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The Use of Radioactive Nanoparticles for the Diagnosis and Treatment of Cancer

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Dedicated to:

Louis Pena

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Ken Czerwinski

Romualdo DeSouza

For inspiring my future in Radiochemistry.

~

And to my fellow students of the Nuclear Chemistry Summer School Program 2013:

Friends who are irradiated together, stay together.

For a list of definitions to relevant terms, see Appendix A

Abstract

Radioactive nanoparticles are emerging in the field of Nuclear Medicine as effective treatments for cancer. This thesis presents the results of a literature review, analyzing current trends in research and postulating future directions for investigation. Chemotherapy used today is non-discriminatory in its effects, harming both healthy and diseased tissue. By attaching antibodies and other vectors to the surface of nanoparticles, increases in preferential accumulation within malignant tumors can be obtained. Targeting methods have the effect of both reducing collateral damage to healthy cells and increasing the effective dose to diseased cells. The surface properties of nanoparticles allow them to be easily modified both structurally and chemically. Different radionuclides can then be attached to these surfaces, functionalizing them either for radiotherapy or diagnostic imaging. The flexibility afforded by nanoplatforms gives radioactive nanoparticles the potential to revolutionize the fields of nuclear medicine and oncology in the treatment of cancer.

An Introduction to Nuclear Medicine

On November 8th, 1895 Wilhelm Roentgen discovered an emission source that could penetrate the flesh of his wife's hand, but not the bone[1]. He was able to generate an image of her skeletal appendage on a photographic plate, creating the world's first medical radiograph. The medical community was soon able to take advantage of this phenomenon and put it to use in diagnosing a host of illnesses. Since then, high-energy emissions have played a crucial part in diagnostic imaging. We see them mostly in radiology, where X-ray images remain a routine procedure. It was eventually found that long-term exposure to these beams could cause burns and blisters, damaging cells simply through their proximity to the emission source.

X-rays were soon conscripted into the fight against cancer; leading to the development of external-beam radiotherapy. Rene Gilbert, a Swiss radiologist, was the first person to apply the use of external radiation to cancer treatment[2]. In the 1930s he used this technique to reduce the size of malignant tissue growth resulting from Hodgkin's Lymphoma. Although his patients eventually relapsed, this treatment was an important step for radiation oncology. In external beam radiation therapy, a high-energy x-ray beam is directed at a tumor. The emission source is then directed at the tumor from a different angle and again fired. This serves to administer the maximum amount of the harmful rays at the tumor, while reducing collateral damage to healthy, surrounding tissue[3-7].

Further innovation came at the hands of Henri Becquerel and Marie and Pierre Curie, who first observed radioactivity in certain elemental isotopes. The damaging effects of radiation were apparent from the premature death of Madame Curie. She died of anemia resulting from damaged bone marrow; a tissue now known to be greatly affected by an

increase in radiation dosage. There was also the widely publicized case of the "Radium Girls". These young women were glow-in-the-dark watch dial painters, who would lick their brushes to form them to fine tips. The paint used was infused with radium, causing it to produce a distinctive glow. Large quantities of radioactive emissions from radium cause tissue damage. These girls developed necrosis in their jaws, teeth, and tongues, as well as anemia and leukemia from their exposure to radium. The grotesque nature of these afflictions brought the dangers associated with radiation to the public.

Though seemingly counter-intuitive that the very thing found to be a cause of cancer could also be used to fight it, this idea paved the way for radioactivity to be used as a treatment of malignant tumors. By injecting certain radioisotopes (Table 1) into the bloodstream, tumors can be irradiated directly without the use of massive accelerators, such as those utilized for external beam therapy[8]. An ideal isotope for this purpose has a short half-life, small emission range, and high linear energy transfer (LET). The short half-life means that the material will decay quickly so that the hazardous material does not remain in the bloodstream for too long.

Table 1: Radioactive Emission Types

Emission Type	Use	Overview	Sample Isotopes
Alpha	Therapy	Helium nucleus from heavy isotopes with a high LET and a short range (approximately 10 micrometers).	Bismuth-213 Actinium-225
Beta-minus	Therapy	Electron emitted from neutron-rich isotope. Moderate LET and medium range (millimeters to a few centimeters).	Yttrium-90 Iodine-131
Beta-plus	Imaging	Positron emitted from a proton-rich isotope with a short range. Annihilates with electron to produce antiparallel gamma photons.	Fluorine-18 Carbon-11
Gamma	Imaging	High-energy photon emitted from a metastable nucleus. Lowest LET, with a range of several meters.	Technetium-99m Iodine 123

A short emission range reduces the effective radius that a particle can reach with its ionizing radiation. A smaller range and shorter half-life mean that less of the patient's healthy cells are irradiated and damaged by the treatment. Alpha and beta radiation both fit this ideal, and are therefore exclusively used for modern radioisotope therapies.

One of the primary advantages to radiation therapy is that the body has natural defenses against chronic low-level doses of ionizing radiation[9]. When exposed to high levels of radioactive emissions, the DNA of a cell can become damaged. Healthy, non-cancerous cells have repair mechanisms present within their nuclei that can restore functionality by fixing damage in the DNA strands. This prevents harmful mutations from being passed down to daughter cells following replication. As a result of these repair mechanisms, a patient who is injected with a radioisotope-based treatment has relatively little damage to their healthy tissue. Molecules called "targeting agents" that are bound to the isotope can carry the treatment to cancerous cells. This effectively concentrates the dose so that only the tumor is permanently damaged. The damage done to the tumor causes the cells to apoptose and prevents them from reproducing. As will be seen, this can result in a drastic reduction of the tumor's mass, either curing the disease or supplementing other treatments.

Standard, non-radioactive or "cold" treatments, such as classic chemotherapy, differ in their functionality. When administered to a patient, these drugs are distributed by the circulatory system throughout the entire body. They affect cells with little or no discrimination, doing a great deal of harm to the patient, both physically and mentally. In successful applications, cancerous cells are killed off more quickly than healthy cells. Treatment becomes a race to eradicate all of the diseased tissue before the patient becomes too ill from the

medicine that was meant to treat them. Targeted radioactive treatments offer an alternative to traditional chemotherapy that may eliminate such detrimental side-effects.

Despite the potential shown by radiotherapeutic treatments, there are many associated and well-documented risks to clinical doses of radiation. The most predominant risk is damage to otherwise healthy tissue. Radiation is a known carcinogen. When the body is exposed to radiation, there is a chance that the cell's genome will become damaged. If damage occurs to certain genes, the patient may once again develop cancer. An excessive dose can give rise to secondary cancers that may manifest years after the initial cancer goes into remission[10].

Radiation sickness is another complication experienced by radiotherapy patients. Long-term exposure to high levels of ionizing radiation can damage sensitive cells, such as those of the immune system[11]. This leaves the afflicted individual vulnerable to opportunistic infections. In addition, symptoms such as nausea and vomiting may result from damage to the gastrointestinal tract. While not as severe as cases seen in the aftermaths of major nuclear events (Hiroshima, Nagasaki, and Chernobyl), radiation sickness can lead to complications in already weakened chemotherapy patients[12]. There are treatments available that can alleviate some of the secondary effects, such as antibiotics to control infection. Unfortunately, full restoration of the immune system must be handled by the patient's own body, a process that can take years[11, 12].

Side effects of radiation can be minimized through the use of highly targeted vectors for the radionuclides. The vectors will cause the radionuclides to accumulate almost exclusively in the afflicted areas and reduce the effective dose to healthy tissue. Another method to reduce side effects is to use isotopes that emit radiation with a very short range due to high LET[7].

Paired with targeted vectors, the emission range on these isotopes is just long enough to penetrate a single cell[13]. The surrounding healthy tissue is left less affected. Modern research in radioimmunotherapy (RIT) revolves around achieving a treatment with these two traits: high specificity and low range.

Nuclear medicine involves more than just the treatment of cancer, as diagnostic imaging procedures are also important. Whereas high-powered alpha and beta emissions are used in radiotherapy, imaging techniques use gamma emissions. Gamma emissions are much lower in energy and have only a nominal interaction with the patient's tissue. Based on the properties of the radionuclide chosen, either a 2D or 3D image of the patient can be generated, highlighting areas of increased radiation levels. Diagnostic nuclides are often chelated to similar vectors that are used in radiotherapy, causing the nuclides to aggregate at the tumor sites. From these images, doctors can obtain detailed information on the size and location of tumors.

Two-dimensional images are gathered by a Single-Photon Emission Computed Tomography (SPECT) scan, whereas three-dimensional images are gathered by Positron Emission Tomography (PET) scans. Either one of these instruments can be paired with other scanning methods – most commonly a CT scan. The resulting overlay highlights tumors, and gives an anatomical frame of reference. This information can be useful to surgeons who are preparing to excise the tissue, so that the tumor can be located rapidly in the operating room.

PET scans may also be used in imaging the brain. One popular method is to tag glucose with a radioactive isotope of fluorine and inject the resultant radiotracer into the patient. Glucose is taken up readily by neurons in the brain. A scan of the brain then shows areas where

there are higher levels of radioactive emissions, which correspond to metabolic rates in the tissue. Areas of higher glucose uptake correspond to a higher metabolism, and therefore more brain activity. Clinicians use glucose-based radiotracers to view the extent of brain damage following a stroke[8, 14].

In the United States, approximately 50% of all nuclear medicine procedures performed in a given year are cardiological scans[14]. One example is a myocardial perfusion scan, during which a radiotracer is injected into the patient's bloodstream. The heart is then scanned before and after a period of exercise. The tracer is pumped around the heart and shows areas that may not be receiving adequate blood flow. Such information can be used in the treatment of heart disease – a major health problem facing the United States[8].

The following review will provide an overview of current research in radiotherapy and nuclear imaging and explore the major trends in research. From this, future trends will be discussed that might provide effective treatment of cancer.

The Genetic and Molecular Basis of Cancer

Every year, over 11 million new people worldwide are diagnosed with cancer[15]. Cancer forms as a result of defects in the patient's genome, either due to errors in genomic replication or through damage done by a mutagen. Mutagenic agents have the ability to cause damage to a DNA strand such that it is misread during transcription, leading to an altered expression of the gene it encodes. These modifications include inserting incorrect nucleotides into the DNA and altering key functional groups. Both of these result in a distortion of the sugar backbone. In normally functioning cells, these distortions are detected through molecular pathways which either fix the damage or force the cell to self-destruct via apoptosis.

What distinguishes healthy from malignant cells is damage to certain genes that encode for aspects of replication. The two most important types of cancer-related genes are oncogenes and tumor suppresser genes. Oncogenes encode for parts of cell survival and proliferation; when damaged, oncogenes tend to be overexpressed, leading to an unhealthy increase in their expression[16]. Tumor suppressors tend to promote apoptosis when they are active, acting as a foil to the cell's normal drive to replicate. When tumor suppressor genes are damaged, the cell is less able to inhibit its replication. It should be noted that damage to more than just one of these genes is required. Typically cancer forms as a cumulative result of multiple damaged genes, each affecting some aspect of cellular regulation[9, 16].

The unchecked replication of damaged cells is the defining characteristic of a malignant tumor. Normally there are a host of cellular checkpoints that must be met before division can occur. When these get bypassed through oncogene and tumor suppressor gene disruption, genome damage is passed down to the daughter cells.

When tumor cells are cultured in a lab dish, they form densely packed layers of tissue stacked on top of each other as a result of losing contact-inhibition. When the same procedure is used with healthy cells, they will form a single layer with no stratification, all receiving the same access to available nutrients. This type of growth occurs in the body during the formation of densely packed tumors. Cancer cells can also enlist the aid of surrounding healthy tissues to stimulate the growth of new blood vessels in a process called angiogenesis. These new vessels help to supply oxygen and nutrients to the starved inner portions of the tumor.

As a result of their damaged chromosome and abnormal growth, tumor cells express certain surface receptors on their membranes that are not expressed in healthy cells[9]. These

receptors are encoded in the cell's genome but are not normally expressed on that type of cell. For example, receptors from a neonatal liver cell may be found on an epithelial skin cell. Over-expression of these receptors is common, allowing for a higher concentration be present on the cell surface[15]. These chemicals provide something chemically unique about cancer cells that can be used to distinguish them from healthy cells. Such proteins can be used in diagnostic tests for certain strains of cancer as well as for targeting therapies using antibodies.

Modern Chemotherapeutic Treatments of Cancer

The first modern treatments for cancer were discovered by clinicians seeking to treat childhood leukemia, a cancer that rapidly progresses in the bone marrow of young children[1]. Small doses of certain types of poisons were found to halt the progression of the disease and in some cases even temporarily remove all traces of the cancer's presence. These poisons were later found to target different factors associated with rapid cellular proliferation. One example of this is with antiangiogenesis drugs, which inhibit the vascularization of tumors and slowly starve the malignant cells to death[17, 18].

Certain drugs may target protein synthesis, a process occurring at a higher level in quickly growing tumor cells[15]. Other drugs may simply inhibit cell growth by targeting key checkpoints in the cell cycle, slowing replication of the cells and the tumor. One drug may slow cell growth but not stop it, whereas another may cause breaks in the DNA strand that are not enough to counter the tumor's rapid growth. If both drugs are administered simultaneously, their effects can be compounded, improving the treatment's efficacy. Oncologists recognized this synergistic need early on and began developing drug "cocktails" that included a variety of different compounds.

Such cocktails are difficult to formulate, and their composition can be unique to each patient's individual situation. Cancer drugs can react quite strongly towards cancerous tissue; however they can also have similar effects on healthy tissue. This effect is often seen in cancer patients who have lost their hair. Trichocyte hair cells divide rapidly; non-targeted chemotherapy drugs interact with cells to a greater degree if they proliferate more rapidly, and they interact with chemotherapy drugs to a greater degree than other cell types. Trichocytes are quickly killed off during therapy, contributing to drastic hair loss. The administering of standard chemotherapy drugs becomes a race against the clock to kill off the tumor cells before the patient's health falters from the treatment.

The Benefits of Radiotherapy

The use of radiotherapy is a promising alternative to chemotherapeutic methods. It offers several benefits over traditional "cold" therapies. The first is the increased range of the treatment. When a radionuclide decays by alpha or beta pathways (the two standards for radiotherapy), its emission can travel between a few millimeters to a few centimeters from its emission source. Anywhere within that range, the emission has the ability to interact with molecules in a cell, generating free radicals that cause breaks in a DNA strand[8]. Such an effective range allows the therapeutic dose of radiation to penetrate to the very center of a tumor.

Even when a tumor has stimulated angiogenesis, large areas of its mass are hypoxic from a lack of nearby blood vessels. Injection methods today rely on intravenous administration of drugs; if blood vessels do not reach a certain area in the body then the medicine will not get there either. This is the case with standard chemo drugs, but not with

radionuclide based treatments. Radiation is able to penetrate tissue regardless of tumor vascularization.

Another benefit to using "hot" radiotherapy treatments is that it is easy to target radionuclides to cancer cells. Targeting agents are molecules or functional groups on a larger structure that interact strongly with some aspect of cells. In this way the treatment is shunted, carried, or bound to a particular location of interest. With standard chemotherapy drugs, the targeting agent must be able to keep the drug localized to its intended destination while still providing the drug with enough steric maneuverability to react with the cancer cell.

Sterics are a non-issue with radionuclides. As discussed above, the distance from an emission source to malignant tissue is less of a factor with respect to therapeutic efficacy, so radionuclides do not need to enter a cell to attack the DNA. A targeting agent can be bound to receptors on the cell's surface and still be close enough to kill the cell.

Disadvantages of Radioactive Treatments

There are disadvantages to using "hot" treatments. A small dose of radiation, such as a single injection with a PET isotope, is not likely to cause any harm to the body[19]. It decays quickly and tends to be spread out over the whole body, limiting the amount of exposure any given cell is likely to experience. Cancer treatments are not discrete events, however. Many injections over an extended period of time are necessary to ensure that the tumor cells have been killed and will not manifest again; therefore the dosage can exceed what healthy cells can endure without consequences.

Targeting Methods: Monoclonal Antibodies

Recently, the idea of a "magic bullet" that selectively targets and binds to the tumor cells in question to deliver treatment has arisen in the form of monoclonal antibodies (immunoglobulin). These complex molecules are produced by the mammalian immune system. They target antigens that are expressed under certain conditions on the surfaces of cells. There are highly specific "antigen-binding fragment" Fab regions on each immunoglobulin molecule that target the expressed factor (Fig. 1). Fab regions bind to their antigen with high selectivity, making them difficult to remove and greatly reducing the chances of accidentally binding the wrong molecule. The rest of the antibody is the non-conserved "complementary fragment" Fc region that can be modified to bind to chemotherapy drugs, chelated radionuclides, and nanoparticles[20, 21].

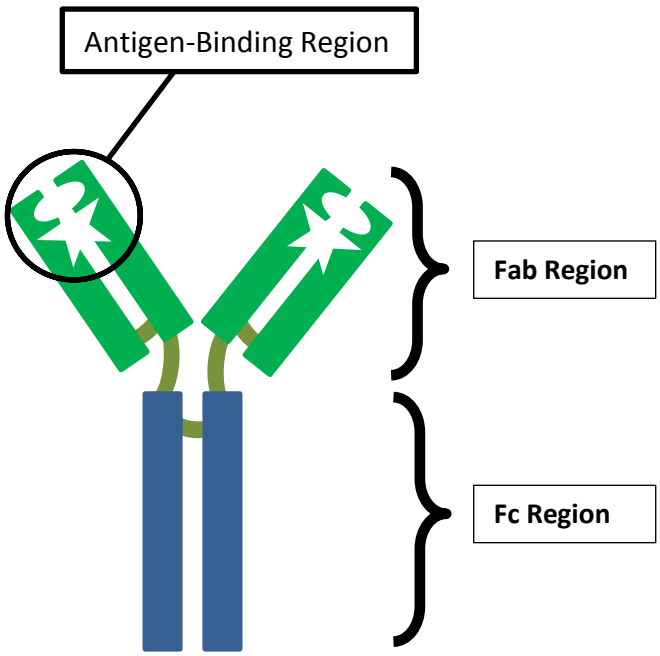

Figure 1: Diagram of an antibody, showing the conserved Fc region, and the antigen binding Fab region

Many lines of cancer cells produce unique proteins and carbohydrates that are expressed on their surfaces. Clinicians are able to identify these cell surface receptors and tailor antibodies to complement them. Surface targeting has seen a degree of success. Several antibodies have been approved for use in hospitals, including Zevalin® and Bexxar®; both have been employed for over a decade[6]. These two antibody-based radioimmunotherapy drugs target phosphoproteins expressed on B-Cells in

some lymphoma patients and have seen some degree of success in clinical trials[22]. The high degree of specificity that is conveyed through antibodies improves the likelihood that newly developed cancer treatments will be effective.

There are still many hurdles that must be overcome before the use of antibodies reaches its full potential. One problem results from the same reason that antibodies are so effective: they bind so specifically to their antigens that unique antibodies must be developed for each type of cancer. In some cases, even the same type of cancer may express different antigens. Cancer can also be caused by mutations. Alterations to the genome at different loci end up producing the same uncontrollable growth. Therefore, if the antibody for a specific cancer is used against a type it was not designed for, the treatment will not be effective because the proper antigen is not present for the drug to target[15].

Every strain of cancer has to be characterized in order to produce antigens that will properly target it. Such tests represent a daunting amount of work; even after an effective treatment is developed, it may still take some time before the correct antigen can be synthesized to treat a particular strain.

Another complication deals with immune response to the antigens designed to target cancer cells. The typical procedure for producing monoclonal antibodies is to use a mouse's spleen cells that have been exposed to the desired antigen. When murine antibodies are injected into humans an immune response is initiated, targeting the foreign (murine) proteins. This response limits the overall effectiveness of the treatment and can cause harm to the patient[21]. Something that has been done in an effort to rectify the immune response is to replace a large section of the mouse-made antibody protein with a section from a human

antibody. The Fc region does not play a part in the antigen binding action and so it can be altered without affecting the Fab region. Mixed sequences can be made similar to human antibodies. A 70% similarity is called chimeric, and 98% similar is referred to as being humanized[22]. Such modifications are effective in reducing or eliminating this risk of immune rejection, but present an extra step in production.

Targeting Methods: Bioaccumulation

The use of monoclonal antibodies exploits a natural mechanism within the body to target foreign proteins. A similar method for targeting uses the body's mechanics to restrict treatment to zones of interest (bioaccumulation). A classic example of bioaccumulation is the use of radioactive iodine salts as a treatment for hyperthyroidism. Iodine will naturally accumulate in the thyroid of patients; the therapeutic dose is administered only to the site of interest. This phenomenon is known as bioaccumulation and can be caused by a number of factors. For example, the material of interest may serve as a metabolite for that particular organ system or cell type.

Cellular metabolism has been exploited extensively in nuclear medicine, and has seen great success with the implementation of the molecule Fluorodeoxyglucose (FDG). FDG is a glucose molecule with an F-18 atom in place of the hydroxyl group at its 2' carbon. This drug is taken up by tumors at a high rate due to their reliance on glycolysis to produce energy. A reliance on glycolysis is due to the rapidly dividing nature of cancer cells[23]. Oxidative phosphorylation and ATP synthesis via the electron transport chain is more efficient but also slower. For maximum energy output, cancer cells will rapidly absorb and break down glucose, as well as glucose analogues such as F-18. Since F-18 is a positron emitter, it lights up tumors in

the body when imaged by a PET scan. F-18 is a powerful and adaptable radiopharmaceutical and is the most commonly used radiotracer in the world[23]. By accumulating preferentially in malignant tissue, F-18 is able to target different types of cancer.

Targeting Methods: Direct Injection as an Alternative to Brachytherapy

The final method of targeting to be discussed here is a down-scaling of a commonly utilized method in oncology. Brachytherapy is a surgical technique in which small "seeds" of radioactive material are surgically implanted within a tumor[14]. During the procedure, a large-bore needle injects pellets from the tip into the tumor, similar to a staple gun. Pellets are left along the line of the injection and this process is repeated until dozens of seeds have been implanted. These radioactive sources are left in place for several months, during which time they are able to constantly irradiate the tumor. While such methods do not remove the tumor altogether, they often lead to a drastic size reduction that allows for eventual resection. The problem with brachytherapy comes from the sources themselves. They do not evenly irradiate the tumor, as there are sections closer to the seeds that are given a very high dose. Other areas are father away and near the edges of the tumor receive only a very small dose.

One solution is to replace the comparatively large and immobile seeds with nanoparticles, small particles with a diameter typically of 1-100 nm. A single injection introduces the particles into the tumor, and their small size allows them to diffuse to all parts of the tumor. Diffusion creates a homogeneous distribution of radiation throughout the tumor. The majority of the particles remain within the confines of the tumor, however a very small number are still able to diffuse out into the rest of the body. To ensure that the particles do not react with healthy body tissue they can be coated with biologically inert compounds, such as

the glycoprotein Gum Arabic (GA). Studies investigating the use of GA-nanoparticles found that the injections caused a drastic reduction in tumor volume and halted any further growth in xenografted prostate cancer in mice[24]. These changes were noted after a single injection. If similar treatments were implemented in human patients to the same degree of success, they could prove to be a much less invasive and time-consuming procedure than traditional brachytherapy.

There are still significant limitations to nanoparticle brachytherapy. To directly inject the nanoparticles, the exact location of the tumor must be known and it must be accessible by injection. Targeting with antibodies or other means allows for a non-specific point of entry, and all cells with the proper antigens will be affected in the same fashion. Direct injection into tumors eliminates the possibility of preemptively treating a metastatic tumor that is too small to have been detected by a CT scan, or those growing in extremely sensitive locations. It also requires a solid-mass tumor, as opposed to "liquid cancer" like leukemia. Uptake into tumor cells is observed; however studies have not been performed to determine if this uptake is exclusive to malignant cells[25]. Regardless of its limitations, direct injection offers promise for treating inoperable tumors.

The capacity for targeted therapy is the paradigm that modern medicine must continue to embrace and advance. The reduction in collateral damage to healthy cells is of great import, especially when dealing with indiscriminately cytotoxic components such as radionuclides. It allows for more potent treatments to be developed without physicians having to worry about side-effects in their patients. While there seems to be great promise in antibody-based treatment, they are not the only solution. Future oncological methods may involve a

combination of direct injection, natural bioaccumulation, and antibody targeting of tumors. A diverse approach to investigating the ideal targeting factor for each disease will be necessary to ensure maximized treatment efficacy.

The Anatomy of a Nanoparticle

Nanoparticles and nanomaterials are an exciting and relatively new innovation. Though procedures for their syntheses have been around since the 1960s, it has only been within the last few decades that their properties have been explored with respect to medical applications[26]. Using their extremely high surface area relative to macroscopic pellets and powders, it is possible to attach thousands of individual atoms and antibodies to each one of these nanoparticles. This serves to increase the binding affinity of the particles to the cancer cells by providing more sites of interaction[20].

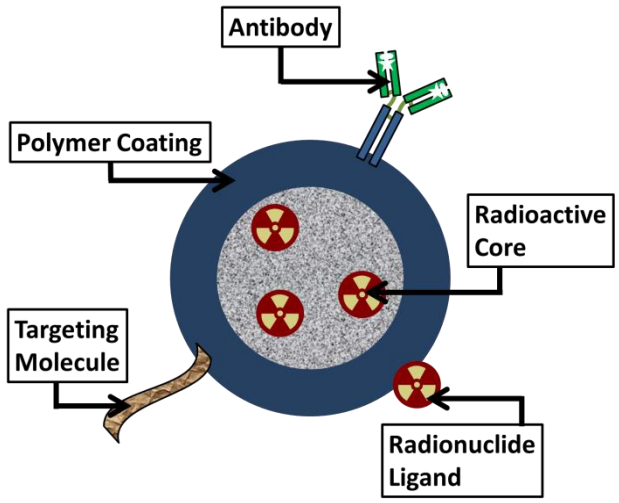

Figure 2: Anatomy of a Nanoparticle – Antibodies, other functional groups and radionuclides can all be attached to the outer coating of a nanoparticle. The core of the nanoparticle can also be made radioactive.

When binding chelated radionuclides, the dose delivered by each successful pairing of antibody to antigen can be increased exponentially. The surface of a nanoparticle can be coated with such functional groups, each of which contains a radionuclide (Fig. 2)[27]. In some cases, the nanoparticles themselves can be made out of a radioisotope, meaning that hundreds or thousands of atoms could be delivered to the tumor

site. Compared to having only a single radionuclide per binding event, the treatment dose delivered could be substantially higher with nanoparticles than with antibodies alone.

Once synthesized, nanoparticles can be stored until needed. Functionalizing nanoparticles is a relatively simple process, making these particles extremely modular and flexible. The same nanoparticle base can be augmented with different antibodies that simultaneously recognize a host of different cancer types. Different radioisotopes could also be attached, depending on the tumor type and size, and whether or not it is intended as a treatment or a PET-active diagnostic tool. Such modular construction is only attainable with nanoplatforms and allows for variable functionality based on the needs of the patient. Cancer manifests differently in every person; the approach to its treatment must be similarly flexible.

Tailoring Nanoparticles for Targeted Radioisotope Therapy

Once the nanoparticle and targeting agent have been synthesized, the next step is to bind the two together. It is common to coat the exterior of a nanoparticle with some sort of biologically inert polymer to trap the metals inside, preventing them from dissolving or reacting with cellular proteins. These coatings vary in functionality, from polyethylene glycol (PEG) to polystyrene (PS) and polycyanoacrylate (PCA)[28]. PEG tends to be the most commonly used coatings because of its duration within the blood stream. It slows clearance of the drug from the blood stream and decreases accumulation in the liver, giving the nanoparticle more time to bind to relevant tumor cells[29]. PEG also serves to increase hydrophilicity of the particle, allowing it to dissolve more easily in aqueous solutions. Finally, the PEG coating provides a substrate onto which antibodies and other targeting ligands can be bound. By modifying the

non-functional end of the ligand, simple reactions can be performed to bind together nanoparticles and targeting ligands.

One downside to traditional methods of chelating a radioisotope directly to a targeting agent is that the isotope eventually decays to a different element, changing its binding chemistry. For example, I-131 is an isotope that is commonly used in medical treatments (Table 2). As a halogen, iodine is very easy to incorporate onto a molecule and can be covalently bonded through standard addition reactions. However, through decay by beta-minus emission, xenon-131 is formed; a noble gas. With full electron orbitals, it cannot sustain chemical bonds and becomes free-floating within the bloodstream. Typically, therapeutic doses of these materials are extremely small and so the small quantity of decay product that forms is often overlooked.

As radiotherapy becomes more prevalent, it would be worth looking into ways of containing these potentially harmful products to minimize any unforeseen side effects. One way to minimize unintentional release is through the use of polymer-coated nanoparticles. Inert polymer coatings keep the body from coming in direct contact with the radioisotopes inside, while also keeping decay products from escaping into the body.

The assumption for such coatings is that the radionuclides will be incorporated into the

Table 2: Common Medical Isotopes

Isotope	Emission	Half-Life ($t_{1/2}$)	Decay Products
Bismuth-213	α	45 min.	Tl-209
Actinium-225	α	10 days	Fr-221, At-217, Bi-213, Po-213
Yttrium-90	β-	64 hr.	Zr-90
Iodine-131	β-	8 days	Xe-131
Fluorine-18	β+	109 min.	O-18
Carbon-11	β+	20 min.	B-11
Technetium-99m	γ	6 hrs.	Tc-99
Iodine-123	γ	13 hr.	Te-123

structure of the nanoparticles themselves. However, some proposed models use non-radioactive nanoparticles that are chelated to organic ligands and then bound to the coated nanoparticle alongside any targeting agents[27]. Nanoparticles are given further flexibility by enabling different radioisotopes to be paired with a variety of targeting agents. For example, the beta emitter Y-90 could be bound if the patient needs a therapeutic drug, while a gamma emitter such as Ga-67 could be used for a SPECT scan[19]. While this added functionality has its benefits, it also removes the isolation between the body and the radionuclide offered by a radioactive nanoparticle.

Current Radioimmunotherapeutic Treatments

Alpha emitters are playing a larger role in modern treatments and research as they are able to deal more damage to tumor cells while reducing damage to healthy tissue. They have an extremely high LET, giving them enormous stopping power at a short range. An alpha particle is relatively large compared to other types of radioactive emissions and has more kinetic energy; typically on the scale of one to tens of MeV. This is enough energy to enter into a cell and break one or both strands of the DNA double-helix with its kinetic energy. Some secondary effects are caused by passage of the highly-ionized particle, such as the formation of free radicals (though they are typically not the most damaging part of alpha emissions). Alpha particles damage cancerous cells but possess a short enough range to minimize damage to adjacent healthy cells. The chief complication is that their short range also prevents them from penetrating deep into the core of the tumor's mass, especially if they were administered into the patient's bloodstream and not through direct injection.

For this reason, beta emitters are still in wide use. While they have less energy, their longer range allows them to irradiate every part of even large tumors. Beta particles do not directly damage the cellular DNA, as they typically have energies on the scale of hundreds of keV, tens to hundreds of times less powerful than an alpha emission. Instead, these electrons will collide with oxygenated molecules within the cell creating highly reactive free radicals. These charged molecules will then indiscriminately react with whatever is nearby. If that happens to be the DNA in the chromosomes of the cell, then severe damage can be a byproduct of the beta emission.

Bexxar® and Zevalin® are antibodies labeled with the beta-emitters I-131 and Y-90 respectively and have been implemented with a degree of success in clinical trials[30]. These antibody drugs target Non-Hodgkin's Lymphoma (NHL). Treatment over the course of three months showed a complete response in 35.5-41.7% of patients, depending on the antibody, and observable responses in 70.9-77.8% of those tested. There were only minor negative toxicological effects, leading Iagaru et. al to conclude that the treatment was safe enough for further clinical trials to be undertaken.

Tumor cells are irradiated in the hopes that their DNA will become too damaged to allow the cell to divide successfully. What is seen in patients is that tumors will either be halted in their growth or begin to shrink. The goal with these treatments is not always to fight the tumors into complete remission, but simply to shrink a dangerously large tumor to a manageable size for resection. This is the case if the tumor is sitting in a location that is too sensitive to operate inside. Other times, the treatment may be given to ensure that any

malignant cells that remain following a tumor removal are killed to encourage a longer-lasting or even complete remission[30].

Radiotherapy Targeting Angiogenesis

The study of angiogenesis that occurs in and around tumor cells is an area of promise for cancer therapy, and may be able to be exploited by targeted nanoparticles. Cancer cells will recruit healthy vascular tissue nearby to construct new vessels to help feed their ever-growing numbers. They do this by excreting hormones such as vascular endothelial growth factor (VEGF) which stimulate growth[32]. Such cellular signals are important for repairing damaged tissue in healthy cells, but can be detrimental when recruited by cancer.

Tumor-induced vascular growth causes certain proteins to be expressed that are not present on normally forming cell walls[15]. One of these in particular, $\alpha_V\beta_3$ integrin, is a transmembrane receptor that can be targeted by both small-molecule antagonists and antibodies. Li et. al attempted to exploit this by attaching such antibodies to the surface of liposome nanoparticles[18]. Y-90 was chosen as the beta emitter and chelated to the outside of the liposome. This layer was then covered with dextran, a polysaccharide that has an affinity to vascular endothelial cells. The antibodies themselves were bound to this outer coating. The particles were injected into murine models of melanoma and colon adenocarcinoma. The results were promising as all test subjects showed a greater reduction in tumor growth over the two-week period over the control. Li concluded that this type of targeting would provide a promising new method for targeting cancers, though they were not yet ready for trials in human patients.

In a much more recent review of antiangiogenesis, Falco was skeptical about its future[17]. One of the hurdles faced by targeting angiogenesis is that tumor cells begin to adapt to inhibition efforts. They can begin to trigger growth without expressing $\alpha_V\beta_3$ on the cell surface. It should be noted that this adaptation was found specifically with angiogenesis inhibiting drugs, not radioisotope therapy. However, it is not difficult to imagine this becoming an issue in human patients. As the drugs begin to target and kill vascular cells that are forced to replicate, the same sorts of adaptive pressures are going to be placed on the malignant cells. If such a treatment were found to be adaptable during clinical trials, the patient may develop a drug-resistant strain of cancer.

Despite this criticism, there is potential for targeting angiogenesis with radionuclides. One potential method is to use small-molecule antagonistic proteins as the targeting agents for nanoparticles. This would allow for a dual-action treatment with a single drug. The antagonist could theoretically bind to one of a number of cell-surface proteins. This would inhibit new vascular growth, as well as place radioactive nanoparticles within close range of tumor cells. The emissions from the radioisotopes then irradiate the nearby tumor while blood flow is cut off from the lack of angiogenesis. There are twelve available antiangiogenesis drugs available on the market today, each of which target an array of different receptors[17]. It is quite likely that at least one of these drugs will be a good fit to pair with radiolabeled nanoparticles. By targeting angiogenesis, researchers can utilize a function relied upon by cancers to inhibit their growth.

Diagnostic Imaging Using Radioisotopes

Though radioisotopes have slowly made their way into the market as a therapeutic drug, they have already been used effectively in diagnostic imaging. Found widely in cardiac, neural, and cancer imaging, radioisotopes are used in dozens of different procedures. The two main types of scans performed are PET and SPECT. Of the two, PET is the most common and has come to be used for a wide range of radiological scans.

A PET scan relies on the properties of a beta-plus emission (a positron), which produces two antiparallel photons when it self-annihilates. These gamma-photons are at a distinctive energy (511 keV) which is picked up by a ring of scintillation detectors. The antiparallel nature of the photons means that they will both hit the detector on opposite sides of the ring at approximately the same time. This coincident event allows the path of the photons to be computed back to their origin at the initial site of annihilation. An output from a PET scan shows highlighted areas of greater decay activity. By functionalizing positron emitters to certain molecules physicians can trace their path through the body. The major problem associated with this scan is that the nature of positrons limits the resolution. When a positron is emitted from its parent nuclide it travels several millimeters from its decay point before running into an electron and being annihilated. As such, there is a certain radius around the actual point of origin where the photons can be traced back to, preventing high-resolution images from being obtained. One way that to overcome this is to utilize a bimodal scanning technique like combined PET/CT instuments.

SPECT works similarly to PET scans, without relying on expensive positron emitters. The patient is injected with a gamma emitting radioisotope such as I-123, which is allowed to

circulate in the bloodstream. A pair of gamma cameras then rotates around the patient, recording absorbance events. The photons are traced back to their origin point to provide information about the level of activity at that site. SPECT is more limited in resolution because it does not have the coincidental detection capability that PET scanners do. A typical SPECT instrument can provide information within 1 centimeter. Higher resolution is not always needed, so the SPECT image suffices. One upside to SPECT is that PET scans tend to be much more expensive. Most positron emitters, such as Tc-99m, require that the clinic be strategically placed within range of a cyclotron. The generators for such nuclides must then be replenished on a weekly basis; otherwise they will decay away into medically useless material. Radiologists must weigh the pros and cons of each procedure before deciding which is best for the particular patient's situation.

Applications of Nuclear Imaging in Oncology

Imaging methods such as PET and SPECT have found wide use in oncology. Traditional scans such as CT and MRI do not have the ability to immediately distinguish healthy from malignant tissue. If an experienced physician knows what they are looking for and where to look for it they may be able to pick out a tumor, but such a method can be unreliable and may result in incorrect diagnosis. Use of a functional imaging technique with a radioactive tracer permits much more accurate diagnosis to be made. One commonly exploited aspect of malignant tumors is that they rely on glycolysis for their metabolism. It has been shown that these malignancies have much higher glucose uptake than the basal metabolic rates of healthy tissue. To this end, FDG has been implemented as an imaging agent. Prior knowledge of tumor locations is not needed for an FDG injection. If the cancer has metastasized throughout the

body, the tracer will still highlight all sites indiscriminately[23]. Unfortunately, FDG is not a solution for every type of cancer. It does not work well for slow growing tumors, which have a lower metabolism and uptake of glucose[19]. It also has a high level of basal metabolic uptake in the brain and gut. This reduces contrast between the background and areas of interest, making the visualization of any tumors in that area very difficult[19].

The low degree of specificity in FDG highlights the need for a new set of targeting agents with which to image cancers. Once again, nanoparticles could serve as options for multi-modal targeting agents. The same antibodies and targeting agents that are used in radioimmunotherapy can be implemented for diagnostic imaging. The only difference is the nature of the radionuclide: a positron or gamma emitter as opposed to an electron or alpha emitter. The targeting agents will bind to cell-surface receptors, concentrating the nanoparticles and leading to areas of higher activity that will light up under PET or SPECT. This method has been shown to be effective in mouse-models, with accumulation occurring at significant levels in tumor cells with a variety of different targeting vectors[29]. In addition to preferential accumulation, experiments with carbon nanotubes have shown rapid system clearance and biocompatibility, something that has been a concern with respect to nanoparticles[33]. These traits are important for reducing side effects and making radiographic scans more routine.

One potential benefit to using nanoparticles for imaging over conventional drugs is that more than one targeting agent can be attached to a nanoparticle. Especially if the patient's cancer is unknown, more than one antibody or ligand may be bound to each nanoparticle, ensuring successful imaging with a single injection.

Radioisotopes for use in Nuclear Imaging

When selecting isotopes for use in diagnostic imaging, there are several factors that must first be considered. The first is the half-life of the radionuclide. Whenever anything radioactive is injected into a human, it is essential that it have a short half-life. Allowing nucleotide decay for a long of a period of time can result in damage to nearby tissue due to chronic radiation exposure and bioaccumulation. However, it must also remain for a long enough period of time for the drug to be synthesized, injected, reach the site of interest, and imaged. It is not currently possible to tailor the decay properties of radioisotopes to fit the needs of the scientific community. Therefore isotopes must be picked from the chart of nuclides and tested to see if they are biologically compatible while having desirable properties *in vivo*. The most commonly used isotope in nuclear medicine today is Technetium-99m, a metastable nucleus that decays by gamma emission to Tc-99[34]. It has a radioactive half-life of just 6 hours, which ensures rapid system clearance and a low dose given to the patient.

These properties make Tc-99m ideal for use in SPECT scans; though it also imposes some significant limitations on its use. Its short half-life means that supplies in hospitals must be consistently replenished from a nearby cyclotron, as its comparatively stable decay product Tc-99 does not have any medical use. Such logistical considerations can become a problem when attempting to apply nuclear technology to radioactive nanoparticles. Typical synthesis procedures for nanoparticles often take hours to fully form the particles to a desirable size - time that is crucial to successful imaging[35]. Positron emitters tend to be the most difficult to deal with, having half-lives typically on the order of minutes to a few hours. Fluorine-18, the isotope in FDG, has a longer half-life of 1.8 hours. Regardless, this fast decay makes it difficult

to obtain a useful amount of nanoparticles from a typical synthesis procedure with F-18 as a reactant. It may prove more useful to modify existing procedures for chelating or bonding these isotopes to ligands and attaching those ligands to non-radioactive, pre-synthesized nanoparticles.

Future Developments Needed for Nanoparticle Applications

The potential for nanoparticles in nuclear medicine is quickly becoming recognized by top researchers in the field, and more studies are coming to light that focus on their use. However, there are some distinct hurdles that must be overcome before nanoparticles will be deemed safe enough for use in humans. Nanoparticles are massive compared to even the largest pharmaceutical macromolecular drugs. This means that their behavior will be vastly different from the decades of traditional medicine biokinetics information that has been gathered. In particular, something that has not been explored is how changing simple properties of nanoparticles will affect their interactions within a biological system. Through some simple procedural changes during synthesis nanoparticles can have pockmarked or smooth surfaces. Their whole geometry can even be altered to form a star-like shell or ellipsoid[35]. Surface charges can also be modified to create more negatively or positively charged particles. These properties can have profound impacts *in vivo*, yet there has been very little done to study their effects. It could be that more selectivity can be gained through modification of these parameters. Toxicity may even be impacted, which could help address health concerns related to nanoparticles. In addition to physical characterization, long-term stability studies need to be undertaken[29]. Micelles, as well as solid-state nanoparticles need to be characterized formally so that such data is available to those conducting research.

Safety Considerations for Nanoparticles

The amount that is not known about nanoparticles in biological systems is staggering, and has led the FDA to only permit nanoparticle-based drug studies on a case-by-case basis[28]. Initial studies have shown that there is a high uptake and bioaccumulation of nanoparticles by the kidneys and liver, but that this can also be altered by changing the particle size[36]. Smaller particles are less likely to accumulate in the renal and hepatic systems, as well as within the alveoli of the lungs. To ensure homogeneous reactivity by the treatment drug, the range of the size distribution of the nanoparticles must be small and consistent. It will take significant research in developing the appropriate protocols to achieve this, especially if the particles have to be synthesized immediately prior to a patient's treatment.

Surface modification through the coating of nanoparticles with polymers such as PEG can also help to reduce undesirable bioaccumulation. PEG has been determined to be non-toxic as it has an LD_{50} of 10g/kg, however it still has the potential to be degraded over time and expose the reactive metals underneath[28]. In addition, normally inert materials have the capacity to become cytotoxic when synthesized on a nanoscale. The extremely high surface area of the particles can drive reaction kinetics beyond what is typically observed. Analysis of this sort of stability and reactivity *in vivo* is essential, especially if bioaccumulation is not able to be eliminated. Nanoparticles need to be able to remain intact and inert long enough for them to be cleared from the patient's system in order to be deemed safe enough for injection.

Learning to modify clearance time in the body can be used to increase bioaccumulation, a desirable effect when using liposomes and micelles. These extremely small nanoparticles are found to clear from biological systems quite rapidly. In cases such as this, it becomes necessary

to find ways to increase the retention time of these particles. Overall, the potential dangers of using nanoparticles for long-term treatments in humans are not truly understood. Standardized rating methods need to be devised so that nanoparticle-based treatments can be evaluated for toxicity in a consistent fashion.

Problems Faced by Nuclear Medicine

Nuclear medicine procedures are being more widely accepted by the medical field as safe and effective methods of diagnosis and treatment. As the number of clinics performing nuclear medicine procedures in the United States grows, the limited number of facilities equipped to produce radioisotopes begin having difficulty keeping up. Cyclotrons are prohibitively expensive, and there is a significant amount of bureaucratic red tape that has to be dealt with before they can be installed. Any clinics must be set up within only a few hours of these cyclotrons by car so that regular shipments of isotope generators can be delivered by a currier before too much of it decays.

As of 2006, there were 70 functional cyclotrons in the U.S., only 39 of which were actively producing radionuclides for use in medicine[37]. With an average of less than one generator per state, access to medical isotopes can be extremely difficult. Patients may have to travel several hours to get access to clinics with the proper staff and equipment. This is a problem that needs to be remedied if new and effective treatments are to be made accessible to those who need them.

Conclusion

The use of nanoparticles in nuclear medicine is an exciting area of research that has the potential to revolutionize modern treatments of cancer. Nanoparticles provide a platform that can be easily tailored for both diagnostic and therapeutic purposes. A nanoparticle can be synthesized as a layered structure with multiple components, each modifying a different aspect of their functionality. The core of the particle is typically metal-based, and in some cases serves as the radiation source. Typically this is then coated with a non-reactive polymeric coating onto which functional groups may be chelated. Targeting vectors are common choices, the most promising of which are monoclonal antibodies. Antibodies bind with high affinity to specific surface receptors, carrying the nanoparticle directly to the sites of cancerous tumors. Targeted nanoparticles are designed to accumulate in such tumors which allow them to be used as tracking agents. Through procedures such as PET and SPECT scans tumors can be located through minimally invasive and reliable means. The bioaccumulation of targeted nanoparticles also allows them to be vectors for radiotherapy. High energy emission isotopes are chosen for these purposes and bombard cancerous cells to the point of apoptosis. The result is a slowed growth rate and decrease in tumor volume. There are some notable safety concerns with respect to nanoparticles; however most of those are a result of not enough being known about their properties. As nanoparticles begin to be recognized for their potential by the research community these uncertainties will be resolved. There is a great deal of potential in functionalized nanoparticles. Their flexibility matches the current need in research and as such would serve well as a new standard for cancer drugs.

Appendix A: Types of Radioactive Decay

α Decay:

Typically occurring within larger nuclei, an alpha decay is characterized by the emission of an alpha particle. Alpha particles are helium nuclei comprised of two neutrons and two protons. When ejected, the particle is moving too fast to accumulate electrons and is therefore highly charged. This charge, and its large mass relative to other emission types, gives alpha decay an extremely high linear energy transfer (LET). Its range in tissue is typically only a few micrometers in tissue.

Ex. $^{213}_{83}\mathrm{Bi} \rightarrow {}^{209}_{81}\mathrm{Tl} + {}^{4}_{2}\mathrm{He}$

β^- Decay:

Nuclei with a large number of neutrons relative to their protons typically exhibit this type of decay. Quantum-mechanical effects cause one of these excess neutrons to convert into a proton. Charge must be conserved in an atom, so an electron is generated to make up for this loss. An atom always has the same number of electrons in its surrounding orbitals as it does protons. Therefore, this loss of a proton means that one of the electrons is ejected from the nucleus. Electrons are also referred to as beta-minus particles. Electrons have a negative charge and can therefore interact with other particles and atoms via Coulombic forces. This give beta particles a moderate LET, though it is significantly less than that of alpha particles. Beta particles have a range of several centimeters in tissue.

Ex. $^{90}_{39}\mathrm{Y} \rightarrow {}^{90}_{40}\mathrm{Zr} + e^- + \mathrm{neutrino}$

β^+ Decay:

Nuclei with a large number of protons relative to their neutrons typically exhibit this type of decay. Quantum-mechanical effects cause one of these excess protons to convert into a neutron. To make up for this loss of positive charge, a positron is emitted. Positrons are the antimatter counterpart to an electron, possessing a charge of +1 instead of -1. This particle is ejected from the nucleus and travels until it comes in contact with an electron. When this happens the two annihilate, their masses converting into light energy. The resultant gamma photons are emitted from the event antiparallel to each other and have a distinctive energy of 511 keV each.

Ex. $^{18}_{9}\mathrm{F} \rightarrow {}^{18}_{8}\mathrm{O} + e^{+} + \text{antineutrino}$

γ Decay:

Gamma decay occurs when a metastable nucleus drops to its ground state, emitting a high-energy gamma photon. A metastable nucleus is typically the result of a previous decay event. For example, Mo-99 decays to Tc-99m which in turn gamma decays to Tc-99. Gamma photons are uncharged and have no mass, therefore interacting very little with surrounding matter. Gamma emissions have the least amount of LET of all the decay types, and can travel through several meters of matter with little attenuation.

Ex. $^{99\mathrm{m}}_{43}\mathrm{Tc} \rightarrow {}^{99}_{43}\mathrm{Tc} + \gamma$

Appendix B: Glossary

Activity: A measure of radioactivity defined as the number of decay events per second. Units can be in Becquerel (Bq), measured as decay per second, or Curies (Ci) which is 3.7×10^{10} decays per second.

Angiogenesis: The growth of new blood vessels from existing ones.

Antigen: A molecule that is the binding complement of the antibody's active Fab region.

Apoptosis: The process by which a cell triggers its own death. A number of chemical signaling pathways exist within every cell to induce this event, some of which are triggered by extensive damage to the DNA.

Bioaccumulation: The accumulation of substances within an organism. This accumulation can sometimes occur in discrete locations, such as the tendency of nanoparticles to settle in the liver.

Chelate: The binding of metal ions, referred to here in reference to the conjugation of radionuclides to an organic ligand.

Cyclotron: A particle accelerator that is used in nuclear medicine to generate radioisotopes. Non-radioactive material is bombarded with high-speed protons to transform it into a different element.

Cytotoxic: The quality of being toxic to cells.

Dose: A measurement of the amount of damaging radiation to which a subject is exposed. The standard unit of measurement is the Gray (Gy) which is measured as Joules of absorbed energy per kilogram of matter.

eV: Symbol for the Electronvolt unit which is defined as the amount of energy gained or lost by the charge of a single electron moving across an electric potential difference of one volt.

Free Radical: This is any molecule that has unpaired electrons in its valence shell. They are highly reactive and can break bonds in nearby molecules. If formed close to a DNA strand, free radicals can cause breaks in the chain.

Glycolysis: The chemical process by which cells break down glucose to form ATP, the chemical energy that fuels most cellular processes.

Half-life: The time it takes for any given number of radionuclides to decay to half of their original number. This is a constant that differs for every known isotope.

Hypoxic: Having a relatively low concentration of oxygen.

Ionizing Radiation: Any form of radiation that has the ability to interact with electrons such that the electron is removed from its corresponding atom. Such radiation can induce the formation of free radicals in cells. Examples of ionizing radiation include alpha, beta, gamma, and UV.

Ligand: A molecular functional group that is formed around a central atom.

Linear Energy Transfer (LET): The transfer of energy from a moving particle to its surroundings. Typically, the higher LET a particle has the more damage it will do to tissue as it passes through. This damage is caused either through knocking electrons out of their orbitals and creating harmful free radicals or by hitting with enough momentum to physically break atomic bonds. Tissues with high concentrations

of oxygen atoms (bone marrow, lungs, etc.) tend to be the most susceptible to free radical formation.

Liposome: A spherical vesicle comprised of a lipid bilayer. Liposomes have an interior vesicle that can be used for containment.

Metastatic Cancer: When cancerous tissue has spread from its site of origin to other parts of the body.

Micelle: Similar to a liposome, micelles are composed of hydrophobic lipids, however they do not form bilayer. As such there is no internal vesicle.

Monoclonal Antibody: Antibodies that are produced by genetically-identical pancreatic cells.

Nanoplatform: A modifiable nanomaterial structure designed to carry functional groups.

Neoplasm: A cancerous tumor.

Nucleotide: A molecular building block of DNA.

Radiograph: An image generated using radioactive emissions.

Radioisotope: An isotope that decays, producing radioactive emissions.

Radiolabel: A radionuclide incorporated into a molecule, often for the purpose of imaging.

Radionuclide: A single atom of a radioisotope.

Radiotracer: A radiolabeled particle that allows the path of said particle to be tracked *in vivo*.

Scintillation Detector: A detector that measures the number of incident gamma rays.

Steric: Having to do with orientation of a molecule.

Vector: The method by which something is delivered to a target location.

Xenograft: The transplantation of tissue to a host of a different species. Xenografted mice are injected with human tumoral cells to better evaluate treatment efficacy.

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