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Peyman (43) **Pub. Date: Jun. 22, 2017**

(54) **METHOD TO VISUALIZE VERY EARLY STAGE NEOPLASM OR OTHER LESIONS**

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(71) Applicant: **Gholam A. Peyman**, Sun City, AZ (US)

(57) **ABSTRACT**

(72) Inventor: **Gholam A. Peyman**, Sun City, AZ (US)

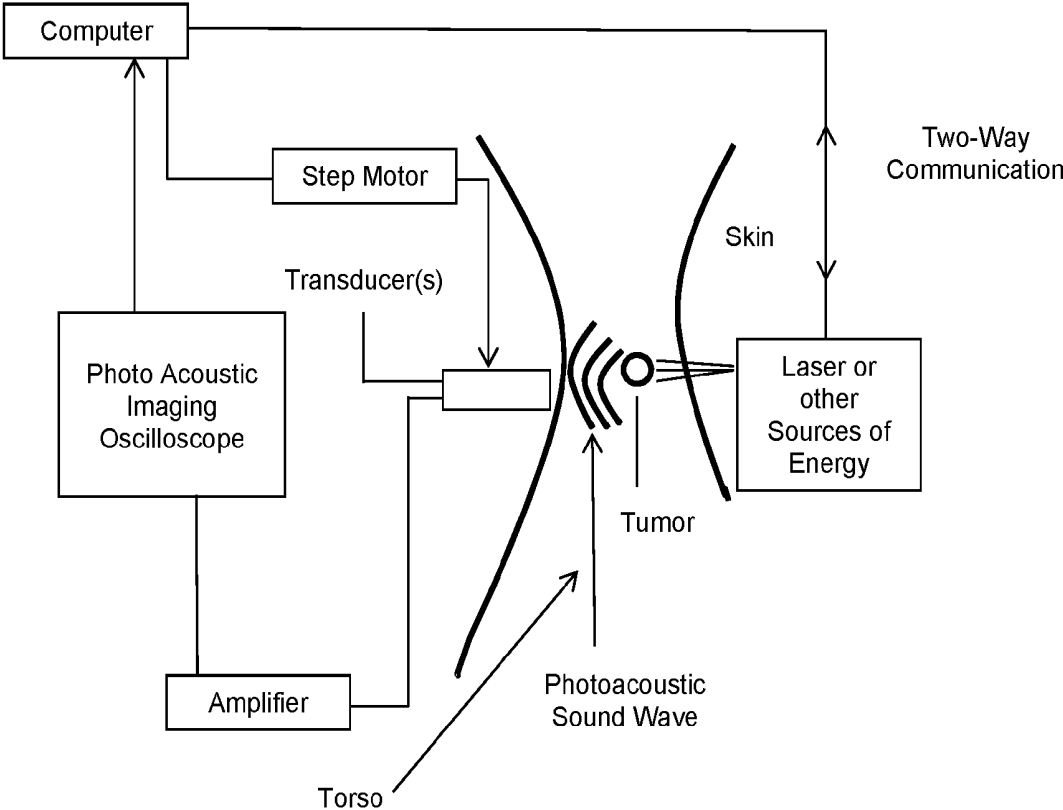
A method for evaluating treatment outcome in a patient having a genetic predisposition for a malignant neoplasm before clinical manifestation of the neoplasm can be seen radiographically. The method permits visualization of any tumor, whether located externally on a patient's body or located internally in the body, and as small as 2 mm in diameter, using a biomarker. The method uses biomarkers conjugated with nanoparticles which include but are not limited to quantum dots, with the conjugated form collectively termed functionalized nanoparticles, that are heated under specified conditions to produce a photoacoustic signal that is then visualized to locate and/or treat the tumor.

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METHOD TO VISUALIZE VERY EARLY STAGE NEOPLASM OR OTHER LESIONS

[0001] A method for evaluating treatment outcome in a patient having a genetic predisposition for a malignant neoplasm before clinical manifestation of the neoplasm can be seen radiographically. The method permits visualization of any tumor, whether located externally on a patient's body or located internally in the body, and as small as 2 mm in diameter, producing a biomarker, either a biomarker specific for the tumor or a general biomarker.

[0002] In general, a biomarker indicates a disease process. As subsequently described, a biomarker can be a protein, antigen, enzyme, hormone, carbohydrate, toxin, DNA, an organism such as bacteria, tumor cell, exosome, or indirectly an antibody, present in a liquid biopsy specimen. It can be produced by the plasma cells, against a tumor antigen, etc.

[0003] The method uses antibodies conjugated with nanoparticles which include but are not limited to quantum dots, with the conjugated form collectively termed functionalized nanoparticles, that are heated under specified conditions to produce a photoacoustic signal that is then visualized to locate the tumor to which the nanoparticles are attached. Nanoparticles may be used for qualitative and quantitative assessment of an analyte in the blood or other tissue using photoacoustic technology, U.S. Pat. No. 8,554,296. As previously stated, as used herein, unless specifically stated otherwise, nanoparticles include but are not limited to quantum dots.

[0004] Early stage small neoplastic cells produce biomarkers that are either specific to the tumor cells or that represent the body's response to the tumor as an antibody. The biomarkers can be proteomic, genetic, epigenetic or glycomic biomolecules. These biomolecules can be recognized in the patient's tissue samples or in the blood. Their existence can be demonstrated thus far chemically using, e.g., immunoassay or PCR methods. Quantitation of these biomarkers is also important to determine disease progression and prognosis.

[0005] Biomarkers for many diseases are found in the blood. As subsequently disclosed, biomarkers detected in a liquid biopsy sample are used to generate antibodies against them using known methods in the art. The anti-tumor antibodies are used to coat nanoparticles in the inventive method, where a lesion can be imaged regardless of the lesion size or location in the body. The method is not limited to tumor detection and/or therapy. As only one example, detecting an antibody against anti- β -amyloid protein plaque present in Alzheimer's disease in a liquid biopsy specimen, the method renders the plaque visible with the nanoparticles and accessible to the inventive treatment. As another example, the method can also be used to detect and/or treat inflammatory processes, etc.

[0006] The inventive method is applicable to any processes or diseases that produce a biomarker detectable in a liquid biopsy specimen. It is applicable to a lesion including an abscess, an ulcer, a tumor either benign or malignant, an ischemic area of stroke and/or an area of the brain affected by a stroke whether visible or microscopically.

[0007] Well over a thousand proteins are differentially expressed in human cancers and thus may serve as biomarkers. Such proteins play a role in cancer-related processes such as angiogenesis, apoptosis, cell differentiation, cell signaling, hematopoiesis, hormonal control, immune reactions, etc. Exemplary biomarkers include, but are not limited

to, carcinoembryonic antigen (CEA) for both malignant pleural effusion and peritoneal cancer dissemination; human epidermal growth factor receptor 2 (HER-2/neu) for stage IV breast cancer; bladder tumor antigen for urothelial cell carcinoma; thyroglobulin for thyroid cancer metastasis; α -fetoprotein for hepatocellular carcinoma; prostate specific antigen (PSA) for prostate cancer; cancer antigen 125 (CA 125) for non-small cell lung cancer; cancer antigen 19.9 (CA 19.9) for pancreatic cancer; cancer antigen 15.3 (CA 15.3) for breast cancer; the combination of leptin, prolactin, osteopontin, and insulin-like growth factor II (IGF-II) for ovarian cancer; the combination of CD98, fascin, secreted chain of the polymeric immunoglobulin receptor (sPIgR), and 14-3-3 eta proteins for lung cancer; troponin I for myocardial infarction, and B-type natriuretic peptide for congestive heart failure. While the previous nine proteins are the only approved markers for cancer testing to date, they are but a small fraction of the total number of available biomarkers, and their sensitivity and specificity vary.

[0008] Other common biomarkers include the estrogen receptor/progesterone receptor (ER/PR), HER-2/neu, and epidermal growth factor receptor (EGFR) for breast cancer, and tissue inhibitor of metalloproteinases (TIMP-1)-associated with serum HER2-positive breast cancer; Kirsten Ras oncogene (KRAS) and UDP glucuronosyltransferase family 1 member A (UGT1A1) for colorectal cancer; HER-2/neu for gastric cancer, c-KIT, CD20 antigen, CD30, and factoril interacting with PAPOLA and CPSF1-platelet-derived growth factor receptor alpha fusion protein (FIP1L1-PDGFR alpha), and platelet-derived growth factor receptor (PDGFR) for gastrointestinal stromal tumor (GIST); Philadelphia Chromosome (BCR/ABL)/PML/RAR alpha and anaplastic lymphoma kinase (TPMT/UGT1A1/ALK EGFR) for leukemia/lymphoma; KRAS/EGFR for lung cancer, and BRAF and S100 for melanoma.

[0009] Other examples of biomarkers include tumor suppressors that are lost in cancers, such as Breast Cancer Gene 1 (BRCA1), Breast Cancer Gene 2 (BRCA2); RNA such as mRNA, microRNA; proteins found in body fluids or tissue such as prostate specific antigen and CA-125; gene and protein based biomarkers; and nonspecific biomarkers such as

glycosaminoglycans in body fluids; alkaline phosphatase and urinary hydroxyproline in skeletal involvement; hyaluronic acid excretion and urinary hydroxyproline in bone disease, and combinations thereof.

[0010] In malignancies, the biomarkers may be released into the circulation either prior to or after the tumor has grown sufficiently to become metastatic. Small tumors (less than about 2 mm) seldom have any clinical manifestations, however even such small tumors can release chemical and/or biomarkers into the circulation.

[0011] The existence of biomarkers in the circulation has been known, but has not met the threshold for locating tumor cells that could not be imaged radiographically or by ultrasound as long as the tumors were asymptomatic. Available imaging methods such as x-ray, magnetic resonance imaging (MRI), functional MRI, computed tomography (CT) scans, CT ultrasound, etc. may not permit visualization of lesions smaller than about 3 mm in diameter. This has been the case for most malignant tumors, or when a malignant tumor is created from a benign precursor lesion such as nevus, breast unspecific cyst or unspecific scar, prostate tumors along with benign prostate hypertrophy or uterus cancer inside the

uterus fibroma, melanoma inside a skin nevus or in a seborrheic keratosis, etc. Moreover, it is often difficult to follow a cancerous tumor which has been irradiated but may still harbor malignant cells, and that can start growing with time and metastasize before it shows a local growth that is detected by conventional imaging or other methods.

[0012] The diagnosis of a malignant tumor may be extremely difficult, even when a tumor is visible clinically or radiologically, e.g. a uterus fibroma that may have some malignant transformation. Moreover, a diagnosis also affects the decision whether or not and also how to remove the tumor. As one example, accessing the uterus through a small incision, and removing the tumor piece by piece using an endoscope and a cutting probe, has a fast post-operative recovery. Such a method is in contrast to completely removing the uterus with the tumor intact out of caution that the tumor may harbor neoplastic cells, but using a large incision with significantly higher operative risks and post-operative complication probabilities. Another, more problematic example, is the decision for a woman having genetic disposition to breast cancer without any physical or radiological manifestation. The woman must endure the stress and fear not knowing if or when she may develop breast cancer, and must consider prophylactic removal of both breasts. As another example, a personal decision whether or not to undergo radiation therapy when a nevus is discovered under the retina, and biopsy results that often do not provide definitive information because of the diversity of the cells in the entire area of the tumor.

[0013] When the tumor site is unknown, locating a biomarker in the circulation may be akin to finding a needle in a hay stack. For any particular tumor or cancer, not all biomarkers are even known. Similarly, finding a micro DNA in the circulation may not provide an answer when the tumor is either invisible or has already metastasized. An example of this occurs in patients with uveal melanomas, having a mortality rate of about 50%, even if the tumors undergoes radiation, at the time the ophthalmologist discovers the tumor. This points to the fact that a malignant tumor can metastasize very early, at times even when the size of the tumor is about 2 mm in diameter which is equal to about one million cells. In general, these lesions do not have any symptoms.

[0014] The inventive method makes it possible to evaluate a patient with genetic predisposition of a malignant neoplasm before its clinical manifestation can be seen radiographically.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 shows a schematic of the treatment evaluation method.

[0016] In one embodiment, the presence of one or more biomarkers is evaluated in any body fluid or organ. Exemplary bodily fluids include, but are not limited to, urine, blood, cerebrospinal fluid (CSF), eye cavity fluid, tear film, sputum, fluid obtained from the trachea, bronchi, abdominal cavity, vagina, uterus etc. The biomarkers are analyzed in vitro by methods known in the art, e.g., immunoassays including enzyme-linked immunoassay (ELISA), Western blots, fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), etc. The biomarkers are then conjugated with functionalized antibody coated nanoparticles and/or quantum dots, as known in the art.

[0017] In one embodiment one obtains a liquid biopsy sample. Such a sample may be obtained from, e.g., blood, urine, cerebrospinal fluid (CSF), aqueous or vitreous or abdominal cavity fluid, lymph node fluid, bladder fluid, milk duct fluid, sputum, gastric fluid, bile duct fluid, sinus fluid, etc. The patient may or may not have any clinical symptom. The patient may or may not have history of a family disposition for tumors in and/or cancer of the breast, brain, lung, prostate, ovary, pancreas, etc., or a genetic abnormality leading to progression in diseases such as, e.g., Alzheimer's, Parkinson's, post traumatic brain syndrome, brain tumor, other neurological disease, age related macular degeneration, an infectious disease, an immune response, etc. The method evaluates the components of the sample for cell free nucleic acid-based biomarkers including but not limited to microRNA and microDNA; protein-based biomarkers, extracellular vesicle (EV)-based biomarkers that are contained within exosomes, extracellular vesicles, or microvesicles, and circulating tumor cell (CTC)-based biomarkers. The method uses methodologies such as next generation sequencing (NGS) or recombinant affinity reagents fabricated into nanostructures such as carbon nanotubes, nanowires, quantum dots, or gold nanoshells, to enhance their detection with the use of, e.g., surface-enhanced Raman scattering (SERS), as known in the art.

[0018] For example, if a known tumor exists and there is a known biomarker for the tumor, one may have or prepare an antibody against the tumor to be used in both imaging and therapy. Large tumors with symptoms can be imaged, but before the inventive method, there was a problem when a biomarker was present in a liquid biopsy specimen but the tumor was invisible, e.g., an early stage of a tumor, and there was no symptomatic or radiographic evidence of the tumor.

[0019] Detecting a tumor biomarker, typically a protein or a glycoprotein, in a liquid biopsy specimen is facilitated by the inventive methods. Once detected, an antibody against that tumor biomarker can be prepared. The antitumor biomarker antibody is used to locate the tumor. Antibody production is a well-known method in the art, and it will be appreciated that the antibody against either or both of the tumor biomarker and the tumor cell may be recombinant, monoclonal, polyclonal, or an aptamer. The prepared anti-tumor cell antibodies are conjugated with nanoparticles and administered to a patient, where they target the tumor cells and can be detected and/or treated. Detection is by photoacoustic imaging technology. Treatment is at least by one of thermal energy. The photoacoustic detection and thermal treatment is described herein.

[0020] In one embodiment, any specific tumor related biomarker may be used. One example uses trastuzumab or herceptin, a recombinant monoclonal antibody, against the oncogene HER-2, previously mentioned, which is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family. Other examples of known monoclonal antibodies or biologics include, but are not limited to, rituximab, cetuximab, racotunomab, obinotuzumab, pertuzumab, belaniatumomab, bevacizumab, nivolumab, ofatumumab, botezomib, daratumumab, ipilimumab, pembrolizumab, and daratumumab.

[0021] In one embodiment, in the absence of a specific biomarker, antibodies against biomarkers that are shared by a number of the tumors may be used. Such biomarkers include glycosaminoglycan, which is specific for a group of cancers such as bladder, gastrointestinal, glioblastoma, etc.

Antibodies against such biomarkers are then conjugated with nanoparticles, termed functionalized nanoparticles. The term “functionalized” indicates nanoparticles that have been coated to render them soluble, biocompatible, and/or targeted by conjugating them with a biomolecule such as an antibody.

[0022] In one embodiment the nanoparticle may be one or more of the following compounds or contain one or more of the following components: quantum dots, nanowires, nanotubes, nanoshells, nanocages, perovskites, nanoparticles that are magnetic such as iron or iron oxide, paramagnetic, or nanoparticles that are non-magnetic such as gold, gold-silica, gold-iron, silica coated gold nanospheres and nanorods, ferritic, quartz, graphene, carbon, zinc oxide, piezoelectric, etc. Any of these nanoparticles, alone or in combination, may be conjugated or otherwise associated with the biomarkers’ antibodies, using methods known in the art.

[0023] In another embodiment, self-assembling bio/nano hybrid material consisting of two constituents at the nanometer or molecular level composed of inorganic and organic compounds, having amphiphilic characteristics, i.e., hydrophilic and lipophilic components or micelles, which may be radioactive (e.g., Cu⁶⁴) or radioactive (e.g., tin) are prepared with biocompatible coatings and administered in the body for both therapy and imaging.

[0024] In one embodiment, the functionalized nanoparticles travel in the body and attach to receptors of desired cells, e.g., tumors, Alzheimer’s plaque, drusen of the retina, etc. These nanoparticles are imaged by applying external thermal energy and/or by applying a reversible or alternating magnetic field. The thermal energy causes the nanoparticles to expand, producing an ultrasound wave in the tissue. The ultrasound wave can be detected by an ultrasonic receiver which is imaged in two to three dimensional formats as a tomogram. In another embodiment the plaques in Alzheimer’s disease, and the drusen in age related macular degeneration, are rendered visible using silica coated nanoparticles <2 nm in diameter administered with turmeric, glycosaminoglycan, amyloid antibody, or percolan, etc. and are quantified. In another embodiment, the nanoparticles are conjugated with antibodies, medications, sterols, antibiotics, antifungals, antibacterials, antiproliferative agents, etc. that can be released from silica coated gold nanoparticles when coated with thermosensitive polymers, e.g., chitosan coated nanoparticles heated to 40° C.-42° C., to treat various diseases including bacteria, fungi, parasites, plaque, drusen, etc. In another embodiment, the plaques and drusen can be quantified by imaging using light, MRI, photoacoustic technology imaging, etc.

[0025] In another embodiment, the functionalized anti-biomarker-conjugated nanoparticle, ranges in size from 1 nm to 900 nm. In another embodiment, the functionalized biomarker ranges in size from 1 nm to 8 nm, chosen to enhance their elimination through the kidney for facilitated clearance.

[0026] In one embodiment, the nanoparticles are rendered magnetic by coating with a thin film of iron oxide prior to their conjugation with biomarkers’ antibodies.

[0027] In one embodiment, the nanoparticles are rendered more biocompatible by coating with a compound, including but not limited to the following: (poly)ethylene glycol, cell

penetrating peptide (CPP), activating CPP (ACPP), biotin, streptavidin, etc., as known in the art, prior to their injection in the body.

[0028] Thermal energy in the form of electromagnetic radiation, ultrasound, or an alternating magnetic field is applied, under the control of a photoacoustic imaging system, to the organ suspected of potentially harboring an as yet invisible neoplasm. The thermal energy applied increases the temperature of the exposed nanoparticle, and creates a photoacoustic image or tomogram of the accumulated heated nanoparticles. This image or tomogram represents a suspected neoplasm in that organ, and is compared to an image taken without the thermal application radiographically.

[0029] In one embodiment, one administers functionalized antibody-coated nanoparticles that, once attached to tumor cells, become visible with a photoacoustic imaging unit that corroborates with an image obtained or not seen with other technology such as ultrasound, MRI, PET, CT scan, etc. In one embodiment, the images obtained with other instruments are either overlapped using a processor or are taken simultaneously during photoacoustic imaging. In one embodiment, after administration of the antibody-coated nanoparticle, an MRI image is overlapped with the photoacoustic image and compared by a processor to verify the changes in the imaged area.

[0030] In one embodiment, the nanoparticles are incorporated in liposomes. In this embodiment, they may contain medications that, upon attainment of a specific tumor temperature, are released. The type of medication is not limited, and can include anti-bacterial, anti-viral, anti-fungal, anti-neoplastic, antiinflammatory such as acetyl cycline, anti-beta-amyloid protein, other antibodies, non-steroidal antiinflammatory drugs, immune stimulating agents, anti-VEGF agents, anti-aggregation agents such as sterols, etc.

[0031] In another embodiment, antibody-coated nanoparticles conjugated with thermosensitive polymers such as chitosan, carrying any medication including but not limited to sterol, squalamine, lanosterol, is administered to a patient having a neurologic pathology such as Alzheimer’s disease, Parkinson’s disease, or age related retinal drusen, etc. In this embodiment, administration is either intravenous or local in the cerebrospinal fluid or vitreous cavity, respectively, or at another local site. After controllably increasing the temperature of the functionalized nanoparticle to between 40° C.-43° C. by increased energy delivery through a delivery source, under the control of the photoacoustic imaging system and a processor, the temperature-sensitive coating polymers such as chitosan melts and release medications specific to the pathology. For example, a medication to dissolve amyloid plaques would be administered to a patient with Alzheimer’s disease; a medication to remove retinal drusen would be administered to a patient with age related retinal disease, etc.

[0032] In one embodiment, the functionalized nanoparticle, e.g., a nanoshell, nanocage, etc., is combined with biotendrimers that are conjugated with biomarkers and monoclonal antibodies and/or genes, e.g., siRNA, mRNA, etc., for simultaneous visualization and therapy.

[0033] In another embodiment, after thermal imaging one increases the temperature of the functionalized nanoparticles. This is achieved by increased energy delivered by a thermal delivery source under the control of the photoacoustic imaging system connected to a processor. The energy

delivery unit increases the temperature of the functionalized nanoparticles to 42° C.-43° C. to melt the temperature-sensitive coating polymers such as chitosan and release anticancer medications, or inhibitory genes, siRNA, miRNA, or checkpoint inhibitors, or introduce missing genes, or add any other genes for gene editing from the thermosensitive coating of the nanoparticles along with a CRISPR complex to modify the genetic composition of the tumor cells, etc. In another embodiment, the temperature of the functionalized nanoparticles is increased, by the thermal delivery unit via a processor under the control of the photoacoustic imaging unit, to image the temperature and control it to 45° C.-47° C., to 47° C., or to 50° C. to kill the suspected tumor to which the antibody-coated nanoparticles are attached.

[0034] In one embodiment, one synthesizes hybrid, very small (1 nm-8 nm) gold silica nanoparticles that have a dual function, the nanoparticles antibody coated for imaging, and having photovoltaic and magnetic properties, to release one or more gene(s) or medication(s) at certain temperatures, creating a photoacoustic response for imaging in the body by light stimulation in the eye for simultaneous imaging and therapy.

[0035] In one embodiment, using antibody coated quantum dots and light of a specific wavelength that is absorbed by the quantum dot and emits light of a different wavelength, one can render the moving tumor cells and extracellular vesicle visible attached to the quantum dots in the retinal or choroidal vessels, or vessels and tumors of the skin, or tumors located beneath the skin and their feeding vessels, by light absorbed by the quantum dots circulating in the vessels, as is done in fluorescence angiography with appropriate filters and camera.

[0036] In another embodiment, a gold quantum dot in a mesoporous silica shell or cage is coated with an antibody or a biomarker to any cell, e.g., neuronal or tumor cells, retinal drusen, Alzheimer plaques, etc. for delivering medication or gene to an organ, e.g., retina or brain.

[0037] In another embodiment, the extent of plaque or drusen, as an indicator of disease progression in the brain or eye, respectively, can be evaluated by conjugating nanoparticles with antibodies to glycosaminoglycan, heparan sulfate, glucosaminoglycan, and/or heparin sulfate proteoglycan, and injecting the composition into the body or locally to adhere to plaques or drusen for diagnosis, quantitation, and/or therapy with antibodies and medication.

[0038] In another embodiment the nanoparticles are used for simultaneous imaging and radiotherapy of very small tumors. The nanoparticles are heated to a temperature ranging from 41° C.-43° C., releasing anti-cancer medication, along with inhibitory siRNA, or modify a gene using the CRISPR cas9 system or another CRISPR system, additionally releasing checkpoint inhibitors such as CTLA-4 or PD-1 along with tumoricidal vectors, etc.

[0039] In one embodiment, the nanoparticles are rendered radioactive by coating with alpha or beta radiators that are antibody specific or nonspecific biomarkers of the tumor. The nanoparticles can also be coated with heat sensitive polymers, including but not limited to chitosan, PEG, poly amino esters, etc.

[0040] In one embodiment, checkpoint inhibitors defined as immune system components that act as co-stimulatory or co-inhibitory molecules, poisons such as bee or snake venom, or other toxic agents that damage tumor cell mem-

branes, or genes that inhibit tumor growth, siRNA, siDNA, mi RNA, mRNA along with the CRISPR cas 9 complex or variations of these may be used.

[0041] In one embodiment, the nanoparticles are coated with a specific or a nonspecific biomarker such as glycosaminoglycan and injected into the circulation, into a body fluid such as the lymphatic system or cerebrospinal fluid (CSF), or inside a body cavity. Examples of injection sites include, but are not limited to, eye, sinuses, abdominal cavity, bladder, uterus, etc. The nanoparticles may also be injected into the breast ducts, e.g., through the nipple, inside the brain, into the prostate or other organ, or may even be applied topically. The injected nanoparticles circulate and seek cells bearing a receptor to their antibody, or perhaps cells with specific receptors or biomolecules, and readily attach within minutes or hours.

[0042] In one embodiment, specific or non-specific biomarkers' antibodies are conjugated with nanoparticles and injected either into circulation or locally into a body cavity. The nanoparticles travel and seek cells bearing specific receptors or biomolecules, and attach within a few hours. The patient's body or organ is then scanned, with the thermal energy producing radiation or an alternating or reversible magnetic field to heat the nanoparticles. Using photoacoustic technology, the sound wave generated by the thermal expansion of the nanoparticle induced by absorption of the thermal energy is recorded. The sound wave signals may originate from any part of the body, or from a specific organ.

[0043] In one embodiment, an alternating magnetic field produces heat in magnetic nanoparticles as a result of rapid circular or semicircular motion of the nanoparticles. The patient's body is scanned within the reversible magnetic field, and the photoacoustic sound is recorded as a temperature profile of the site of the nanoparticle/cell membrane imaged and location of the lesion is verified.

[0044] In another embodiment, other source of thermal energy are used. Such sources include, but are not limited to, electromagnetic radiation, visible light, invisible light, infrared radiation, microwaves, or radiofrequency waves, etc. The nanoparticles are heated from body temperature of 37° C. to 40° C. or 43° C., or if needed to 45° C. At the desired temperature, e.g., 41° C.-43° C., the heat sensitive coating of the nanoparticle melts, releasing its cargo of, e.g., medication, gene, etc., thus facilitating or enhancing passage of these compounds through the membrane of the neoplastic cells.

[0045] In another embodiment, use of a photoacoustic technology unit controls the thermal delivery unit and the thermal energy delivered to the nanoparticles to maintain or reach a predetermined temperature for a desired time.

[0046] In one embodiment, the temperatures rise of the nanoparticles expands them, producing a photoacoustic sound wave. This photoacoustic sound wave is recorded by one or multiple ultrasonic receivers located on the patient's skin. The signal can be obtained from any part of the body, or from a specific organ, since the signal travels through the body as a wave. The signal or sound pulse is converted to an electric pulse in the receiver, then is amplified and imaged on a monitor. A processor produces a two- or three-dimension image of the lesion, localizing the location of the sound and indicating the size of a lesion and its temperature by the amplitude of the sound pulse,

[0047] In one embodiment, photoacoustic imaging is used for a very early stage diagnosis of cancerous lesion that are

less than 2 mm in diameter, which are radiographically invisible without knowing their exact location in the body.

[0048] In one embodiment using photoacoustic technology and a specific or non-specific tumor biomarker, a very small lesion (<2 mm in diameter) is imaged in the body when the tumor has not caused any clinical symptom. The inventive method thus is used to differentiate a malignant lesion from a benign lesion, even if the cancerous lesion is inside a benign lesion. It is noteworthy that biopsy of these very small tumors, even when the lesion is visible, e.g., on skin or under the retina, may not yield malignant cells if the biopsy is performed on a part of the lesion that contains benign cells. With tumors in the brain, it is most often the case that the tumors will not be noted, absent a neurological symptom.

[0049] In one embodiment, the inventive method is used with specific biomarkers of a tumor such as breast cancer, prostate cancer, glioma, pancreatic malignancies, along with nonspecific biomarkers. The location and size of a malignant tumor in any organ is imaged in a patient with a genetic propensity to develop a tumor. The thermal energy may also be applied, if desired, to treat the lesion simultaneously with providing the photoacoustic effect. Subsequent evaluation of

the level of these biomarkers in the blood indicate if the lesion was damaged or eliminated by the method, including use of medicaments and/or other treatment agents delivered by the method as cargo in the nanoparticles.

[0050] In one embodiment, a combination of biomarkers can be used in an early stage. For example, specific or nonspecific bio-markers such as glycosaminoglycans can be used in imaging a malignant lesion using antibody-coated nanoparticles to photoacoustically image the presence of a very small early stage tumor anywhere in the body.

[0051] In another embodiment, the inventive method is employed to determine residual tumor cells that may have left at the site of a tumor resection or elsewhere in the body, and to treat or eliminate the residual tumor cells.

[0052] In another embodiment, the functionalized nanoparticles are conjugated with one of the recombinant, monoclonal, or polyclonal antibodies or aptamers known in the art and administered along with either one or more toxin(s) or antibodies, along with a medication that is provided at a much lower dose systemically to kill the already compromised tumor cells. Monoclonal antibodies that may be used include, but are not limited to, those shown in Table 1, e.g., rituximab, obinuzumab, ofatumumab, etc.

TABLE 1

Name	Trade name	Type	Source	Target	Use
3F8		mab	mouse	GD2	neuroblastoma
8H9		mab	mouse	B7-H3	neuroblastoma, sarcoma, metastatic brain cancers
Abagovomab		mab	mouse	CA-125 (imitation)	ovarian cancer
Abciximab	ReoPro	Fab	chimeric	CD41 (integrin alpha-IIb)	platelet aggregation inhibitor
Abituzumab		mab	humanized	CD51	cancer
Abrilumab		mab	human	integrin $\alpha 4\beta 7$	inflammatory bowel disease, ulcerative colitis, Crohn's disease
Actoxumab		mab	human	<i>Clostridium difficile</i>	<i>Clostridium difficile</i> infection
Adalimumab	Humira	mab	human	TNF- α	Rheumatoid arthritis, Crohn's Disease, Plaque Psoriasis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile Idiopathic Arthritis, Hemolytic disease of the newborn
Adecatumumab		mab	human	EpCAM	prostate and breast cancer
Aducanumab		mab	human	beta-amyloid	Alzheimer's disease
Afelimumab		F(ab') ₂	mouse	TNF- α	sepsis
Afutuzumab		mab	humanized	CD20	lymphoma
Alacizumab pegol		F(ab') ₂	humanized	VEGFR2	cancer
ALD518		?	humanized	IL-6	rheumatoid arthritis
Alemtuzumab	Campath, MabCampath	mab	humanized	CD52	Multiple sclerosis
Alirocumab		mab	human	NARP-1	hypercholesterolemia
Altumomab pentetate	Hybri-ceaker	mab	mouse	CEA	colorectal cancer (diagnosis)
Amatuximab		mab	chimeric	mesothelin	cancer
Anatumomab mafenatox		Fab	mouse	TAG-72	non-small cell lung carcinoma
Anetumab ravtansine		mab	human	MSLN	cancer
Anifrolumab		mab	human	interferon α/β receptor	systemic lupus erythematosus
Anrukizumab (=IMA-638)		mab	humanized	IL-13	?

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
Apolizumab		mab	humanized	HLA-DR ?	hematological cancers
Arcitumomab	CEA-Scan	Fab'	mouse	CEA	gastrointestinal cancers (diagnosis)
Ascrinivacumab		mab	human	activin receptor-like kinase 1	cancer
Aselizumab		mab	humanized	L-selectin (CD62L)	severely injured patients
Atezolizumab		mab	humanized	CD274	cancer
Atinumab		mab	human	RTN4	?
Atlizumab (=tocilizumab)	Actemra, RoActemra	mab	humanized	IL-6 receptor	rheumatoid arthritis
Atorolimumab		mab	human	Rhesus factor	hemolytic disease of the newborn[citation needed]
Bapineuzumab		mab	humanized	beta amyloid	Alzheimer's disease
Basiliximab	Simulect	mab	chimeric	CD25 (α chain of IL-2 receptor)	prevention of organ transplant rejections
Bavituximab		mab	chimeric	phosphatidyserine	cancer, viral infections
Bectumomab	LymphoScan	Fab'	mouse	CD22	non-Hodgkin's lymphoma (detection)
Begelomab		mab	mouse	DPP4	?
Belimumab	Benlysta, LymphoStat-B	mab	human	BAFF	non-Hodgkin lymphoma etc.
Benralizumab		mab	humanized	CD125	asthma
Bertilimumab		mab	human	CCL11 (eotaxin-1)	severe allergic disorders
Besilesomab	Scintimun	mab	mouse	CEA-related antigen	inflammatory lesions and metastases (detection)
Bevacizumab	Avastin	mab	humanized	VEGF-A	metastatic cancer, retinopathy of prematurity
Bezlotoxumab		mab	human	<i>Clostridium difficile</i>	<i>Clostridium difficile</i> infection
Bicirimab	FibriScint	Fab'	mouse	fibrin II, beta chain	thromboembolism (diagnosis)
Bimagrumab		mab	human	ACVR2B	myostatin inhibitor
Bimekizumab		mab	humanized	IL17A and IL17F	?
Bivatuzumab mertansine		mab	humanized	CD44 v6	squamous cell carcinoma
Blinatumomab		BiTE	mouse	CD19	cancer
Blosozumab		mab	humanized	SOST	osteoporosis
Bococizumab		mab	humanized	neural apoptosis-regulated proteinase 1	dyslipidemia
Brentuximab vedotin		mab	chimeric	CD30 (TNFRSF8)	hematologic cancers
Briakinumab		mab	human	IL-12, IL-23	psoriasis, rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis
Brodalumab		mab	human	IL-17	inflammatory diseases
Brolucizumab		mab	humanized	VEGFA	?
Brontictuzumab		mab	humanized	Notch 1	cancer
Canakinumab	Ilaris	mab	human	IL-1?	rheumatoid arthritis
Cantuzumab mertansine		mab	humanized	mucin CanAg	colorectal cancer etc.
Cantuzumab ravtansine		mab	humanized	MUC1	cancers
Caplacizumab		mab	humanized	VWF	thrombotic thrombocytopenic purpura, thrombosis
Capromab pendetide	Prostascint	mab	mouse	prostatic carcinoma cells	prostate cancer (detection)
Carlumab		mab	human	MCP-1	oncology/immune indications
Catumaxomab	Removab	3funct	rat/mouse hybrid	EpCAM, CD3	ovarian cancer, malignant ascites, gastric cancer

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
cBR96-doxorubicin immunoconjugate		mab	humanized	Lewis-Y antigen	cancer
Cedelizumab		mab	humanized	CD4	prevention of organ transplant rejections, treatment of autoimmune diseases
Certolizumab pegol	Cimzia	Fab'	humanized	TNF- α	Crohn's disease
Cetuximab	Erbix	mab	chimeric	EGFR	metastatic colorectal cancer and head and neck cancer
Ch.14.18		mab	chimeric	???	neuroblastoma
Citatumumab bogatox		Fab	humanized	EpCAM	ovarian cancer and other solid tumors
Cixutumumab		mab	human	IGF-1 receptor	solid tumors
Clazakizumab		mab	humanized	Oryctolagus cuniculus	rheumatoid arthritis
Clenoliximab		mab	chimeric	CD4	rheumatoid arthritis
Clivatuzumab tetraxetan	hPAM4-Cide	mab	humanized	MUC1	pancreatic cancer
Codrituzumab		mab	humanized	glypican 3	cancer
Coltuximab ravtansine		mab	chimeric	CD19	cancer
Conatumumab		mab	human	TRAIL-R2	cancer
Concizumab		mab	humanized	TFPI	bleeding
Crenezumab		mab	humanized	1-40- β -amyloid	Alzheimer's disease
CR6261		mab	human	Influenza A hemagglutinin	infectious disease/influenza A
Dacetuzumab		mab	humanized	CD40	hematologic cancers
Daclizumab	Zenapax	mab	humanized	CD25 (α chain of IL-2 receptor)	prevention of organ transplant rejections
Dalotuzumab ^[39]		mab	humanized	insulin-like growth factor I receptor	cancer etc.
Dapirolizumab pegol		mab	humanized	CD40 ligand	?
Daratumumab		mab	human	CD38 (cyclic ADP ribose hydrolase)	cancer
Dectrekumab		mab	human	IL-13	?
Demcizumab		mab	humanized	DLL4	cancer
Denintuzumab mafodotin		mab	humanized	CD19	cancer
Denosumab	Prolia	mab	human	RANKL	osteoporosis, bone metastases etc.
Derlotuximab biotin		mab	chimeric	histone complex	recurrent glioblastoma multiforme
Detumomab		mab	mouse	B-lymphoma cell	lymphoma
Dinutuximab		mab	chimeric	ganglioside GD2	neuroblastoma
Diridavumab		mab	human	hemagglutinin	influenza A
Dorlimomab aritox		F(ab') ₂	mouse	?	?
Droxitumab		mab	human	DR5	cancer etc.
Duligotumab		mab	human	HER3	?
Dupilumab		mab	human	IL4	atopic diseases
Durvalumab		mab	human	CD274	cancer
Dusigitumab		mab	human	ILGF2	cancer
Ecomeximab		mab	chimeric	GD3 ganglioside	malignant melanoma
Eculizumab	Soliris	mab	humanized	C5	paroxysmal nocturnal hemoglobinuria
Edobacomab		mab	mouse	endotoxin	sepsis caused by Gram-negative bacteria
Edrecolomab	Panorex	mab	mouse	EpCAM	colorectal carcinoma
Efalizumab	Raptiva	mab	humanized	LFA-1 (CD11a)	psoriasis (blocks T-cell migration)
Efungumab	Mycograb	scFv	human	Hsp90	invasive <i>Candida</i> infection
Eldelumab		mab	human	interferon gamma-induced protein	Crohn's disease, ulcerative colitis
Elgentumab		mab	human	ERBB3	cancer
Elotuzumab		mab	humanized	SLAMF7	multiple myeloma
Elsilimomab		mab	mouse	IL-6	?
Emactuzumab		mab	humanized	CSF1R	cancer
Emibetuzumab		mab	humanized	HHGFR	cancer

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
Enavatuzumab		mab	humanized	TWEAK receptor	cancer etc.
Enfortumab vedotin		mab	human	AGS-22M6	cancer expressing Nectin-4
Enlimomab pegol		mab	mouse	ICAM-1 (CD54)	?
Enoblituzumab		mab	humanized	B7-H3	cancer
Enokizumab		mab	humanized	IL9	asthma
Enoticumab		mab	human	DLL4	?
Ensituximab		mab	chimeric	5AC	cancer
Epitumomab cituxetan		mab	mouse	episialin	?
Epratuzumab		mab	humanized	CD22	cancer, SLE
Erlizumab		F(ab') ₂	humanized	ITGB2 (CD18)	heart attack, stroke, traumatic shock
Ertumaxomab	Rexomun	3funct	rat/mouse hybrid	HER2/neu, CD3	breast cancer etc.
Etaracizumab	Abegrin	mab	humanized	integrin $\alpha v \beta 3$	melanoma, prostate cancer, ovarian cancer etc.
Etolizumab		mab	humanized	integrin $\alpha 7 \beta 7$	inflammatory bowel disease
Evinacumab		mab	human	angiopoietin 3	dyslipidemia
Evolocumab		mab	human	PCSK9	hypercholesterolemia
Exbivirumab		mab	human	hepatitis B surface antigen	hepatitis B
Fanolesomab	NeutroSpec	mab	mouse	CD15	appendicitis (diagnosis)
Faralimomab		mab	mouse	interferon receptor	?
Farletuzumab		mab	humanized	folate receptor 1	ovarian cancer
Fasinumab		mab	human	HNGF	acute sciatic pain
FBTA05	Lymphomun	3funct	rat/mouse hybrid	CD20	chronic lymphocytic leukaemia
Felvizumab		mab	humanized	respiratory syncytial virus	respiratory syncytial virus infection
Fezakinumab		mab	human	IL-22	rheumatoid arthritis, psoriasis
Ficlatuzumab		mab	humanized	HGF	cancer etc.
Figitumumab		mab	human	IGF-1 receptor	adrenocortical carcinoma, non-small cell lung carcinoma etc.
Firivumab		mab	human	influenza A virus hemagglutinin	?
Flanvotumab		mab	human	TYRP1(glycoprotein 75)	melanoma
Fletikumab		mab	human	IL 20	rheumatoid arthritis
Fontolizumab	HuZAF	mab	humanized	IFN- γ	Crohn's disease etc.
Foralumab		mab	human	CD3 epsilon	?
Foravirumab		mab	human	rabies virus glycoprotein	rabies (prophylaxis)
Fresolimumab		mab	human	TGF- β	idiopathic pulmonary fibrosis, focal segmental glomerulosclerosis, cancer
Fulranumab		mab	human	NGF	pain
Futuximab		mab	chimeric	EGFR	?
Galiximab		mab	chimeric	CD80	B-cell lymphoma
Ganitumab		mab	human	IGF-1	cancer
Gantenerumab		mab	human	beta amyloid	Alzheimer's disease
Gavilimomab		mab	mouse	CD147 (basigin)	graft versus host disease
Gemtuzumab ozogamicin	Mylotarg	mab	humanized	CD33	acute myelogenous leukemia
Gevokizumab		mab	humanized	IL-1 β	diabetes etc.
Girentuximab	Rencarex	mab	chimeric	carbonic anhydrase 9 (CA-IX)	clear cell renal cell carcinoma[81]
Glembatumumab vedotin		mab	human	GPNMB	melanoma, breast cancer
Golimumab	Simponi	mab	human	TNF- α	rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis
Gomiliximab		mab	chimeric	CD23 (IgE receptor)	allergic asthma
Guselkumab		mab	human	IL23	psoriasis
Ibalizumab		mab	humanized	CD4	HIV infection

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
Ibritumomab tiuxetan	Zevalin	mab	mouse	CD20	non-Hodgkin's lymphoma
Icnucumab		mab	human	VEGFR-1	cancer etc.
Idarucizumab		mab	humanized	dabigatran	reversal of anticoagulant effects of dabigatran
Igovomab	Indimacis- 125	F(ab') ₂	mouse	CA-125	ovarian cancer (diagnosis)
IMAB362		mab	human	CLDN18.2	gastrointestinal adenocarcinomas and pancreatic tumor cancer
Imalumab	Myoscint	mab	human	MIF	cancer
Imciromab		mab	mouse	cardiac myosin	cardiac imaging
Imgatuzumab		mab	humanized	EGFR	cancer
Inclacumab		mab	human	selectin P	?
Indatuximab ravtansine		mab	chimeric	SDC1	cancer
Indusatumab vedotin		mab	human	GUCY2C	cancer
Infliximab	Remicade	mab	chimeric	TNF- α	rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease, ulcerative colitis
Intetumumab		mab	human	CD51	solid tumors (prostate cancer, melanoma)
Inolimomab		mab	mouse	CD25 (α chain of IL-2 receptor)	graft versus host disease
Inotuzumab		mab	humanized	CD22	cancer
ozogamicin	Yervoy				
Ipilimumab		mab	human	CD152	melanoma
Iratumumab		mab	human	CD30 (TNFRSF8)	Hodgkin's lymphoma
Isatuximab		mab	chimeric	CD38	cancer
Itolizumab		mab	humanized	CD6	?
Ixekizumab		mab	humanized	IL-17A	autoimmune diseases
Keliximab	CEA-Cide	mab	chimeric	CD4	chronic asthma
Labetuzumab		mab	humanized	CEA	colorectal cancer
Lambrolizumab		mab	humanized	PDCD1	antineoplastic agent
Lampalizumab		mab	humanized	CFD	?
Lebrikizumab		mab	humanized	IL-13	asthma
Lemalesomab		mab	mouse	NCA-90 (granulocyte antigen)	diagnostic agent
Lenzilumab		mab	human	CSF2	?
Lerdelimumab		mab	human	TGF beta 2	reduction of scarring after glaucoma surgery
Lexatumumab		mab	human	TRAIL-R2	cancer
Libivirumab		mab	human	hepatitis B surface antigen	hepatitis B
Lifastuzumab vedotin		mab	humanized	phosphate-sodium co-transporter	cancer
Ligelizumab		mab	humanized	IGHE	severe asthma and chronic spontaneous urticaria
Lilotomab		mab	mouse	CD37	cancer
satetraxetan					
Lintuzumab		mab	humanized	CD33	cancer
Lirilumab		mab	human	KIR2D	?
Lodelcizumab		mab	humanized	PCSK9	hypercholesterolemia
Lokivetmab		mab	veterinary	<i>Canis lupus familiaris</i> IL31	?
Lorvotuzumab mertansine		mab	humanized	CD56	cancer
Lucatumumab		mab	human	CD40	multiple myeloma, non-Hodgkin's lymphoma,
Lulizumab pegol		mab	humanized	CD28	Hodgkin's lymphoma autoimmune diseases
Lumiliximab		mab	chimeric	CD23 (IgE receptor)	chronic lymphocytic leukemia

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
Lumretuzumab		mab	humanized	ERBB3	cancer
Mapatumumab		mab	human	TRAIL-R1	cancer
Margetuximab		mab	humanized	ch4D5	cancer
Maslimomab		?	mouse	T-cell receptor	?
Mavrilimumab		mab	human	GMCSF receptor α -chain	rheumatoid arthritis
Matuzumab		mab	humanized	EGFR	colorectal, lung and stomach cancer
Mepolizumab	Bosatria	mab	humanized	IL-5	asthma and white blood cell diseases
Metelimumab		mab	human	TGF beta 1	systemic scleroderma
Milatumzumab		mab	humanized	CD74	multiple myeloma and other hematological malignancies
Minretumomab		mab	mouse	TAG-72	tumor detection (and therapy?)
Mirvetuximab soravtansine		mab	chimeric	folate receptor alpha	cancer
Mitumomab		mab	mouse	GD3 ganglioside	small cell lung carcinoma
Mogamulizumab		mab	humanized	CCR4	cancer
Morolimumab		mab	human	Rhesus factor	?
Motavizumab	Numax	mab	humanized	respiratory syncytial virus	respiratory syncytial virus (prevention)
Moxetumomab pasudotox		mab	mouse	CD22	cancer
Muromonab-CD3	Orthoclone OKT3	mab	mouse	CD3	prevention of organ transplant rejections
Nacolomab tafenoatox		Fab	mouse	C242 antigen	colorectal cancer
Namilumab		mab	human	CSF2	?
Naptumomab estafenoatox		Fab	mouse	5T4	non-small cell lung carcinoma, renal cell carcinoma
Narnatumab		mab	human	RON	cancer
Natalizumab	Tysabri	mab	humanized	integrin α 4	multiple sclerosis, Crohn's disease
Nebacumab		mab	human	endotoxin	sepsis
Necitumumab		mab	human	EGFR	non-small cell lung carcinoma
Nemolizumab		mab	humanized	IL31RA	?
Nerelimomab		mab	mouse	TNF- α	?
Nesvacumab		mab	human	angiopoietin 2	cancer
Nimotuzumab	Theracim, Theraloc	mab	humanized	EGFR	squamous cell carcinoma, head and neck cancer, nasopharyngeal cancer, glioma
Nivolumab		mab	human	PD-1	cancer
Nofetumomab merpentan	Verluma	Fab	mouse	?	cancer (diagnosis)
Obiltoxaximab		mab	chimeric	<i>Bacillus anthracis</i> anthrax	<i>Bacillus anthracis</i> spores
Obinutuzumab	Gazyva	mab	humanized	CD20	Chronic lymphatic leukemia
Ocaratuzumab		mab	humanized	CD20	cancer
Ocrelizumab		mab	humanized	CD20	rheumatoid arthritis, lupus erythematosus etc.
Odulimomab		mab	mouse	LFA-1 (CD11a)	prevention of organ transplant rejections, immunological diseases
Ofatumumab	Arzerra	mab	human	CD20	chronic lymphocytic leukemia etc.
Olaratumab		mab	human	PDGF-R α	cancer
Olokizumab		mab	humanized	IL6	?
Omalizumab	Xolair	mab	humanized	IgE Fc region	allergic asthma
Onartuzumab		mab	humanized	human scatter factor receptor kinase	cancer
Ontuxizumab		mab	chimeric/humanized	TEM1	cancer
Opicinumab ¹		mab	human	LINGO-1	multiple sclerosis

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
Oportuzumab monatox		scFv	humanized	EpCAM	cancer
Oregovomab	OvaRex	mab	mouse	CA-125	ovarian cancer
Orticumab		mab	human	oxLDL	?
Otelixizumab		mab	chimeric/humanized	CD3	diabetes mellitus type 1
Otlertuzumab		mab	humanized	CD37	cancer
Oxelumab		mab	human	OX-40	asthma
Ozanezumab		mab	humanized	NOGO-A	ALS and multiple sclerosis
Ozoralizumab		mab	humanized	TNF- α	inflammation
Pagibaximab		mab	chimeric	lipoteichoic acid	sepsis (<i>Staphylococcus</i>)
Palivizumab	Synagis, Abbosynagis	mab	humanized	F protein of respiratory syncytial virus	respiratory syncytial virus (prevention)
Panitumumab	Vectibix	mab	human	EGFR	colorectal cancer
Pankomab		mab	humanized	tumor specific glycosylation of MUC1	ovarian cancer
Panobacumab		mab	human	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i> infection
Parsatuzumab		mab	human	EGFL7	cancer
Pascolizumab		mab	humanized	IL-4	asthma
Pasotuxizumab		mab	chimeric/humanized	folate hydrolase	cancer
Pateclizumab		mab	humanized	LTA	TNF
Patritumab		mab	human	HER3	cancer
Pembrolizumab		mab	humanized	PDCD1	cancer etc.
Pemtumomab	Theragyn	?	mouse	MUC1	cancer
Perakizumab		mab	humanized	IL17A	arthritis
Pertuzumab	Ommitarg	mab	humanized	HER2/neu	cancer
Pexelizumab		scFv	humanized	C5	reduction of side effects of cardiac surgery
Pidilizumab		mab	humanized	PD-1	cancer and infectious diseases
Pinatuzumab vedotin		mab	humanized	CD22	cancer
Pintumomab		mab	mouse	adenocarcinoma antigen	adenocarcinoma (imaging)
Placulumab		mab	human	human TNF	?
Polatuzumab vedotin		mab	humanized	CD79B	cancer
Ponezumab		mab	humanized	human beta-amyloid	Alzheimer's disease
Priliximab		mab	chimeric	CD4	Crohn's disease, multiple sclerosis
Pritoxaximab		mab	chimeric	<i>E. coli</i> shiga toxin type-1	?
Pritumumab		mab	human	vimentin	brain cancer
PRO 140		?	humanized	CCR5	HIV infection
Quilizumab		mab	humanized	IGHE	asthma
Racotumomab		mab	mouse	N-glycolylneuraminic acid	cancer
Radretumab		mab	human	fibronectin extra domain-B	cancer
Rafivirumab		mab	human	rabies virus glycoprotein	rabies (prophylaxis)
Ralpancizumab		mab	humanized	neural apoptosis-regulated proteinase 1	dyslipidemia
Ramucirumab	Cyramza	mab	human	VEGFR2	solid tumors
Ranibizumab	Lucentis	Fab	humanized	VEGF-A	macular degeneration (wet form)
Raxibacumab		mab	human	anthrax toxin, protective antigen	anthrax (prophylaxis and treatment)
Refanezumab		mab	humanized	myelin-associated glycoprotein	recovery of motor function after stroke
Regavirumab		mab	human	cytomegalovirus glycoprotein B	cytomegalovirus infection
Reslizumab		mab	humanized	IL-5	inflammations of the airways, skin and gastrointestinal tract

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
Rilotumumab		mab	human	HGF	solid tumors
Rinucumab		mab	human	platelet-derived growth factor receptor beta	neovascular age-related macular degeneration
Rituximab	MabThera, Rituxan	mab	chimeric	CD20	lymphomas, leukemias, some autoimmune disorders
Robatumumab		mab	human	IGF-1 receptor	cancer
Roledumab		mab	human	RHD	?
Romosozumab		mab	humanized	sclerostin	osteoporosis
Rontalizumab		mab	humanized	IFN- α	systemic lupus erythematosus
Rovelizumab	LeukArrest	mab	humanized	CD11, CD18	haemorrhagic shock etc.
Ruplizumab	Antova	mab	humanized	CD154 (CD40L)	rheumatic diseases
Sacituzumab		mab	humanized	tumor-associated calcium signal transducer 2	cancer
govitecan					
Samalizumab		mab	humanized	CD200	cancer
Sarilumab		mab	human	IL6	rheumatoid arthritis, ankylosing spondylitis
Satumomab		mab	mouse	TAG-72	cancer (diagnosis)
pendetide					
Secukinumab		mab	human	IL-17A	uveitis, rheumatoid arthritis psoriasis
Seribantumab		mab	human	ERBB3	cancer
Setoxaximab		mab	chimeric	<i>E. coli</i> shiga toxin type-2	?
Sevirumab		?	human	cytomegalovirus	cytomegalovirus infection
Sibrotuzumab		mab	humanized	FAP	cancer
SGN-CD19A		mab	humanized	CD19	acute lymphoblastic leukemia and B-cell non-Hodgkin lymphoma
SGN-CD33A		mab	humanized	CD33	Acute myeloid leukemia
Sifalimumab		mab	humanized	IFN- α	SLE, dermatomyositis, polymyositis
Siltuximab		mab	chimeric	IL-6	cancer
Simtuzumab		mab	humanized	LOXL2	fibrosis
Siplizumab		mab	humanized	CD2	psoriasis, graft-versus-host disease (prevention)
Sirukumab		mab	human	IL-6	rheumatoid arthritis
Sofituzumab		mab	humanized	CA 125	ovarian cancer
vedotin					
Solanezumab		mab	humanized	beta amyloid	Alzheimer's disease
Solitomab		mab	mouse	EpCAM	?
Sonepcizumab		?	humanized	sphingosine-1-phosphate	choroidal and retinal neovascularization
Sontuzumab		mab	humanized	episialin	?
Stamulumab		mab	human	myostatin	muscular dystrophy
Sulesomab	LeukoScan	Fab'	mouse	NCA-90 (granulocyte antigen)	osteomyelitis (imaging)
Suvizumab		mab	humanized	HIV-1	viral infections
Tabalumab		mab	human	BAFF	B-cell cancers
Tacatuzumab	AFP-Cide	mab	humanized	alpha-fetoprotein	cancer
tetraxetan					
Tadocizumab		Fab	humanized	integrin α IIb β 3	percutaneous coronary intervention
Talizumab		mab	humanized	IgE	allergic reaction
Tanezumab		mab	humanized	NGF	pain
Taplitumomab		mab	mouse	CD19	cancer[citation needed]
paptox					
Tarextumab		mab	human	Notch receptor	cancer
Tefibazumab	Aurexis	mab	humanized	clumping factor A	Staphylococcus aureus infection
Telimomab aritox		Fab	mouse	?	?
Tenatumomab		mab	mouse	tenascin C	cancer
Teneliximab		mab	chimeric	CD40	?

TABLE 1-continued

Name	Trade name	Type	Source	Target	Use
Teplizumab		mab	humanized	CD3	diabetes mellitus type 1
Teprotumumab		mab	human	CD221	hematologic tumors
Tesidolumab		mab	human	C5	?
TGN1412		?	humanized	CD28	chronic lymphocytic leukemia, rheumatoid arthritis
Ticilimumab (=tremelimumab)		mab	human	CTLA-4	cancer
Tildrakizumab		mab	humanized	IL23	immunologically mediated inflammatory disorders
Tigatuzumab		mab	humanized	TRAIL-R2	cancer
TNX-650		?	humanized	IL-13	Hodgkin's lymphoma
Tocilizumab[6] (=atlizumab)	Actemra, RoActemra	mab	humanized	IL-6 receptor	rheumatoid arthritis
Toralizumab		mab	humanized	CD154 (CD40L)	rheumatoid arthritis, lupus nephritis etc.
Tosatoxumab		mab	human	Staphylococcus aureus	?
Tositumomab	Bexxar	?	mouse	CD20	follicular lymphoma
Tovetumab		mab	human	CD140a	cancer
Tralokinumab		mab	human	IL-13	asthma etc.
Trastuzumab	Herceptin	mab	humanized	HER2/neu	breast cancer
TRBS07	Ektomab	3funct	?	GD2	melanoma
Tregalizumab		mab	humanized	CD4	?
Tremelimumab		mab	human	CTLA-4	cancer
Trevogrumab		mab	human	growth differentiation factor 8	muscle atrophy due to orthopedic disuse and sarcopenia
Tucotuzumab		mab	humanized	EpCAM	cancer
celmoleukin					
Tuvirumab		?	human	hepatitis B virus	chronic hepatitis B
Ublituximab		mab	chimeric	MS4A1	cancer
Ulocuplumab		mab	human	C—X—C chemokine receptor type 4	hematologic malignancies
Urelumab		mab	human	4-1BB	cancer etc.
Urtioxazumab		mab	humanized	<i>Escherichia coli</i>	diarrhoea caused by <i>E. coli</i>
Ustekinumab	Stelara	mab	human	IL-12, IL-23	multiple sclerosis, psoriasis, psoriatic arthritis
Vandortuzumab		mab	humanized	STEAP1	cancer
vedotin					
Vantictumab		mab	human	Frizzled receptor	cancer
Vanucizumab		mab	humanized	angiopoietin 2	cancer
Vapaliximab		mab	chimeric	AOC3 (VAP-1)	?
Varlilumab		mab	human	CD27	?
Vatelizumab		mab	humanized	ITGA2	?
Vedolizumab		mab	humanized	integrin $\alpha 4\beta 7$	Crohn's disease, ulcerative colitis
Veltuzumab		mab	humanized	CD20	non-Hodgkin's lymphoma
Vepalimomab		mab	mouse	AOC3 (VAP-1)	inflammation
Vesencumab		mab	human	NRP1	?
Visilizumab	Nuvion	mab	humanized	CD3	Crohn's disease, ulcerative colitis
Volociximab		mab	chimeric	integrin $\alpha 5\beta 1$	solid tumors
Vorsetuzumab		mab	humanized	CD70	cancer
mafodotin					
Votumumab	HumaSPECT	mab	human	tumor antigen CTAA16.88	colorectal tumors
Zalutumumab	HuMax-EGFr	mab	human	EGFR	squamous cell carcinoma of the head and neck
Zanolimumab	HuMax-CD4	mab	human	CD4	rheumatoid arthritis, psoriasis, T-cell lymphoma
Zatuximab		mab	chimeric	HER1	cancer
Ziralimumab		mab	human	CD147 (basigin)	?
Zolimomab aritox		mab	mouse	CD5	systemic lupus erythematosus, graft-versus-host disease

[0053] In another embodiment using photoacoustic technology, the circulating tumor, exosomes, or extracellular vesicles in the blood are quantified non-invasively by having a thermal energy source such as laser-microwave, RF, or other unit mounted on the patient's wrist, neck, etc. and a receiver to count and record the sound wave generated by circulating cells to which the antibody-coated nanoparticles are attached.

[0054] In another embodiment, the ultrasonic receiver of the photoacoustic unit is an array of ultrasonic receivers mounted on a hand held probe. The hand held probe contacts the patient's skin via a gel placed over the area suspected to contain a tumor or lesion. It simultaneously records multiple photoacoustic signals from the lesion during thermal energy application. Thermal energy applied pulses can range from one per second to a million times or more per second. Each time a thermal pulse reaches the nanoparticles, the nanoparticles expand and create a photoacoustic response that is recorded by the photoacoustic receiver.

[0055] The probe can be moved in any direction, e.g., up and down, side to side, etc., over the skin while recording the sound waves from the nanoparticles. Using a processor in the photoacoustic unit, one uses the photoacoustic response data to construct a two- or three-dimensional image of the tumor. The hand held probe permits scanning any bodily surface, including but not limited to breast, eye, CNS, spinal cord, extremities, internal organs, eye, nose, chest, trachea, throat, abdomen, and urogenital organs. The data from the ultrasonic array probe of the photoacoustic unit is stored in a computer during the probe's motion, permitting video construction showing tumor shape, structure, location, etc. for video presentation, evaluation, and archiving.

[0056] In one embodiment, the unit is capable of storing vast quantities of data from photoacoustic signals. The unit is also capable of storing vast quantities of data from non-stationary tissues, e.g., circulating tumor cells and exosomes in blood vessels, that have accumulated antibody coated nanoparticles on their cell membranes. The targeted cells can also be any normal or abnormal circulating cell in the blood or lymphatic system. The photoacoustic unit reproduces signals from these mobile cells and/or exosomes as photoacoustic cinematography/angiography or video.

[0057] In one embodiment, the cinematography or video recording is done by the photoacoustic unit recording at least 30 frames/second of photoacoustic signals, and converting them into an image of a moving object. A cinematography or video is performed by obtaining at least 30 frames of photos of a moving object per second. In photoacoustic videography or photoacoustic angiography, 30 or more frames of pulse signals from the heated nanoparticles per second are needed to reproduce or convert the still images to a moving object, e.g., blood flow, etc. by the unit. Use of such a system is known: Peyman et al. *Ophthalmic Surg Laser Imaging* 43 (2012) 143-51 doi: 10.3928.15428877-20120105-01 showing, however, lower resolution because no nanoparticles or photoacoustic imaging system was employed, and expressly incorporated by reference herein in its entirety.

[0058] In one embodiment the photoacoustic processor converts the microscopic still images to a video or photoacoustic angiography; since the only moving parts in the vessels that are targeted with antibody coated nanoparticles are the circulating tumor cells or exosomes, extracellular vesicles or bubbles covered with antibody coated nanopar-

ticles that are heated by a pulse of thermal energy produces an internal ultrasonic pulse signal recorded by the photoacoustic receiver. A moving image of the cells and exosomes can be created by the unit whether the cells are on the tumor interior or exterior.

[0059] Nanoparticle assisted photoacoustic video-angiography or nanoparticle assisted photoacoustic cinematography is novel and inventive. All "photoacoustic" terminology has previously been used for describing tissue heating or the difference in the temperature between two tissues, vessels vs. skin, and has been done with light alone, not in combination with nanoparticles. In one embodiment, the method is performed for therapy by providing the patient with at least one antibody-coated functionalized nanoparticle having a detectable property, with the antibody targeting the functionalized nanoparticle to a specific patient site, then heating the nanoparticles to generate a photoacoustic signal, i.e., thermal therapy, and imaging to visualize any localized nanoparticle at the site. The ultrasonic receiver of the photoacoustic unit is an array of ultrasonic receivers mounted on a hand held probe simultaneously recording multiple photoacoustic signals from the lesion during thermal energy application which in one embodiment is pulsating. The array of ultrasonic receivers of the photoacoustic unit mounted on a hand held probe simultaneously records multiple photoacoustic signals from the lesion or vessels during thermal energy application, reproducing motion of moving nanoparticles and/or cells as a nanoparticle assisted photoacoustic video-angiography or nanoparticle assisted photoacoustic cinematography.

[0060] In another embodiment, software associated with the photoacoustic unit can enhance either or both the photoacoustic signals and resulting images. Enhancement may facilitate differentiating exosomes from circulating cells due to the smaller exosome size. All exosomes or other types of extracellular vesicles are less than one micron; in contrast, tumor cells are five to twenty times larger than exosomes. The inventive system for the first time permits in vivo observation and separation of exosomes from tumor cells, and separation of circulating tumor cells from a tumor mass. The separated cells or cell structures can be observed, counted, and quantified to assess the therapeutic effect of a procedure on tumor cells.

[0061] In another embodiment, after imaging and therapy, the biomarkers are collected from liquid biopsies and compared with those obtained prior to therapy in different post-operative periods to confirm the therapeutic effect of the procedure and prognosticate the condition.

[0062] In another embodiment, the antibody coated nanoparticles are conjugated and administered with checkpoint inhibitors along with known immune therapy agents and vaccines to facilitate circulating killer cells attack and removal of tumor cells.

[0063] In another embodiment, polymeric nanoparticles or polysaccharide or synthetic polymers conjugated with biomarkers are administered to enhance a vaccination effect and are taken up by antigen presenting cells.

[0064] The embodiments shown and described in the specification are only specific embodiments of inventors who are skilled in the art and are not limiting in any way. Therefore, various changes, modifications, or alterations to those embodiments may be made without departing from the

spirit of the invention in the scope of the following claims. The references cited are expressly incorporated by reference herein in their entirety.

What is claimed is:

1. A treatment evaluation method comprising administering, to a patient with a genetic predisposition for but without any clinical manifestation or radiographic evidence of a lesion that has at least one detectable biomarker in a liquid biopsy specimen, functionalized antibody-coated nanoparticles having a detectable property, heating the nanoparticles with an energy source to generate photoacoustic signals, performing photoacoustic imaging with a photoacoustic imaging unit to visualize any locally accumulated nanoparticle at a body site in the patient, imaging the lesion at the site, treating the patient for the lesion, and performing the method at least one time post-treatment to evaluate the treatment outcome quantifying the presence or absence of circulating cells or exosomes in the patient having a genetic predisposition for the lesion.
2. The method of claim 1 where the lesion is a malignant lesion interior to a benign lesion.
3. The method of claim 2 where the lesion is inside a uterine fibroma, or is a melanoma inside a skin nevus or a seborrheic keratosis.
4. The method of claim 1 where the functionalized antibody-coated nanoparticles further comprise at least one of
 - (i) one toxin and/or medicament present in an amount to provide the patient a dose lower than a conventional dose of the toxin and/or medicament due to the presence of tumor cells previously compromised by the method, or
 - (ii) at least one checkpoint inhibitor and the method is performed on a patient receiving immunotherapy and/or a vaccine, the method facilitating killer cells attack and removal of the tumor cells.
5. The method of claim 1 wherein the biomarker is selected from the group consisting of CEA for both malignant pleural effusion and peritoneal cancer dissemination; estrogen receptor/progesterone receptor (ER/PR), HER-2/neu, EGFR for breast cancer, and TIMP-1 associated with serum HER2-positive breast cancer; bladder tumor antigen for urothelial cell carcinoma; thyroglobulin for thyroid cancer metastasis; α -fetoprotein for hepatocellular carcinoma; PSA for prostate cancer; CA 125 for non-small cell lung cancer; CA 19.9 for pancreatic cancer; CA 15.3 for breast cancer; the combination of leptin, prolactin, osteopontin, and IGF-II for ovarian cancer; the combination of CD98, fascin, sPIgR, and 14-3-3 eta for lung cancer; troponin I for myocardial infarction, B-type natriuretic peptide for congestive heart failure; KRAS and UGT1A1 for colorectal cancer; HER-2/neu for gastric cancer; c-KIT, CD20 antigen, CD30, FIP1L1-PDGFR alpha, and PDGFR for GIST; Philadelphia Chromosome (BCR/ABL)/PML/RAR alpha and TPMT/UGT1A1/ALK EGFR for leukemia/lymphoma; KRAS/EGFR for lung cancer, BRAF and S100 for melanoma; loss of BRCA1 and BRCA2 in breast cancer; RNA such as mRNA, microRNA; glycosaminoglycans, alkaline phosphatase and urinary hydroxyproline in skeletal involvement; hyaluronic acid excretion and urinary hydroxyproline in bone disease, and combinations thereof.
6. The method of claim 1 where heating the functionalized antibody-coated nanoparticles uses an alternating magnetic field producing heat as a result of rapid circular or semicircular motion of the nanoparticles.
7. The method of claim 1 where heating the functionalized antibody-coated nanoparticles uses a source of thermal energy.
8. The method of claim 7 where the thermal energy is selected from the group consisting of electromagnetic radiation, visible light, invisible light, infrared radiation, microwaves, radiofrequency waves, and combinations thereof.
9. The method of claim 1 further comprising heating the functionalized antibody-coated nanoparticles from body temperature of 37° C. to at least one of
 - 40° C. to 43° C. to release at least one medication from a thermosensitive polymer coating the antibody coated functionalized nanoparticles; or
 - 45° C. to 47° C. to kill a suspected tumor to which the functionalized antibody-coated nanoparticles are attached.
10. The method of claim 9 where heating of the functionalized antibody-coated nanoparticles is controlled by a processor, the processor under the control of the photoacoustic imaging unit.
11. The method of claim 1 where the nanoparticles in the functionalized antibody-coated nanoparticles are selected from the group consisting of
 - nanoparticles rendered magnetic by coating with a thin film of iron oxide prior to their conjugation with biomarkers;
 - nanoparticles with increased biocompatibility by coating with at least one of (poly)ethylene glycol, cell penetrating peptide (CPP), activating CPP (ACPP), biotin, or streptavidin prior to patient administration;
 - nanoparticles incorporated in liposomes containing at least medication;
 - nanoparticles combined with biodendrimers that are conjugated with biomarkers and monoclonal antibodies and/or genes for simultaneous visualization and therapy;
 - nanoparticles rendered radioactive by coating with alpha or beta radiators that are antibody specific or nonspecific biomarkers of the tumor;
 - nanoparticles rendered heat sensitive by coating with a heat sensitive polymer; and
 - combinations thereof.
12. A patient evaluation method comprising
 - analyzing a liquid biopsy sample from a patient for the presence of a biomarker for a lesion, the biomarker selected from the group consisting of a cell free nucleic acid-based biomarker, a protein-based biomarker, an extracellular vesicle (EV)-based biomarker, a circulating tumor cell (CTC)-based biomarker, and combinations thereof;
 - performing next generation sequencing or providing recombinant affinity reagents fabricated into nanostructures to enhance detection of the biomarkers in the sample; and
 - administering at least a specific antibody or multiple-nonspecific antibodies to the patient to detect the lesion.
13. The method of claim 12 where the lesion is either detected in a diagnostic method or is evaluated post-treatment in a therapeutic method.

- 14.** The method of claim **13** further comprising
(a) providing an antibody against the tumor;
(b) conjugating the anti-tumor antibody with a nanoparticle to form a functionalized antibody-coated nanoparticle;
(c) providing the result of step (b) to a patient to bind the tumor, and
(d) detecting the tumor by detecting the nanoparticle/lesion by a defined thermal energy and/or treating the tumor at the tumor site.
- 15.** The method of claim **14** where detecting is by photoacoustic technology and treatment is by applying thermal energy.
- 16.** The method of claim **12** where the antibody is selected from the group consisting of a recombinant antibody, a monoclonal antibody, a polyclonal antibody, an aptamer, and combinations thereof.
- 17.** The method of claim **14** where the antibody is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, an aptamer, and combinations thereof.
- 18.** The method of claim **12** where the liquid biopsy sample is selected from the group consisting of blood, urine, cerebrospinal fluid (CFS), aqueous or vitreous or abdominal cavity fluid, lymph node fluid, bladder fluid, milk duct fluid, sputum, gastric fluid, bile duct fluid, sinus fluid, and combinations thereof.
- 19.** A method of patient therapy comprising providing the patient in need thereof with at least one antibody-coated functionalized nanoparticle having a detectable property, the antibody targeting the functionalized nanoparticle to a specific patient site; heating the nanoparticles to generate a photoacoustic signal, and imaging to visualize any localized nanoparticle at the site.
- 20.** The method of claim **19** where imaging is by applying external thermal energy and/or by applying a reversible or alternating magnetic field.
- 21.** The method of claim **19** where visualizing occurs from thermally generated nanoparticle expansion, producing an ultrasound wave detectable by an ultrasonic receiver or array to create a two- or three-dimensional image of the lesion.
- 22.** The method of claim **21** where visualization is in a two or three dimensional format as a tomogram.
- 23.** The method of claim **19** where the patient has Alzheimer's disease and plaques are visualized using silica coated nanoparticles administered with amyloid antibody, or where the patient has age related macular degeneration and drusen is visualized and/or treated with simultaneously delivery of medication.
- 24.** The method of claim **23** further comprising quantitating the plaques or the drusen during imaging to determine disease progression or therapy.
- 25.** The method of claim **19** performed using as the ultrasonic receiver of the photoacoustic unit an array of ultrasonic receivers mounted on a hand held probe simultaneously recording multiple photoacoustic signals from the lesion during thermal energy application.
- 26.** The method of claim **25** performed using a pulsating source of thermal energy and an array ultrasonic receiver of the photoacoustic unit mounted on a hand held probe simultaneously recording multiple photoacoustic signals from the lesion or vessels during thermal energy application reproducing motion of moving nanoparticles and/or cells as a nanoparticle assisted photoacoustic video-angiography or nanoparticle assisted photoacoustic cinematography.
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专利名称(译)	可视化非常早期肿瘤或其他病变的方法		
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申请(专利权)人(译)	PEYMAN, 古拉姆A.		
当前申请(专利权)人(译)	PEYMAN, 古拉姆A.		
[标]发明人	PEYMAN GHOLAM A		
发明人	PEYMAN, GHOLAM A.		
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其他公开文献	US10136820		
外部链接	Espacenet USPTO		

摘要(译)

在放射学上可以看到在肿瘤临床表现之前评估具有恶性肿瘤遗传易感性的患者的治疗结果的方法。该方法允许使用生物标记物可视化任何肿瘤，无论是位于患者体外还是位于体内，并且直径小至2mm。该方法使用与纳米颗粒缀合的生物标记物，所述纳米颗粒包括但不限于量子点，其中共轭形式统称为功能化纳米颗粒，其在特定条件下加热以产生光声信号，然后使其可视化以定位和/或治疗肿瘤。

