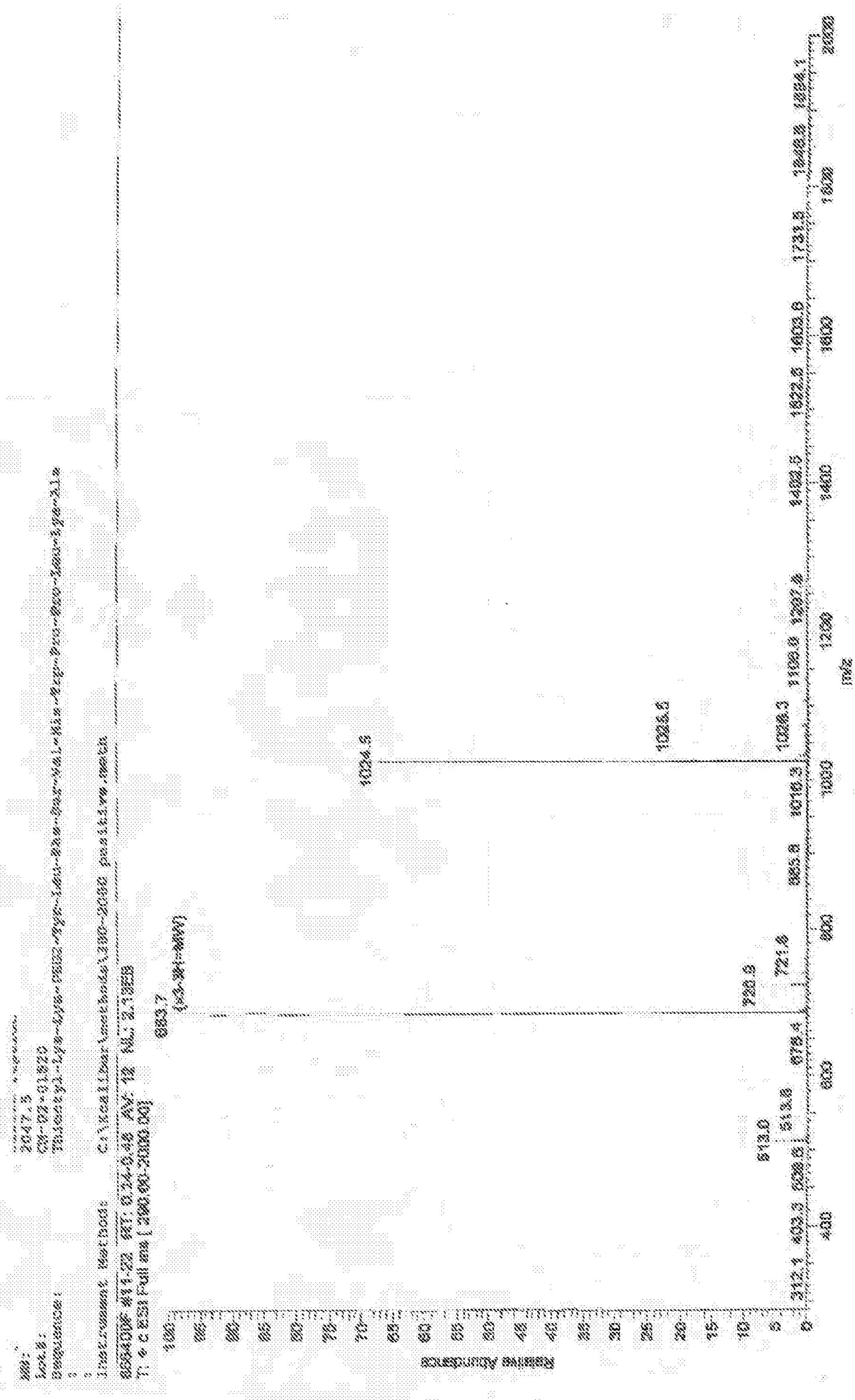

Injection Date : 3/1/2013
Sample Name : final
Acq Operator : MSW
Acq. Method : FINAL.M
Analysis Method : C:\HPCHEM\1\METHODS\FINAL.M
Location : Vial 11
Inj. Vol. : 50 µl
mAU
1200
1000
800
600
400
200
0
min

FIGURE 4



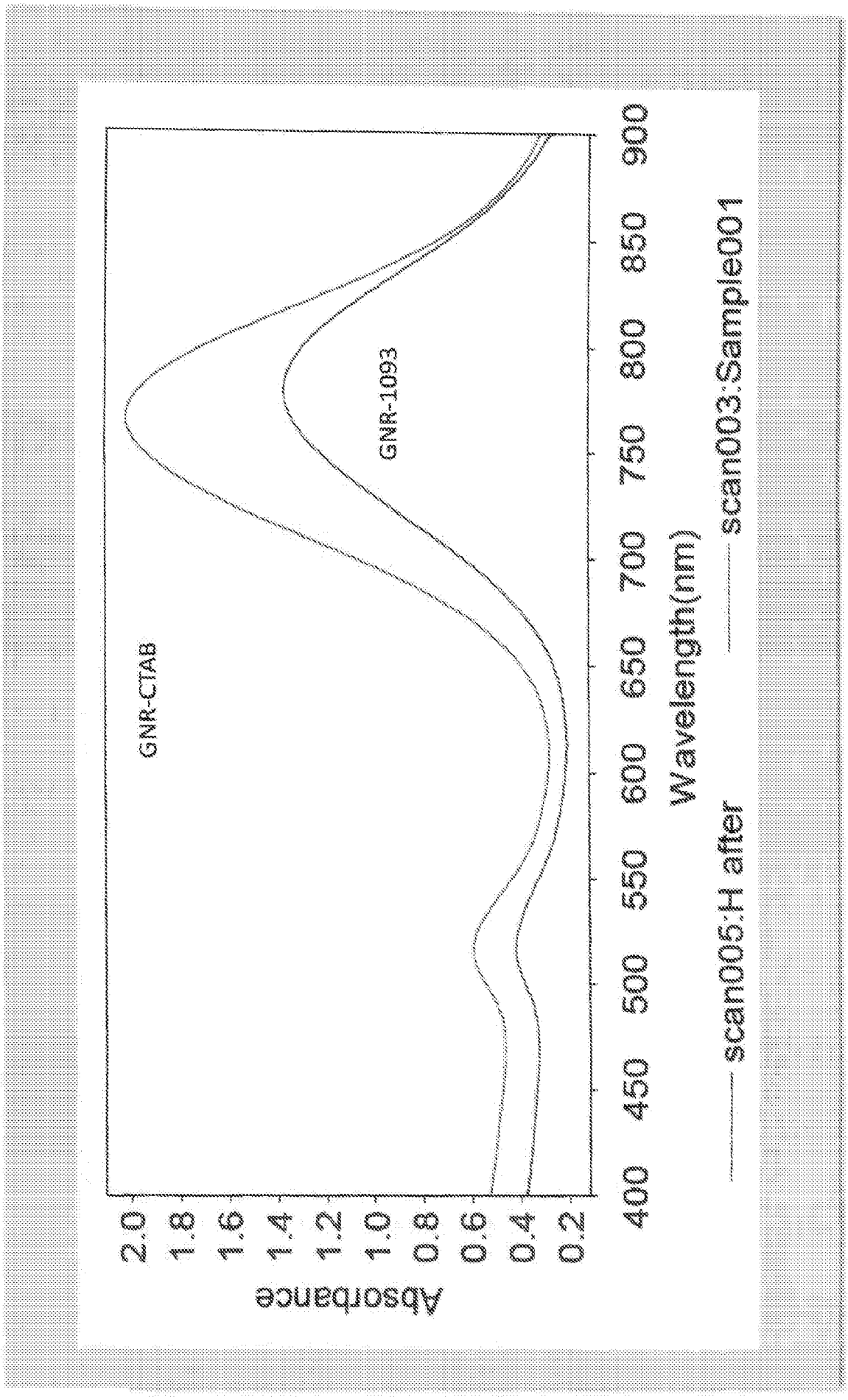
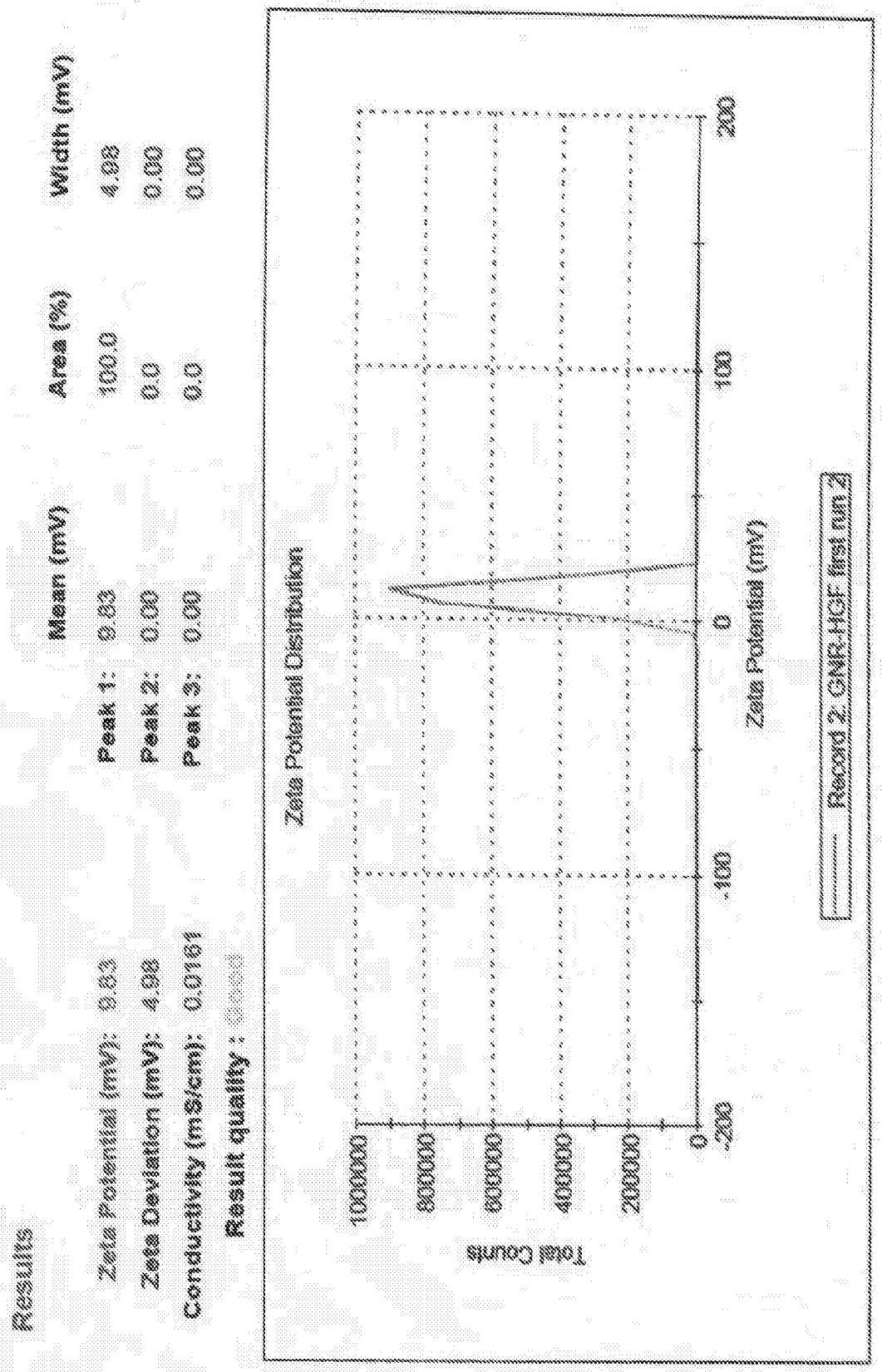

Absorbance
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
400
450
500
550
600
650
700
750
800
850
900
Wavelength(nm)
GNR-CTAB
GNR-1093
scan005.H after
scan003.Sample001

FIGURE 5a

FIGURE 5b


Results
Mean (mV)
Area (%)
Width (mV)
Zeta Potential (mV): 0.83
Peak 1: 0.83
100.0
4.08
Zeta Deviation (mV): 4.08
Peak 2: 0.00
0.0
0.00
Conductivity (mS/cm): 0.0101
Peak 3: 0.00
0.0
0.00
Result quality : Good
Zeta Potential Distribution
Total Counts
1000000
800000
600000
400000
200000
0
-200
-100
0
100
200
Zeta Potential (mV)
Record 2: GNR-HGF first run 2

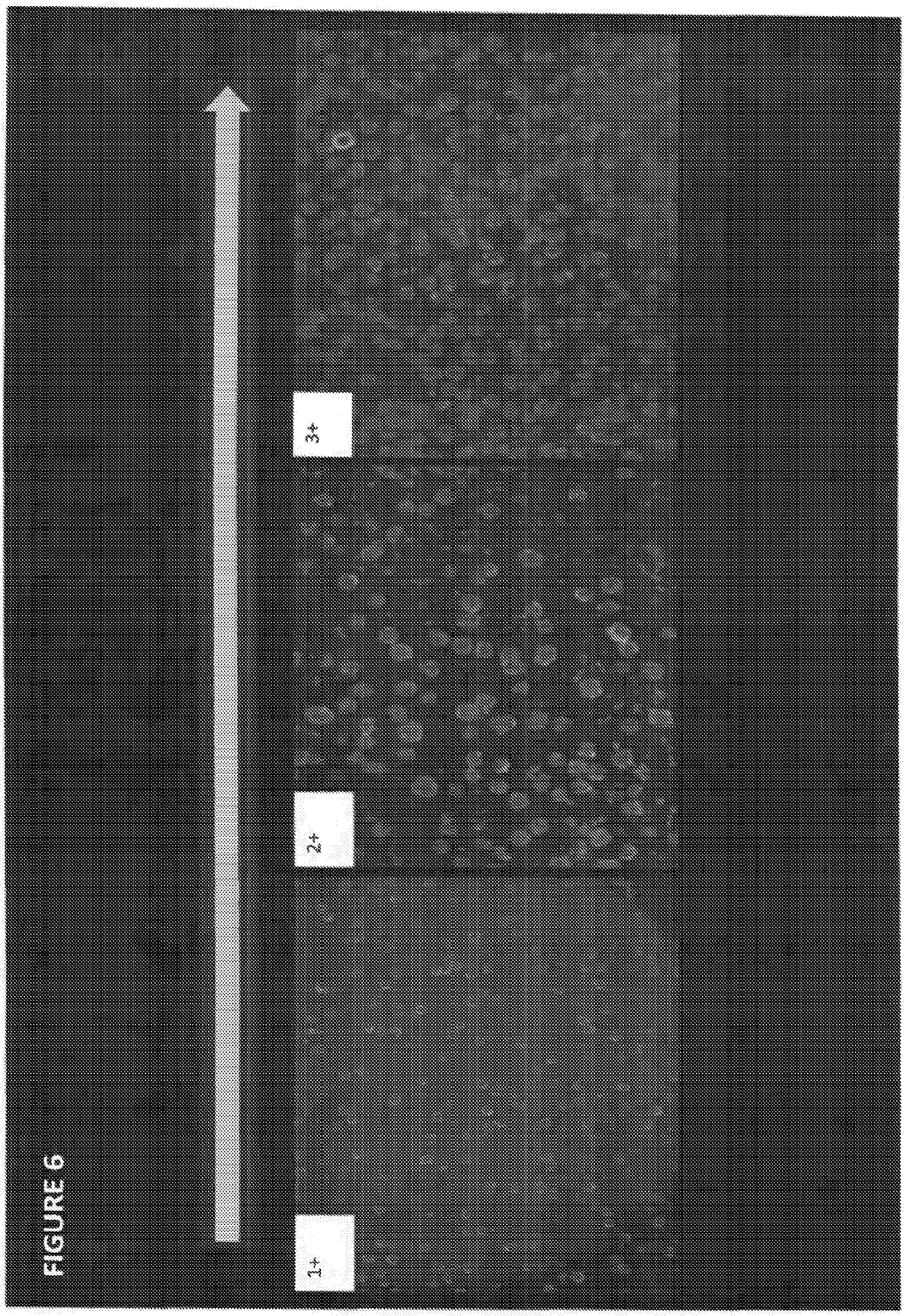
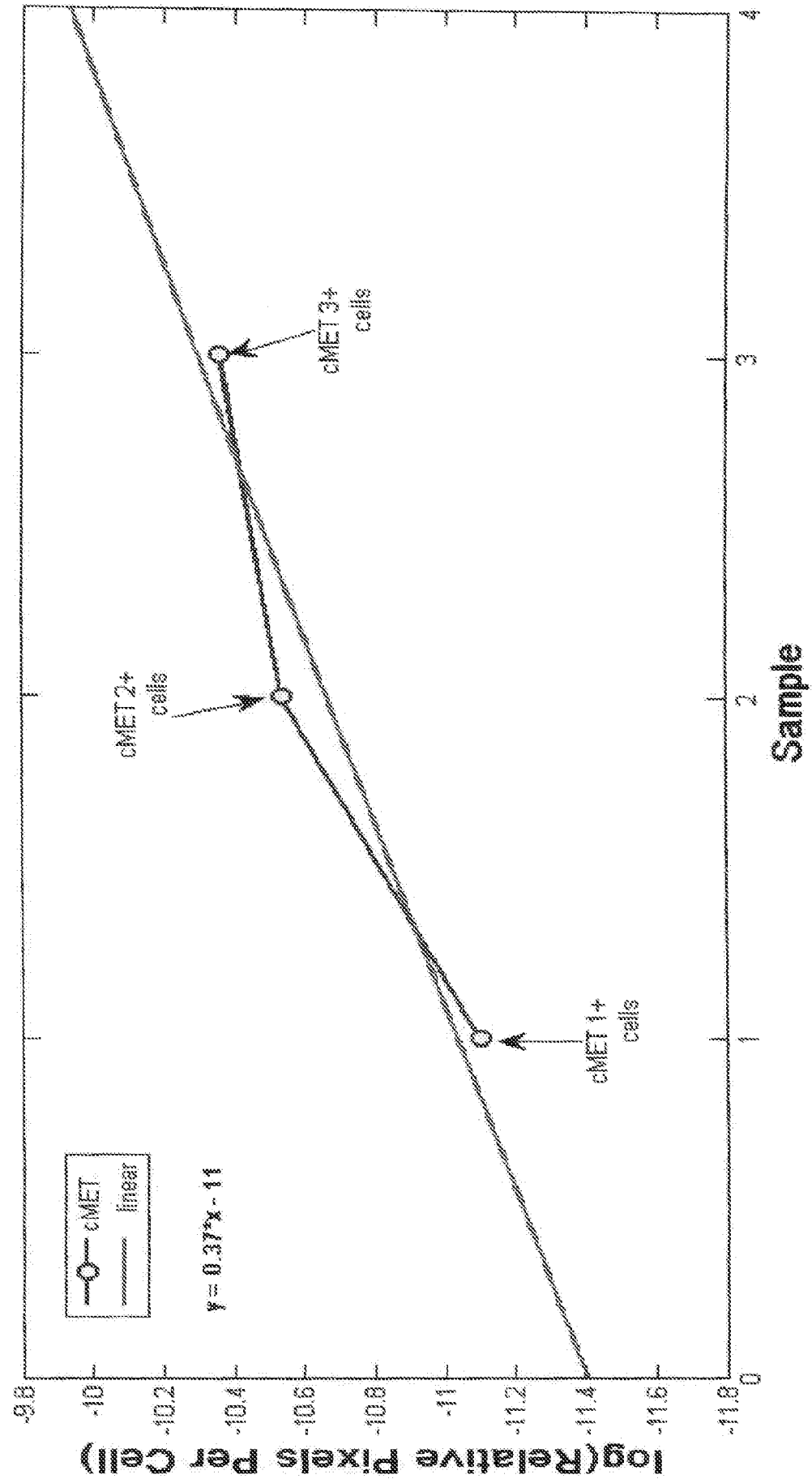


FIGURE 6

FIGURE 7


log(Relative Pixels Per Cell)
Sample
cMET
linear
y = 0.37*x - 11
cMET 1+ cells
cMET 2+ cells
cMET 3+ cells
-9.8
-10
-10.2
-10.4
-10.6
-10.8
-11
-11.2
-11.4
-11.6
-11.8
0
1
2
3
4

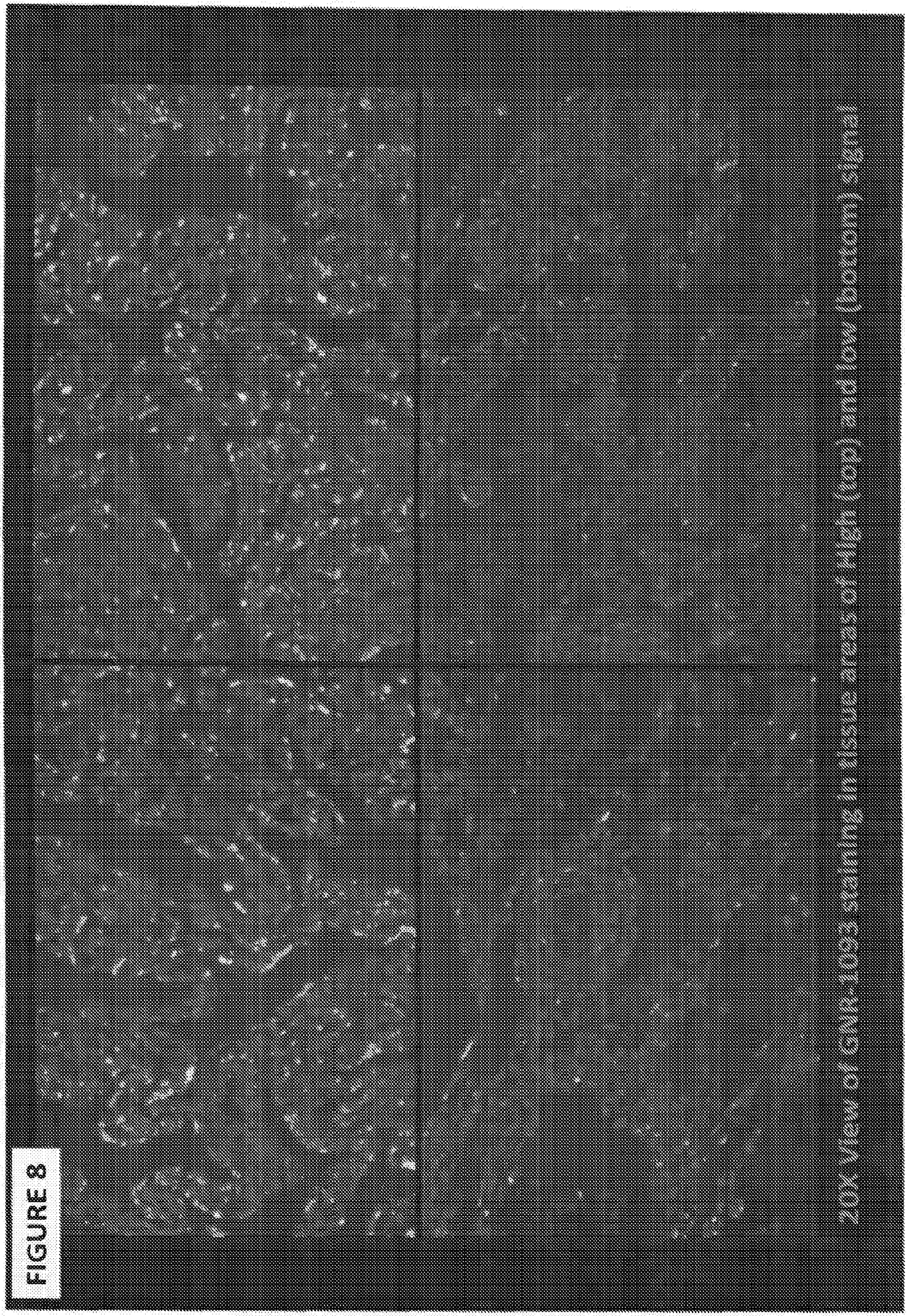


FIGURE 8

20X View of GNR-1093 staining in tissue areas of High (top) and low (bottom) signal

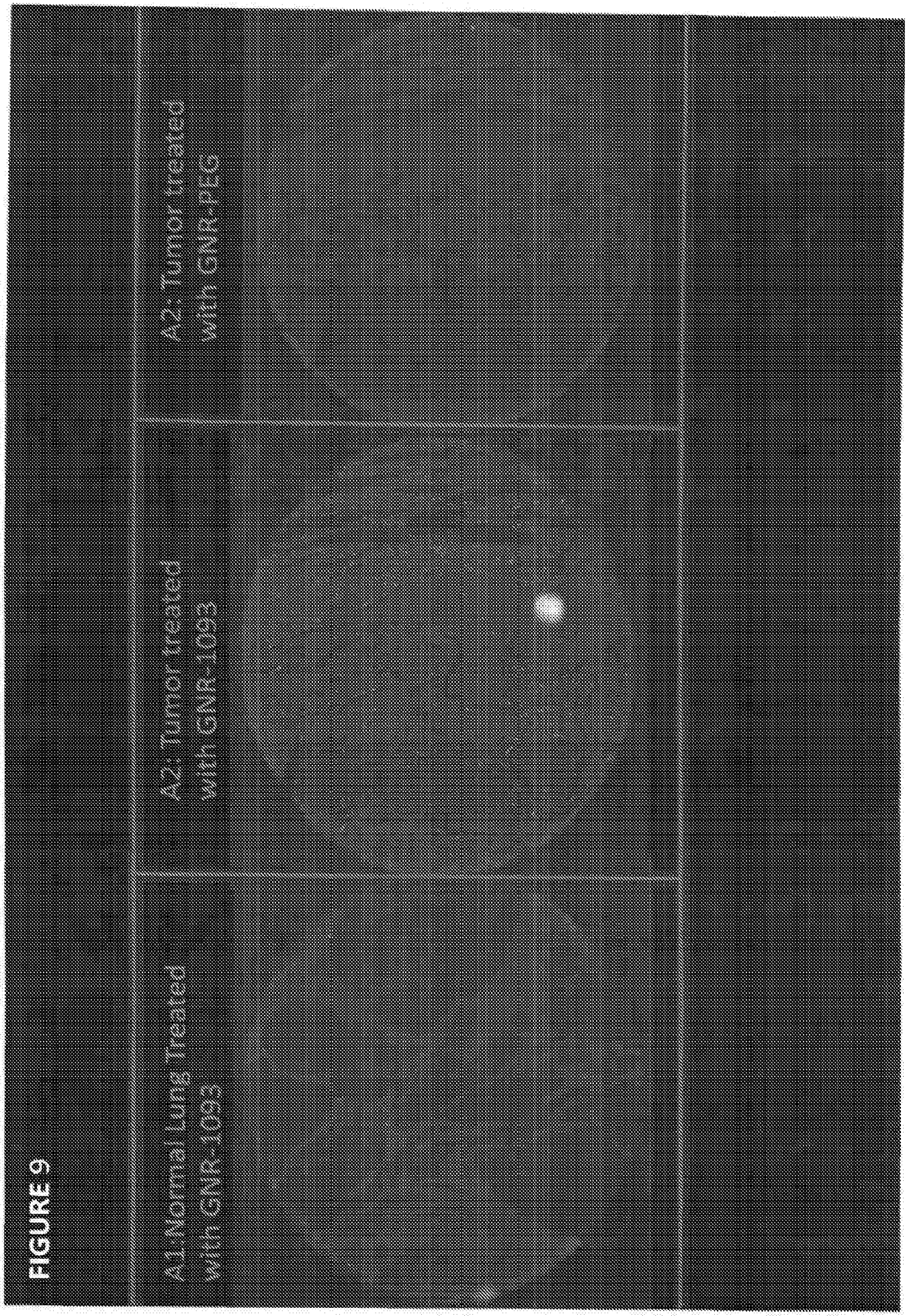

A1 Mammal (mouse) treated with GNR-1063
A2: Tumor treated with GNR-PEG
A3 Mammal (mouse) treated with GNR-1063
A4: Tumor treated with GNR-PEG

FIGURE 9

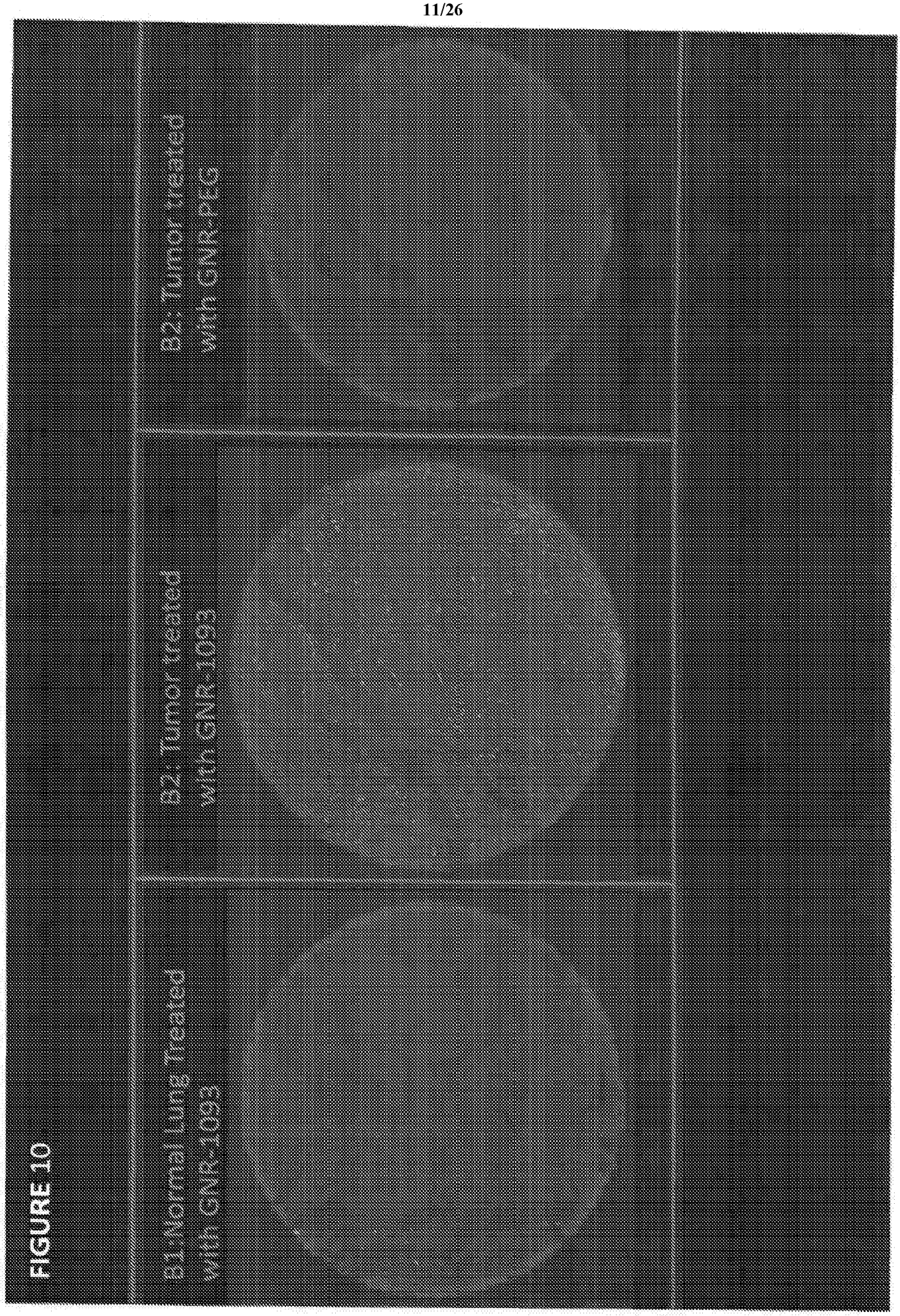

B7-H3 Tumor treated with GNR-126
B7-H3 Tumor treated with GNR-126 and laser
B7-H3 Tumor treated with GNR-126 and laser

FIGURE 10

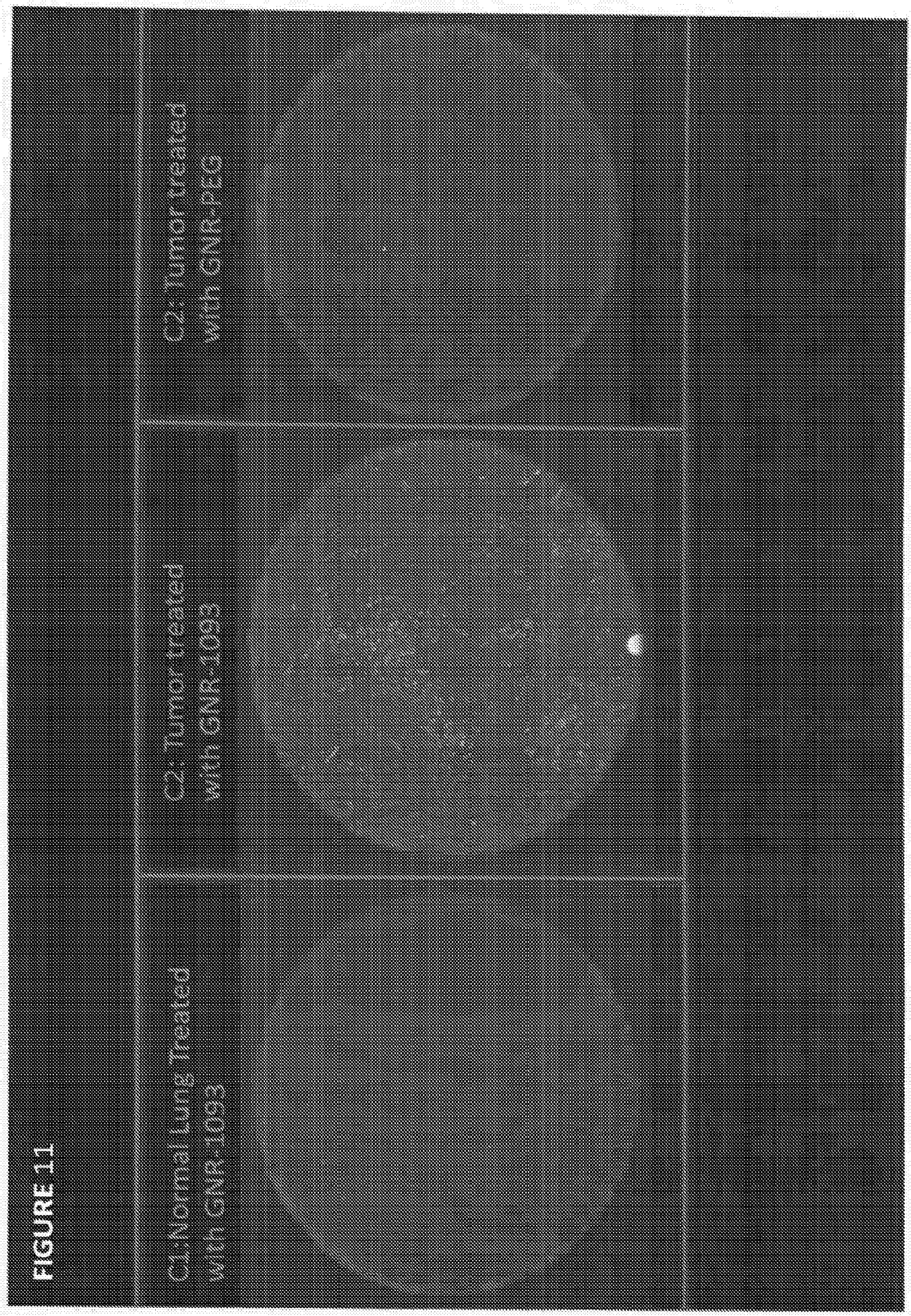

C1-Normal tissue treated with GNA-1053
C2-Tumor tissue treated with GNA-1053
C2-Tumor tissue treated with GNA-53

FIGURE 11

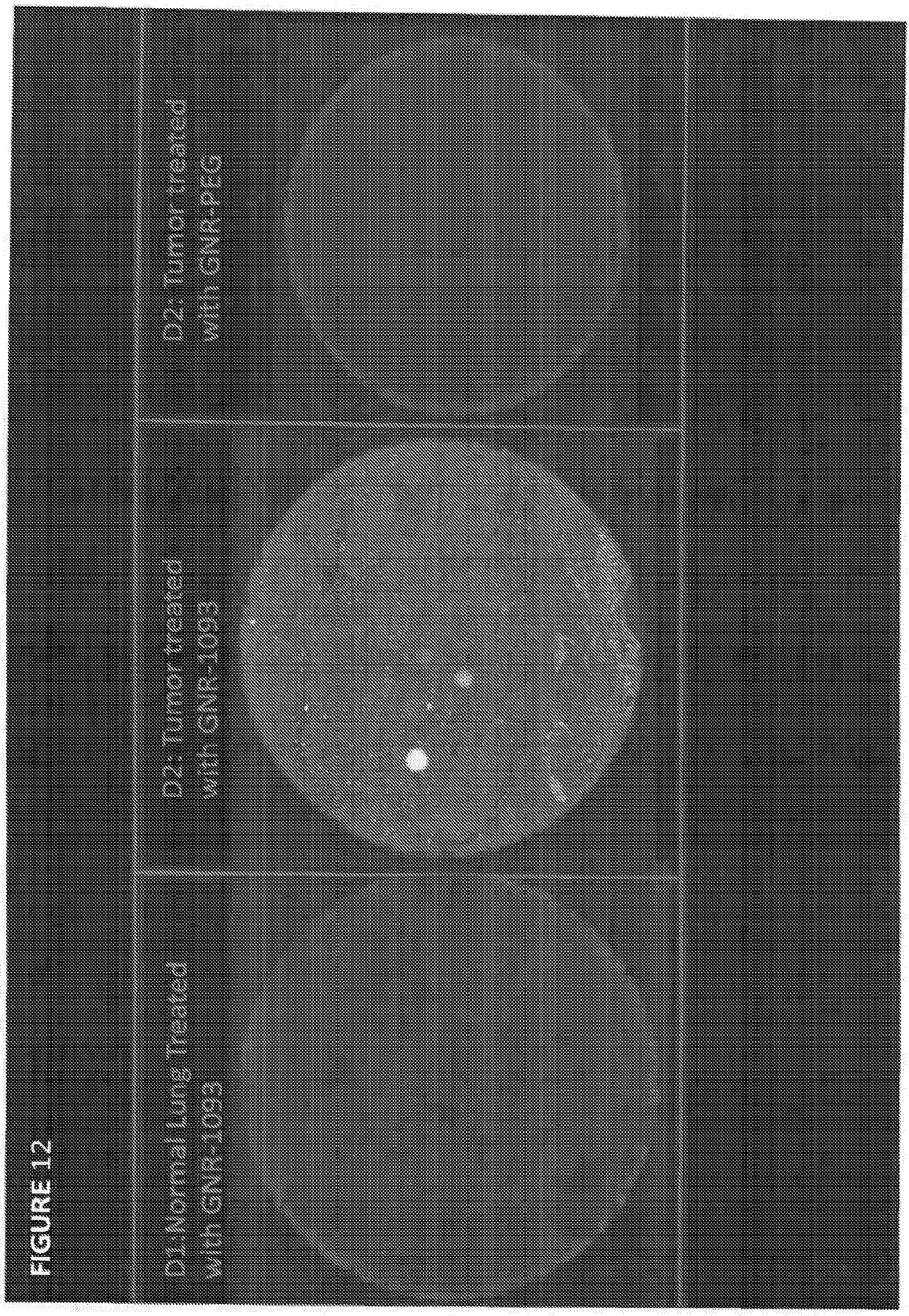

D2 tumor treated with GNR-1063
D2 tumor treated with GNR-1063
D2 tumor treated with GNR-1063

FIGURE 12

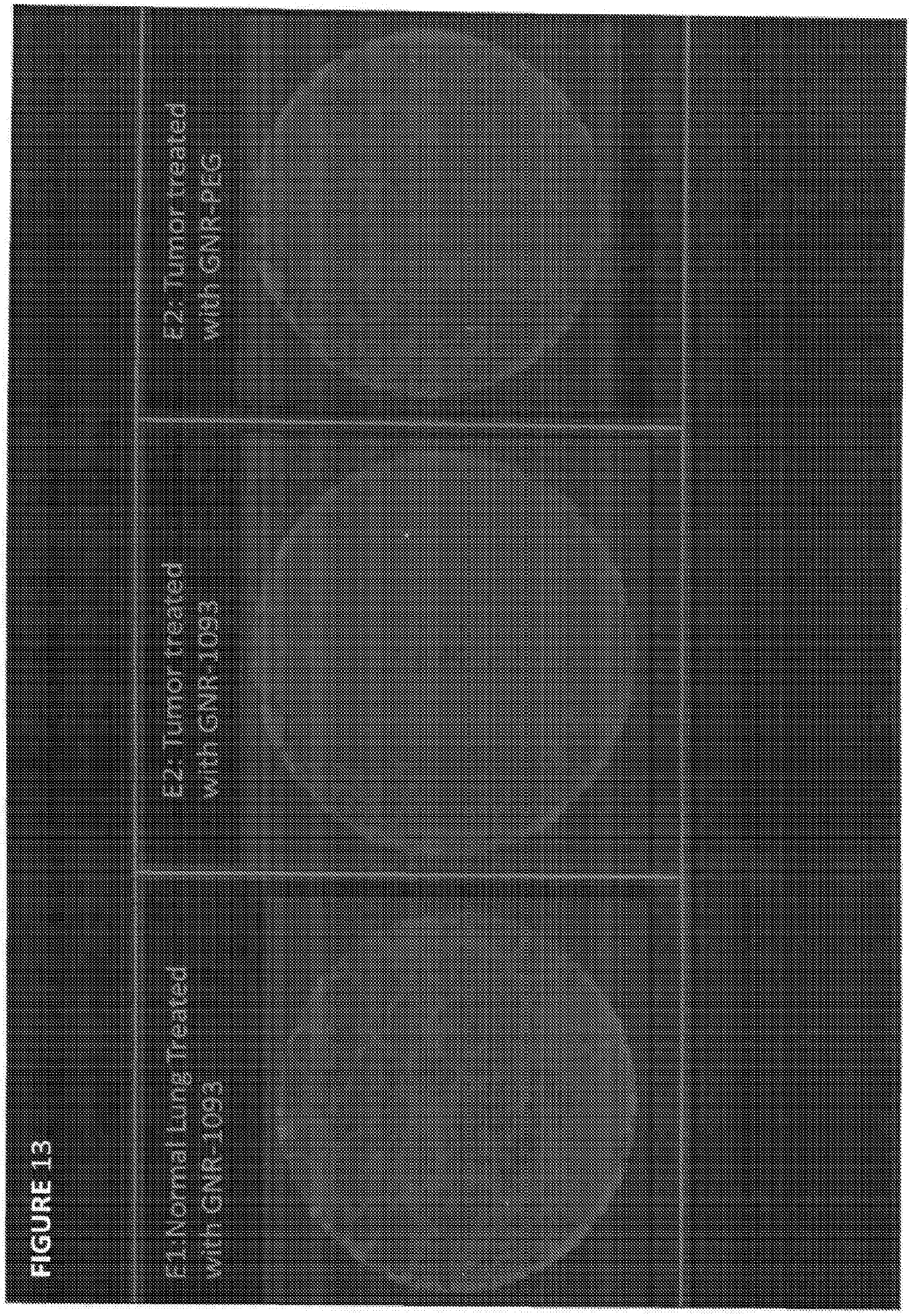

E2: Tumor treated with GNR-E3
E2: Tumor treated with GNR-1053
E1: Tumor treated with GNR-1053

FIGURE 13

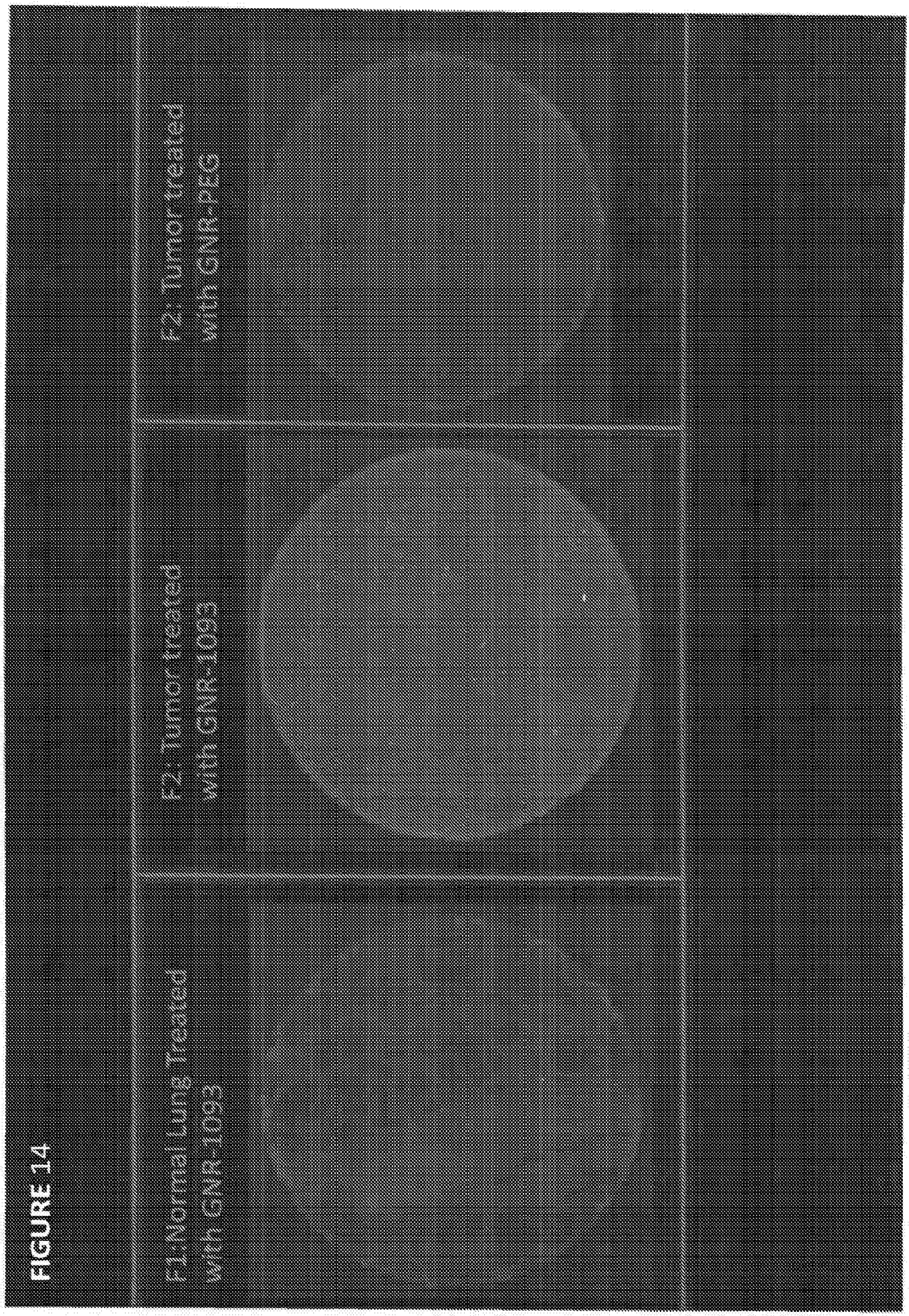

F1 Normal Lung Treated with GNR-1053
F2 Tumor treated with GNR-1053
F2 Tumor treated with GNR-649

FIGURE 14

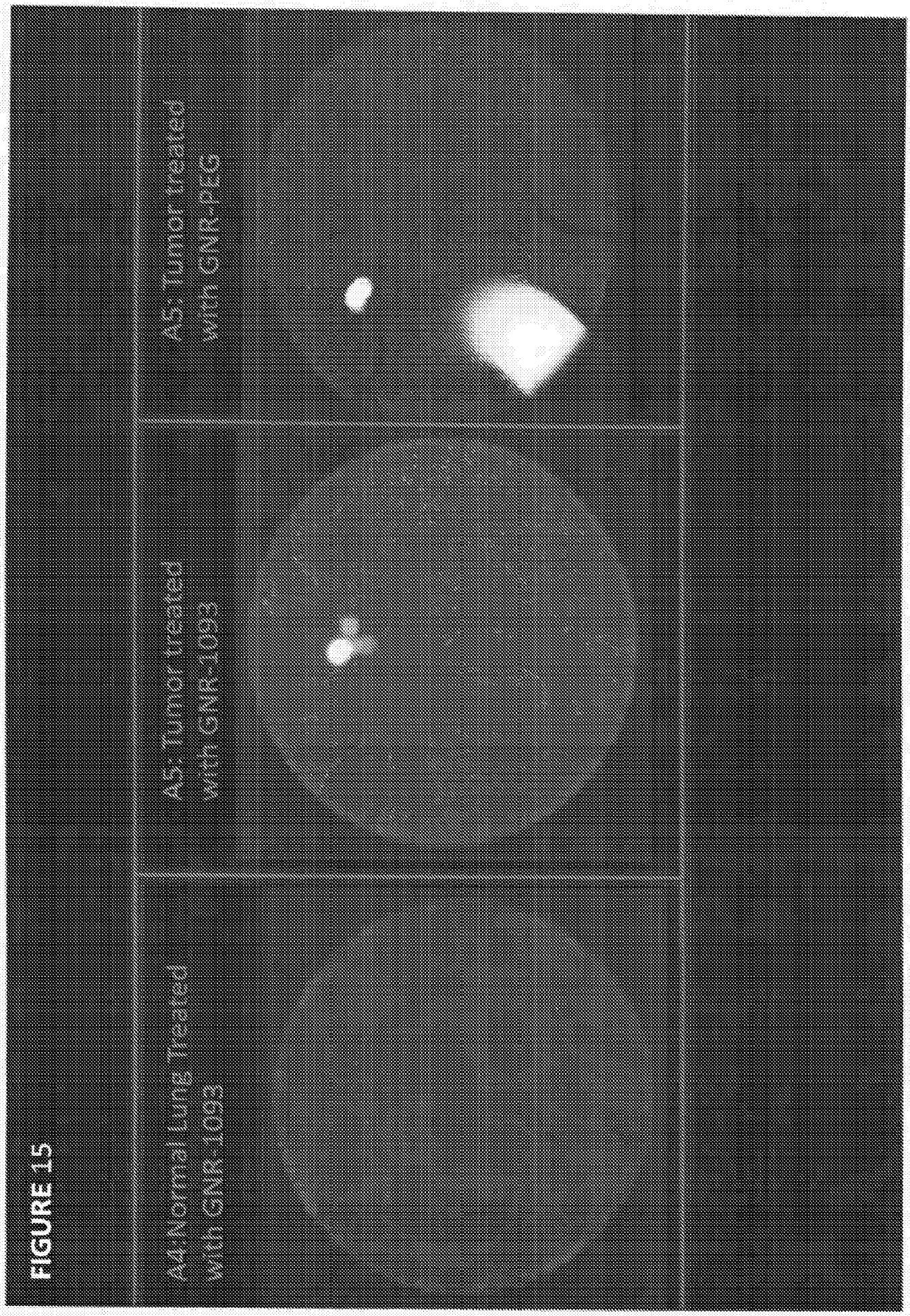

A5 Tumor treated with GMR-6293
A5 Tumor treated with GMR-1093
Animal Tumor Treated with GMR-1093

FIGURE 15

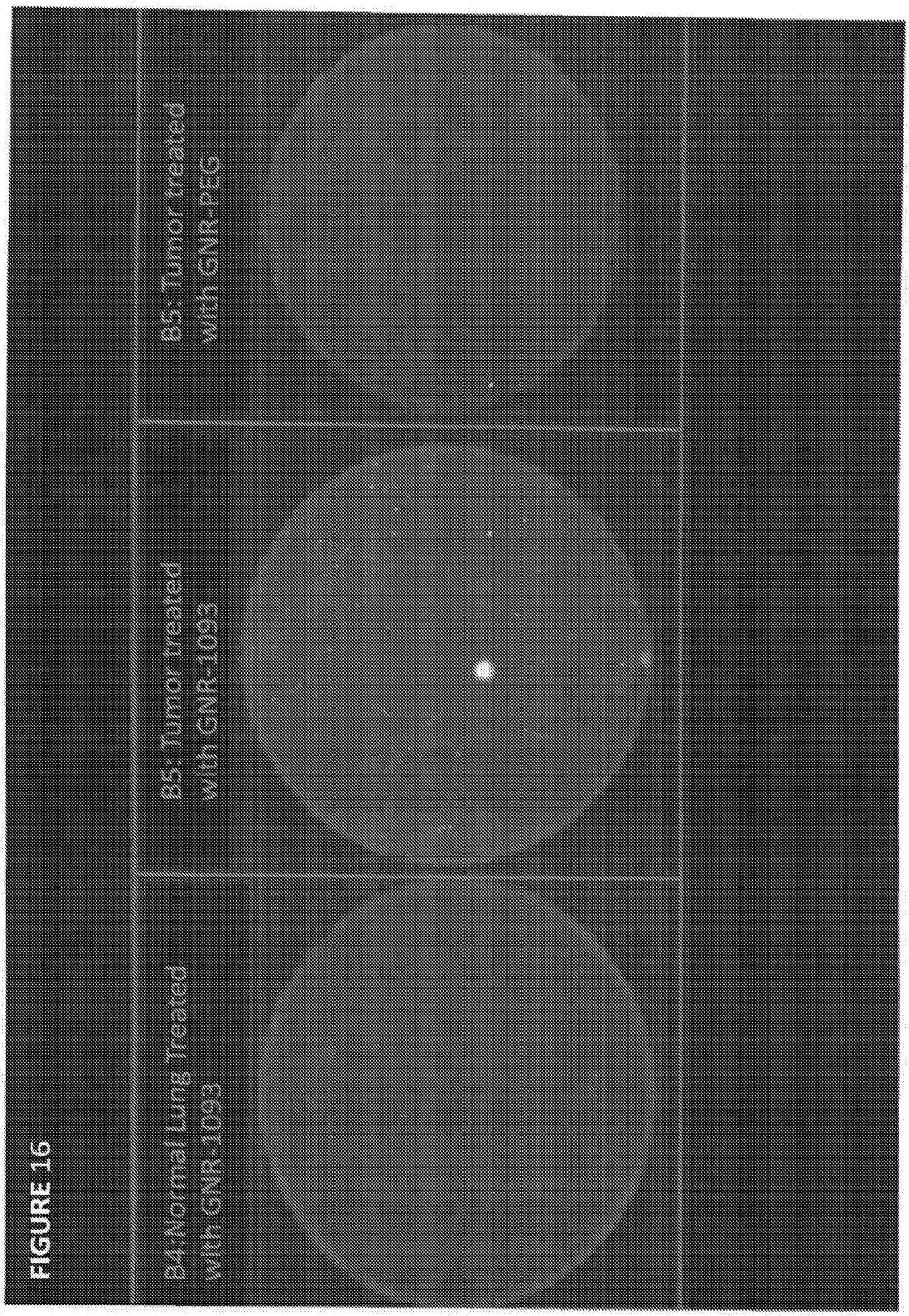

Day 0 Tumor Treated with GNR-pegs
Day 15 Tumor Treated with GNR-pegs
Day 0 Tumor Treated with GNR-TDS
Day 15 Tumor Treated with GNR-TDS

FIGURE 16

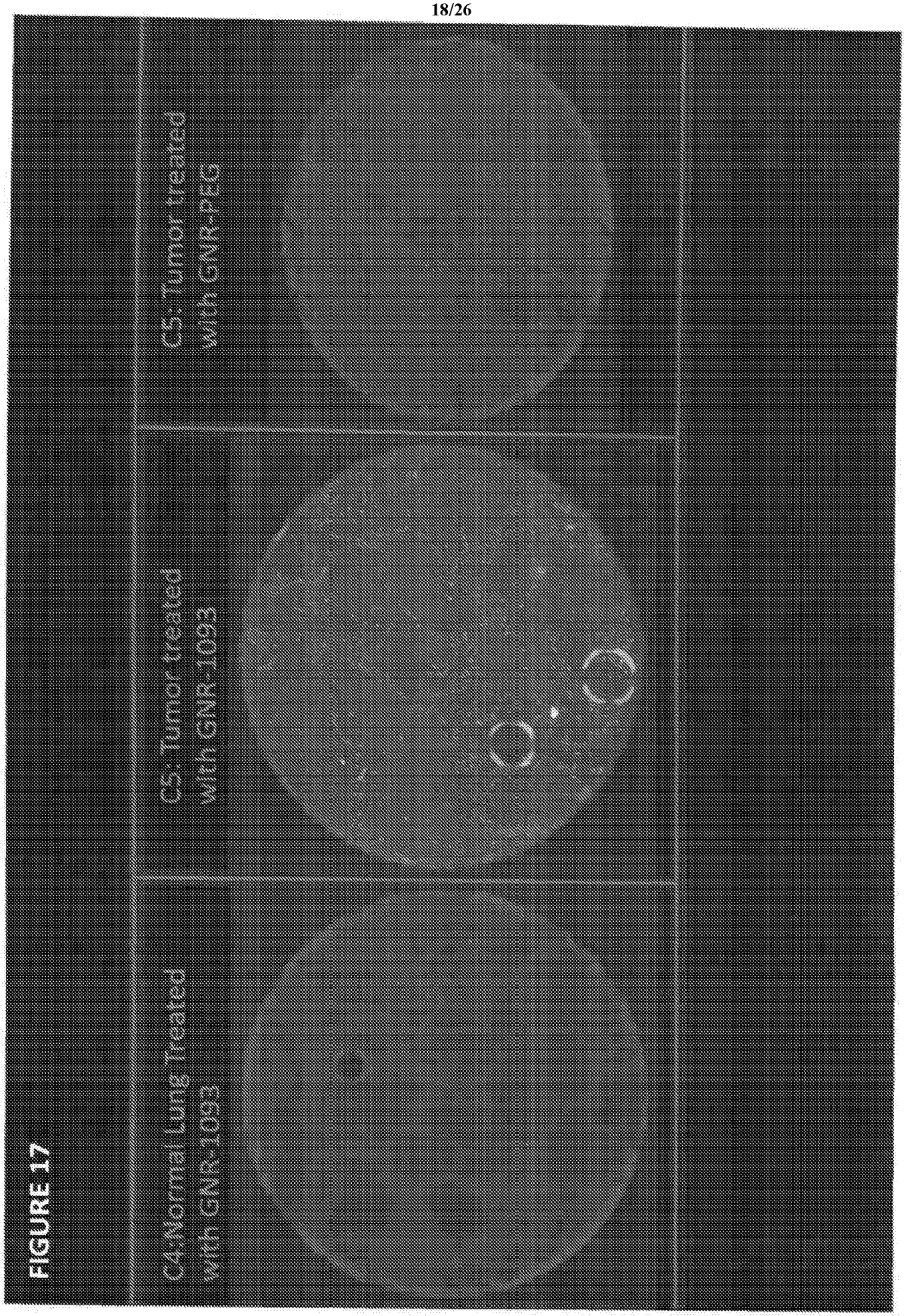

C4:Normal tissue treated with GNR-1093
C5:Tumor treated with GNR-1093
C6:Tumor treated with GNR-PEG

FIGURE 17

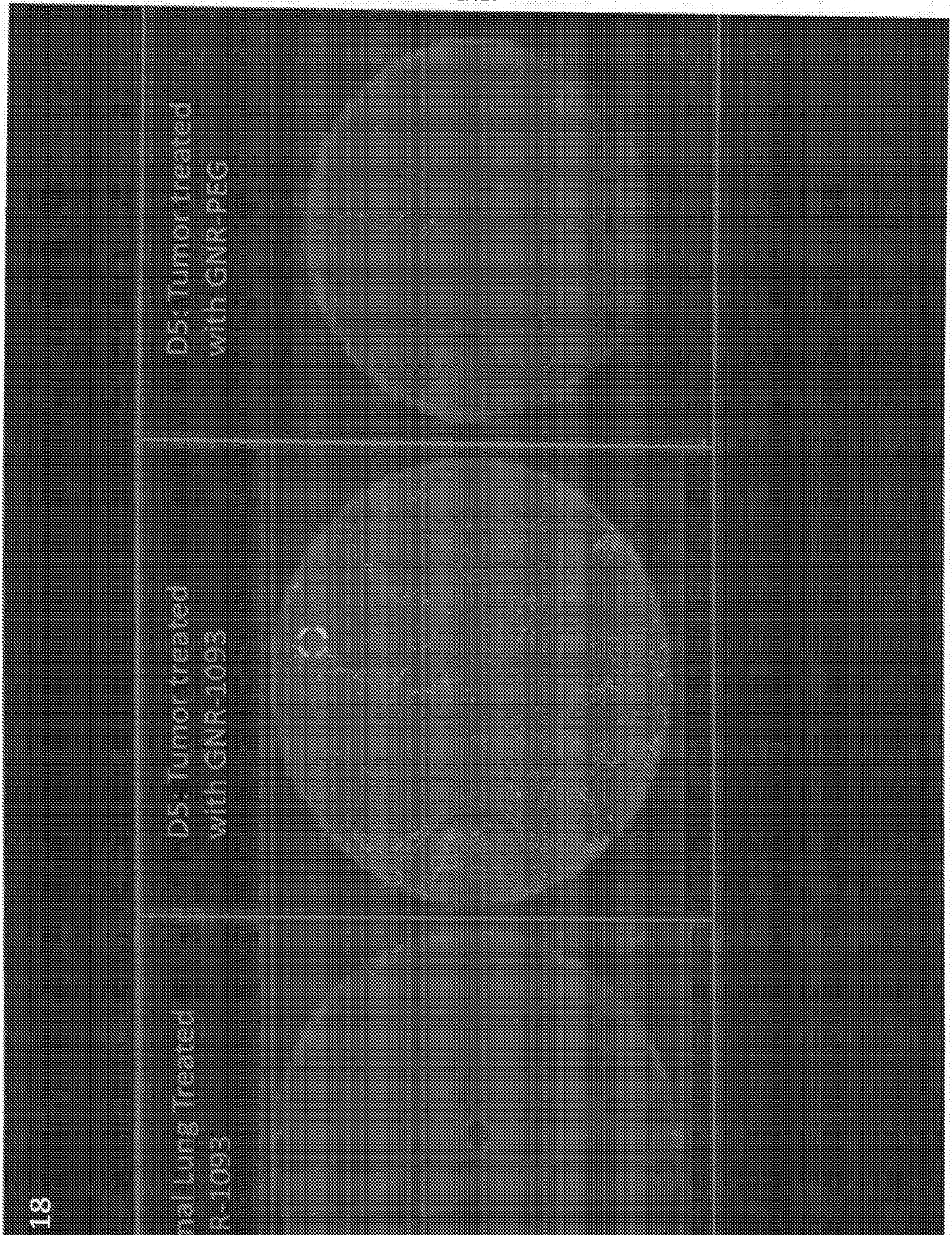


FIGURE 18
D4:Normal Lung Treated with GNR-1093
D5: Tumor treated with GNR-1093
D5: Tumor treated with GNR-PEG

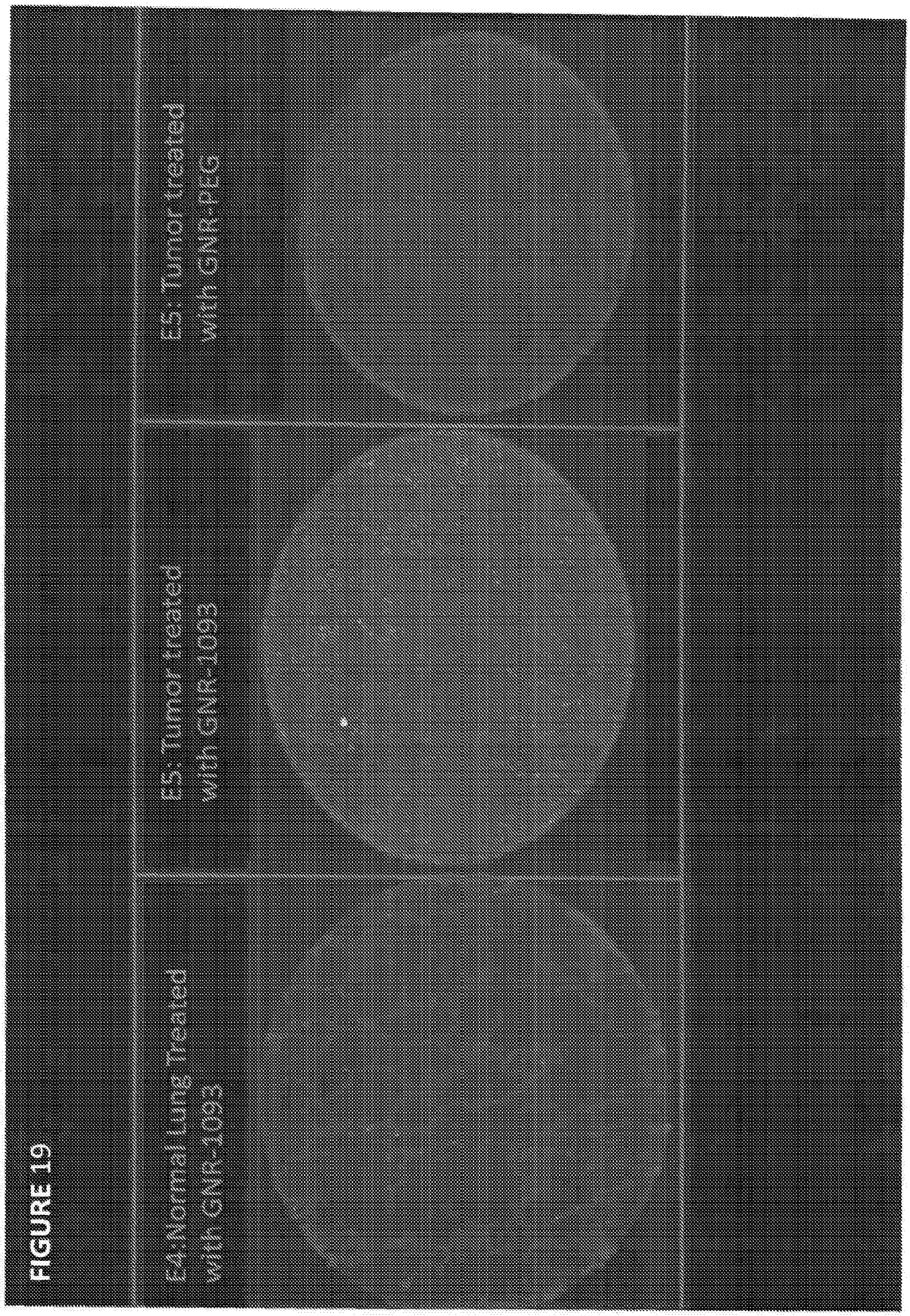


FIGURE 19
E4 Normal Lung Treated with GNR-1033
E5 Tumor treated with GNR-1033
E5 Tumor treated with GNR-PEG

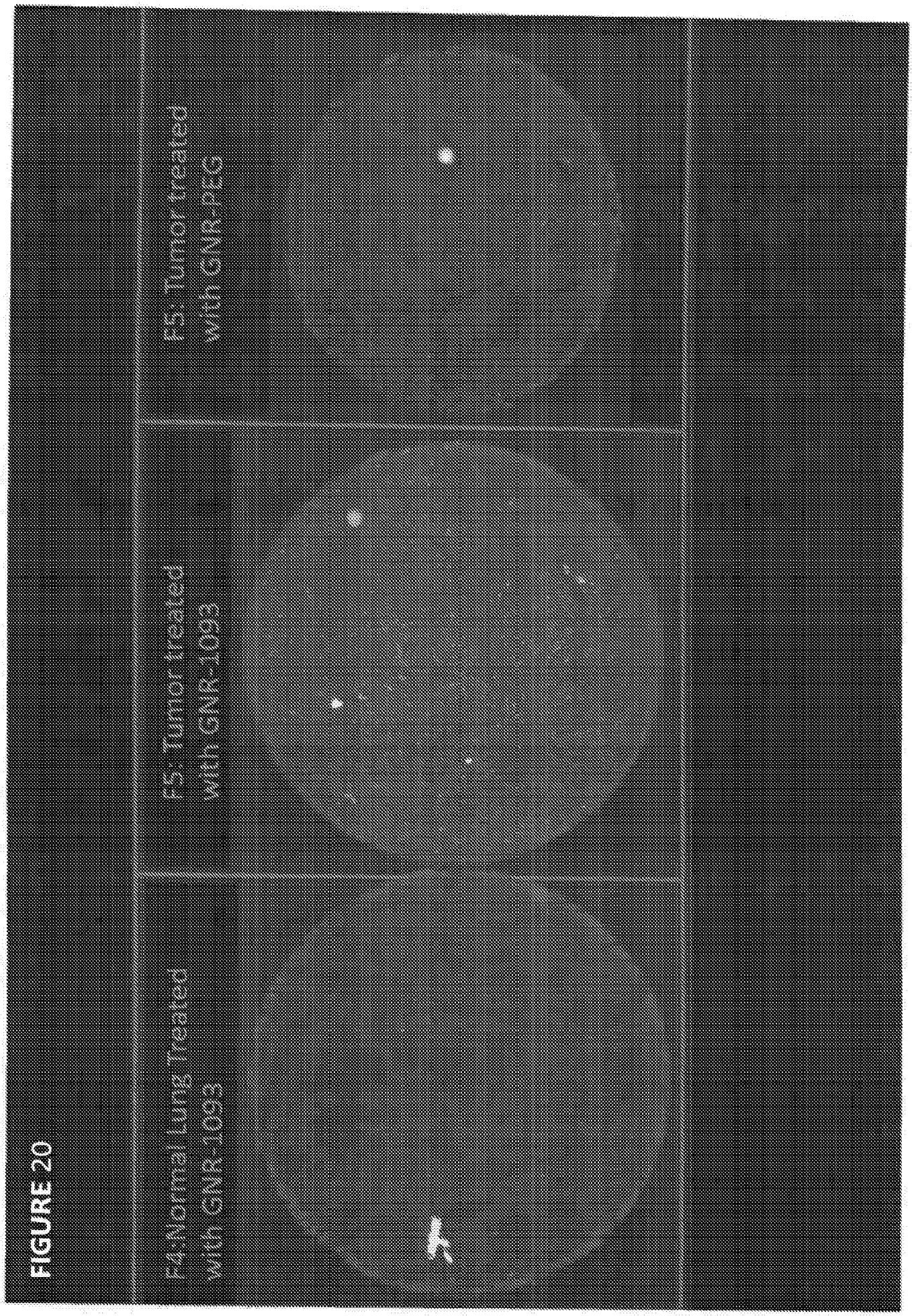

FS: Tumor treated
with GNR-PEG
FS: Tumor treated
with GNR-3E3
FS: Tumor treated
with GNR-3E3

FIGURE 20

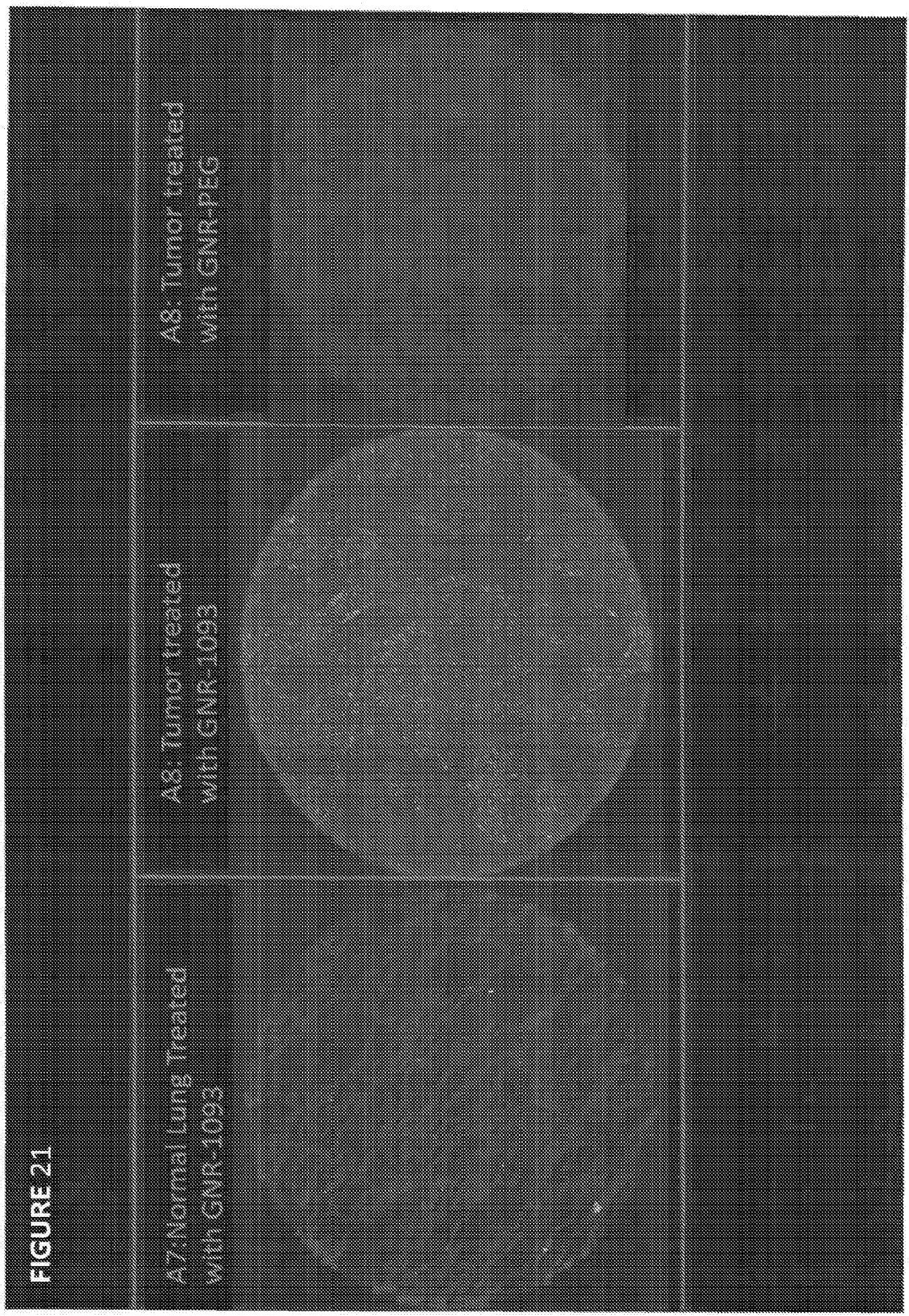
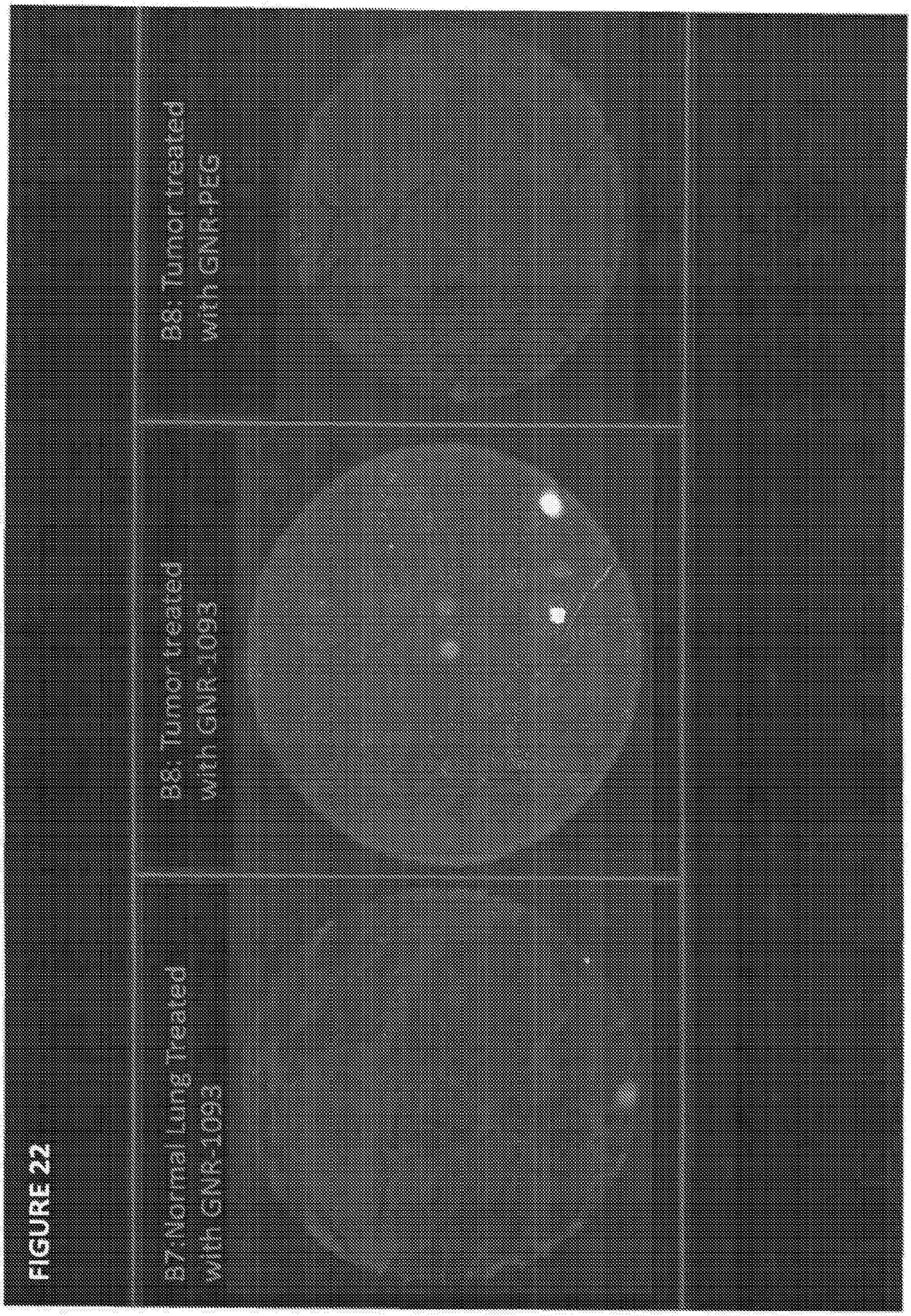

A7 Normal Tumor Tissue Paired
With GNR-1033
A7 Tumor Paired
With GNR-1033
A3 Tumor Paired
With GNR-1033
A3 Tumor Paired
With GNR-PEG

FIGURE 21

FIGURE 22


B7: Normal Lung Treated with GNR-1093
B8: Tumor treated with GNR-1093
B8: Tumor treated with GNR-PEG

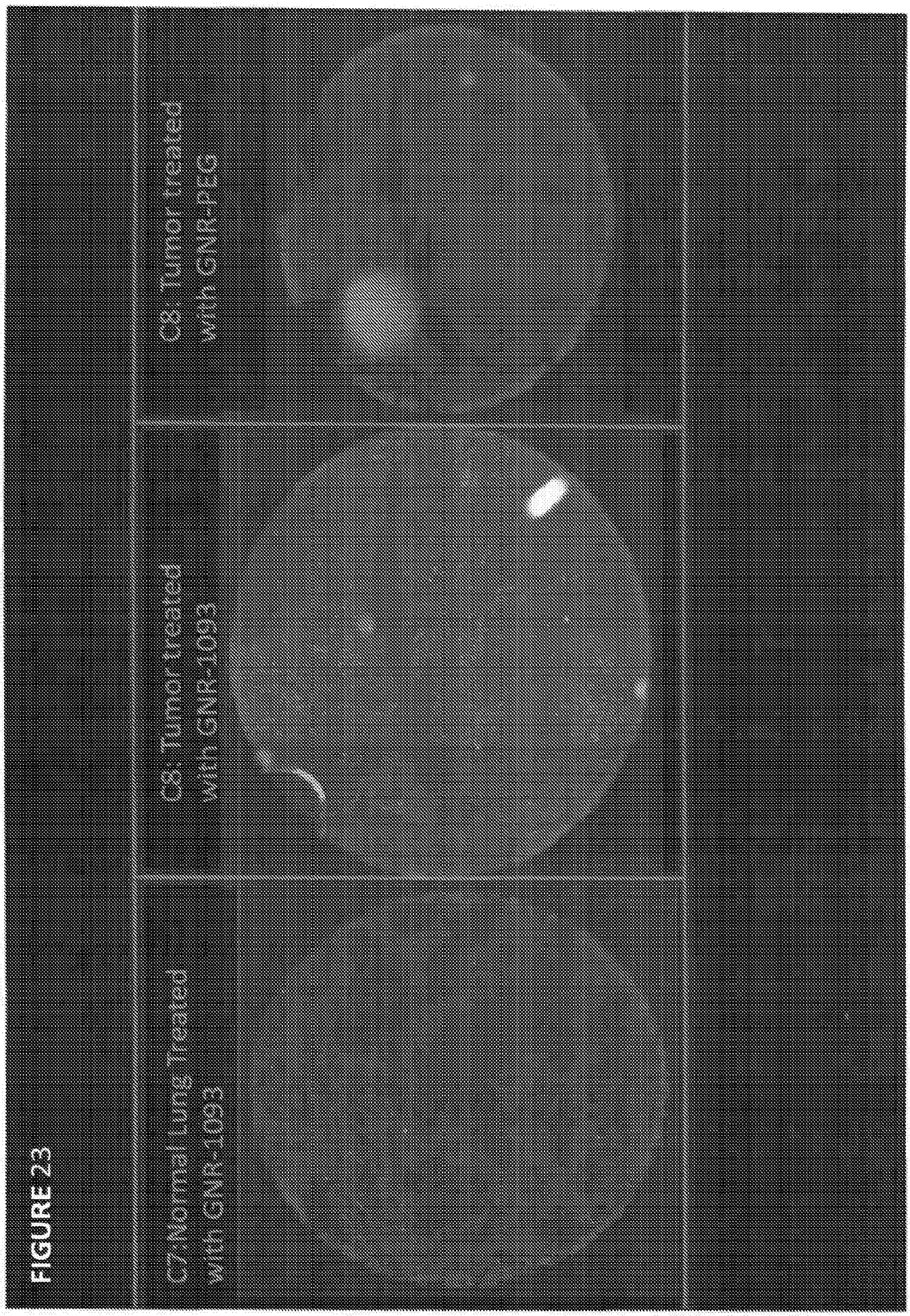

CT Normal Lung Tissue
with GNP-1093
CT Normal Lung Tissue
with GNP-1093
CT Tumor Tissue
with GNP-1093
CT Tumor Tissue
with GNP-PEG

FIGURE 23

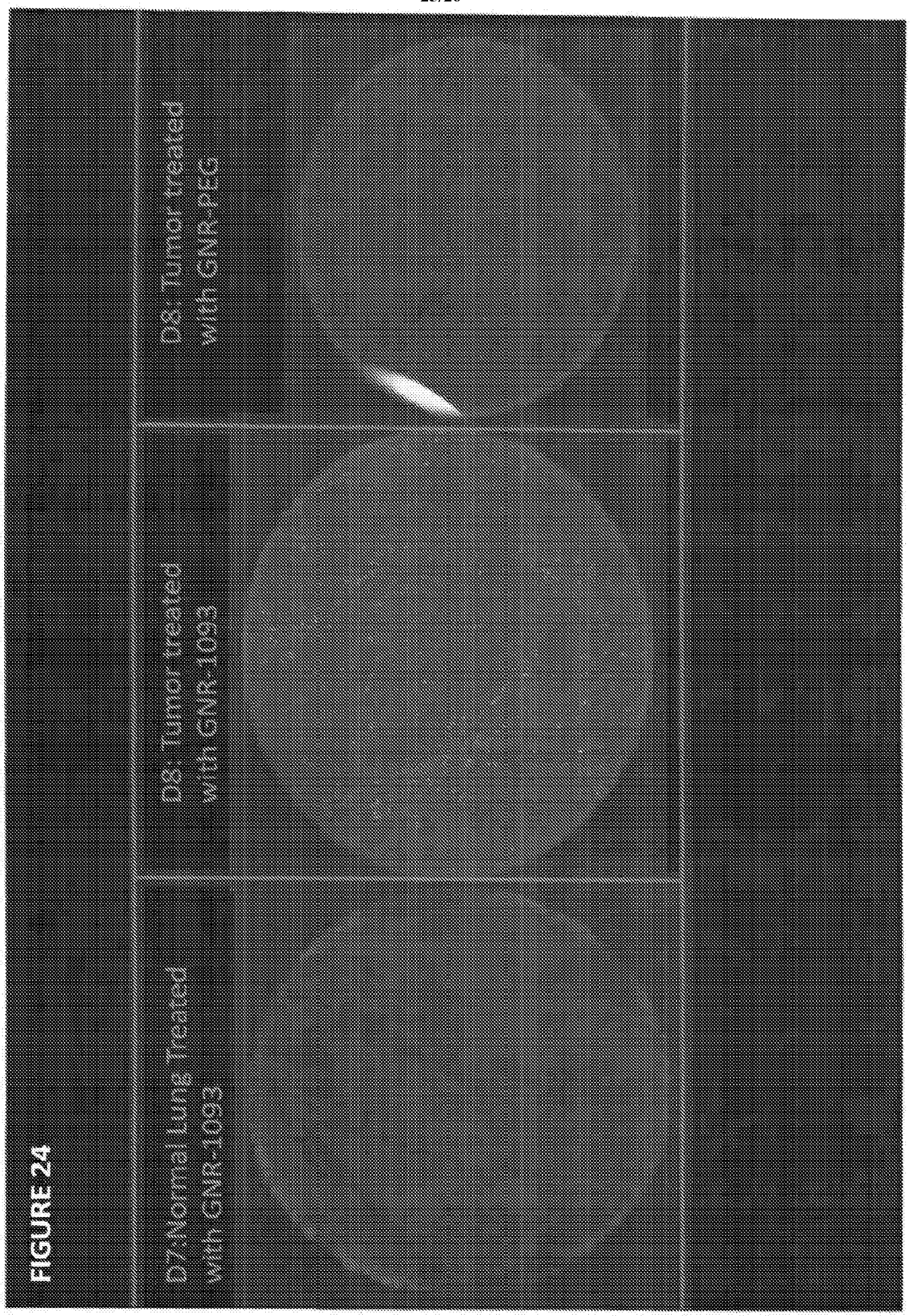

D4 Tumor treated with GNR-pSG
D3 Tumor treated with GNR-1093
D7 Normal lung treated with GNR-1093

FIGURE 24

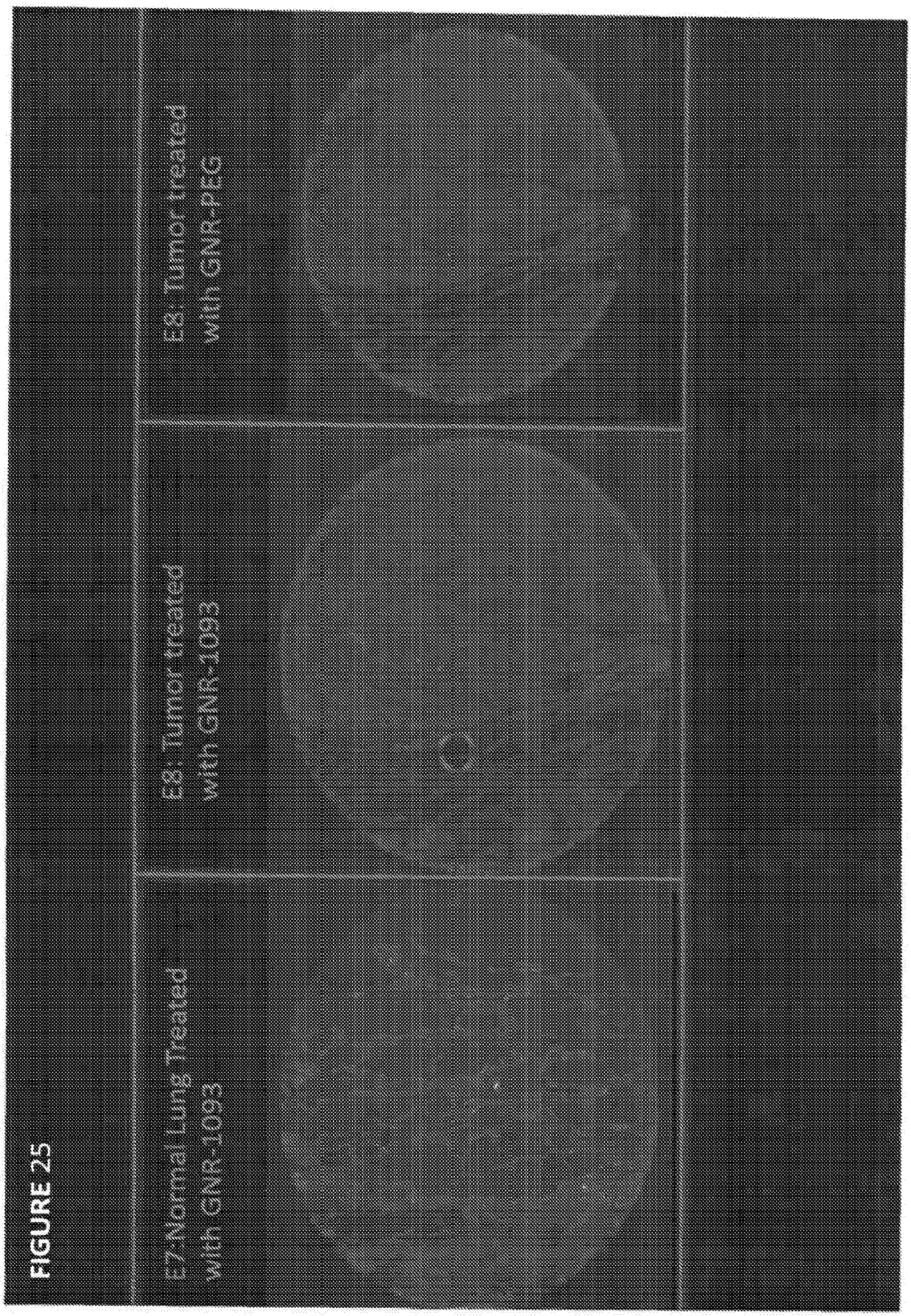

E3 Tumor treated with GNR-PEG
E3 Tumor treated with GNR-PEG and 1064 nm laser
E7 Mammary Tumor treated with GNR-PEG
E7 Mammary Tumor treated with GNR-PEG and 1064 nm laser

FIGURE 25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/37874

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 47/48, A61K 49/00, A61K 51/06, A61K 9/127, A61K 38/00 (2016.01)
CPC - A61K 47/48884, A61K 49/0054, A61K 51/06, A61K 9/1271, A61K 38/00
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)
IPC(8)- A61K 47/48, A61K 49/00, A61K 51/06, A61K 9/127, A61K 38/00 (2016.01)
CPC- A61K 47/48884, A61K 49/0054, A61K 51/06, A61K 9/1271, A61K 38/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- A61K 47/48884, A61K 49/0054, A61K 51/06, A61K 9/1271, A61K 38/00 (keyword search, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar
Search Terms Used: Nanoparticle, nanorod, c-Met, HGF, thioctic, lysine, PEG, length, linker

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 2014/0079774 A1 (Brinker et al.) 20 March 2014 (20.03.2014) abstract, para [0024], [001], [0193], SEQ ID NO: 18, Fig. 5	1-3, 8-9 --- 4-7, 10
Y	US 2014/0329089 A1 (The Regents of University of California) 06 Noember 2014 (06.11.2014) abstact, para [0032]	4, 5, 10
Y	Dixit et al. "Synthesis and Grafting of Thioctic Acid-PEG-Folate Conjugates onto Au Nanoparticles for Selective Targeting of Folate Receptor-Positive Tumor Cells" Bioconjugate Chem. 2006, 17, 603-609; abstract, Fig. 1	5-7
Y	Kos et al. "Histidine-rich stabilized polyplexes for c-Met directed tumor-targeted gene transfer" Nanoscale, 2015, 7, 5350 (18 February 2015) pg 5354, col 2, para 1	6
A,P	Caldwell et al. "Gold nanorod immunoassay to quantify C-Met expression in NSCLC patients selected for anti-EGFR therapy" Journal of Thoracic Oncology Vol. 11 No. 2S, page S18, February, 2016; [retrieved on 10 August 2016 from http://www.jto.org/article/S1556-0864(15)00141-0/pdf] whole doc.	1

☐ Further documents are listed in the continuation of Box C. ☐

* 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
03 October 2016	26 OCT 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/37874

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.:
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:

3. ☐ Claims Nos.:
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.

Group I: claims 1-10, drawn to a nanoparticle conjugate comprising a target molecule with the specific affinity for c-MET receptor.

Group II: claims 11-14, drawn to a method of treating a cancer patient with an anti-c-MET drug.

Group III: claims 15-17, drawn to a method of forming a nanoparticle conjugate.

- Please see extra sheet for continuation -

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.:
1-10

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 16/37874

Continuation of:

Box NO III. Observations where unity of invention is lacking

The inventions listed as Groups I through III 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:

Special Technical Features

Group I includes the special technical feature of a composition comprising a nanoparticle conjugate, not required by Groups II and III.

Group II includes the special technical feature of a method of treating a cancer patient with an anti-c-MET drug, not required by Groups I and III.

Group III includes the special technical feature of a method of forming a nanoparticle conjugate, not required by Groups I and II.

Common Technical Features

The inventions of Groups I-III share the technical feature of a nanoparticle conjugate comprising a nanoparticle linked via a linker to a target molecule with the specific affinity for c-MET receptor.

The inventions of Groups II-III share the technical feature of a nanorod linked to a peptide with the specific affinity to human c-MET receptor.

However, these shared technical features do not represent a contribution over prior art in view of US 2014/0079774 A1 to Brinker et al. (hereinafter 'Brinker') and US 2014/0329089 A1 to The Regents of University of California (hereinafter 'UC'). Brinker teaches a nanoparticle conjugate comprising a nanoparticle linked via a linker to a target molecule with the specific affinity for c-MET receptor (abstract, protocells for specific targeting of hepatocellular and other cancer cells which comprise a nanoporous silica core with a supported lipid bilayer;.....a targeting peptide which targets cancer cells in tissue to be treated such that binding of the protocell to the targeted cells is specific and enhanced; para [0024], five (5) different 7 mer peptides which show activity as novel binding peptides for MET receptor (a.k.a. hepatocyte growth factor receptor, expressed by gene c-MET), see SEQ ID NOs: 1-5; [0031], FIG. 5 depicts the synthesis of MC40-targeted mesoporous silica nanoparticle-supported lipid bilayers (protocells) loaded with histone-packaged pCB.....A sulfhydryl-to-amine crosslinker (spacer arm=9.5 nm) was used to conjugate peptides, modified with a C-terminal cysteine residue, to DOPE moieties in the SLB (supported lipid bilayer); see Fig. 5, Crosslinker in the upper left hand side of Fig. 5). Brinker does not specifically teaches that said nanoparticle is in the form of a nanorod. UC teaches a method of preparing a metal nanorod, comprising: seeding a metal nanoparticle within the lumen of a nanotube; and growing a metal nanorod from the seeded metal nanoparticle to form a metal nanorod-nanotube composite (claim 1). Given that the the method of synthesis of nanorod is well known in the art, for example, as taught by UC, it would have been obvious to one of ordinary skill in the art to have applied nanorod to the method of Brinker, and thus, to have provided a nanorod linked to a peptide with the specific affinity to human c-MET receptor.

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

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