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Transforming Nuclear Medicine with Nanoradiopharmaceuticals

Indrajit Roy¹, Sunil Krishnan², Andrei V. Kabashin^{3,4}, Irina N. Zvestovskaya^{4,5}, and Paras N. Prasad^{4,6,*}

¹ Department of Chemistry, University of Delhi, Delhi-110007, India

² Department of Radiation Oncology, Mayo Clinic Florida, Jacksonville, Florida 32224, USA

³ Aix Marseille University, CNRS, LP3, Campus de Luminy – Case 917, 13288, Marseille, France

⁴ MEPhI, Institute of Engineering Physics for Biomedicine (PhysBio), 115409 Moscow, Russia

⁵ LPI of RAS, Nuclear Physics and Astrophysics Department, 119991 Moscow, Russia

⁶ Department of Chemistry and Institute for Lasers, Photonics, and Biophotonics, University at Buffalo, The State University of New York, Buffalo, New York 14260, USA.

ABSTRACT:

Nuclear medicine is expected to make major advances in cancer diagnosis and therapy; tumor-targeted radiopharmaceuticals preferentially eradicate tumors, while causing minimal damage to healthy tissues. The current scope of nuclear medicine can be significantly expanded by integration with nanomedicine, which utilizes nanoparticles for cancer diagnosis and therapy by capitalizing on the increased surface area-to-volume ratio, the passive/active targeting ability and high loading capacity, the greater interaction cross-section with biological tissues, the rich surface properties of nanomaterials, the facile decoration of nanomaterials with a plethora of functionalities, and the potential for multiplexing several functionalities within one construct. This review provides a comprehensive coverage of nuclear nanomedicine using tumor-targeted nanoparticles for cancer radiation therapy with either pre-embedded radionuclides or non-radioactive materials which can be extrinsically triggered using various external nuclear particle sources to produce in-situ radioactivity. In addition, it describes the prospect of combining nuclear nanomedicine with other modalities to enable synergistically enhanced combination therapies. The review also discusses

advances in the fabrication of radionuclides as well as describes laser ablation technologies for producing nanoradiopharmaceuticals, which combine the ease of production with exceptional purity and rapid biodegradability, along with additional imaging or therapeutic functionalities. From a practical standpoint, these attributes of nanoradiopharmaceuticals may provide distinct advantages in diagnostic/therapeutic sensitivity and specificity, imaging resolution, and scalability of turnkey platforms. Coupling image-guided targeted radiation therapy with the possibility of in-situ activation of nanomaterials as well as combining with other therapeutic modalities using a multifunctional nanoplatform could herald an era of exciting technological and therapeutic advances to radically transform the landscape of nuclear medicine. The review concludes with a discussion of current challenges and presents the authors' views on future opportunities to stimulate further research in this rewarding field of high societal impact.

Keywords: nuclear medicine; nanomedicine; nanoradiopharmaceuticals; multimodality; theranostics; radiation therapy; ion-beam therapy; neutron capture therapy; laser ablation.

Table of Contents

1. Introduction

2. Conventional radionuclide delivery systems

3. Nanocarriers for radionuclides

Soft' or 'organic' nanocarriers

'Hard' or 'inorganic' nanocarriers

4. *In situ* activation *via* external trigger

X-ray radiosensitization

Ion beam therapy

Neutron capture therapy

5. Recent approaches and concepts

Laser ablation for synthesis of nanomaterials for nuclear nanomedicine

Other nuclear and combination therapies

Theranostics using Cerenkov radiation

6. Nanoradiopharmaceuticals in clinics

7. Challenges and future perspectives

1. Introduction

Continuing advances in nuclear technologies have led to the emergence of exciting drugs and radiopharmaceuticals, which demonstrate high efficiency in imaging and therapy of cancer and other diseases. In the traditional approach, radiopharmaceuticals accumulate in tumors and preferentially eradicate them by spontaneous radioactive emission, while causing minimal damage to healthy tissues.¹ In such cases, they are already prepared in a radioactive state and then administered to the patient. An emerging paradigm of nuclear medicine is irradiation of tumors with nuclear particles such as neutrons and protons to directly destroy the tumor by inducing in-situ radioactivity.^{2,3} Although radiotherapy using x-rays, γ -rays, or charged particles do not involve interaction with the atomic nucleus to produce radioactivity, they can also be included in a broader definition of nuclear medicine. Indeed, the interaction of radiation beams with high atomic number elements such as hafnium, gold, and gadolinium leads to increased localized ionizations around these elements, which can also be used in imaging and therapy.³ Thus, for this review, we define and cover nuclear medicine as a field of medicine, which employs particle radiation (α , β , neutrons, protons, etc) and electromagnetic waves such as x-rays and γ -rays from external sources (accelerator, reactor, etc.), as well as internal radioactivity generated by tumor-localizing radionuclides, for diagnosis and treatment. In particular, the imaging functionality implies the use of x-rays for large-view imaging of organs and tissues, while γ and β^+ (positron) emission from radionuclides can be used to precisely locate the distribution of radionuclides in the whole organism. On the other hand, the therapeutic functionality implies either the focusing of external radiation sources (*e.g.* high energy x-rays, ion beams) directly on tumors to cause cancer cell death, or the injection of internal therapeutic radionuclides (*e.g.* α , β^- , auger electron emitters) through a direct catheter or *via* suitable carriers (*e.g.* peptides, antibodies) to deliver the radionuclide to the tumor and initiate radiation-induced DNA damage of actively proliferating cancer cells leading to their selective elimination.⁴ Methods of nuclear

medicine have demonstrated spectacular progress over the last few decades and become one of the key tools for the diagnosis and treatment of tumors, with Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) being the main methods to diagnose tumors and metastases,⁵ and radiation therapy (or radiotherapy) being employed for cancer treatment.^{1,6}

Until recently molecular delivery vehicles such as peptides, antibodies, and antibody fragments and their therapeutic payload had been primarily used to target radionuclides to the tumor.^{7,8} Despite significant progress being made in this field, the clinical adoption of such agents has been limited due to a lack of robust overall survival data and logistical/financial challenges. However, their application prospects have recently been re-energized by the appearance of promising radiopharmaceuticals, including radium-223 chloride (Alpharadin/Xofigo) for treatment of bone metastases from hormone-refractory prostate cancer,⁹ and lutetium Lu-177 dotatate (Lutathera) for treatment of gastroenteropancreatic neuroendocrine tumors.¹⁰ Overall, there are a series of problems and challenges which complicate the widespread application of radiopharmaceuticals based on molecular delivery vehicles in clinical practice. Such vehicles are relatively small (typically, less than 60-65 kDa) and can carry only a few linked radionuclides.^{7,8} Therefore, high concentrations of radionuclide-carrying molecules are required to achieve a substantial therapeutic outcome, but this can lead to severe side effects, taking into account relatively low efficiency of molecular targeting (typically, less than 10-12%). As another problem, the size of molecular targeting agents is within the renal glomerular filtration range (< 7-8 nm), which leads to immediate accumulation of radionuclides in the kidneys. Since the radionuclides are in a highly radioactive state at the time of infusion, they typically cause severe kidney damage, including interstitial nephritis or complete renal failure.¹⁰ Other problems include altered biodistribution and pharmacodynamics profile upon radionuclide incorporation, potential immunogenic effects, inability to co-incorporate additional active agents for multiple biological effect, *etc.*

Paralleling recent advances in the development of radiopharmaceuticals, there has been a burgeoning interest in combining nuclear medicine with nanomedicine to produce nanopradiopharmaceuticals. Nanomedicine employs functional nanoscale objects (nanoparticles) in the diagnosis and therapy of cancer and other diseases.¹¹⁻¹⁴ Depending on

physico-chemical properties of nanomaterials, they can be employed in different tasks of nuclear medicine. As an example, exhibiting strong absorption of x-rays, nanoparticles of non-radioactive elements with high atomic numbers (Au, Bi, *etc.*) can be used as contrast agents in radioimaging,¹⁵ and as sensitizers of radiotherapies.⁶ Additionally, a large surface area-to-volume ratio and high loading capacity enable nanoparticles as efficient transport vehicles of radionuclides in order to enhance image contrast and maximize therapeutic outcome.¹⁶ Furthermore, many inorganic nanoparticles can provide additional imaging and therapeutic functionalities (optical, magnetic, *etc.*) or combine several functionalities within one construct, thus producing multi-modal theranostics. Several review articles already exist highlighting this rapidly developing research field; but they have considered only particular aspects of nuclear nanomedicine, such as the development of nanoparticle-based carriers for radionuclides,¹⁷⁻²¹ nanoparticles as contrast agents for Computed Tomography (CT) imaging,²² or nanotechnologies for radiotherapy.²³ This review article is an attempt to describe a broader perspective of nuclear medicine, from nanoparticles as contrast agents and sensitizers of therapies using external radiation or particle beams, to nanomaterials as carriers of diagnostic and/or therapeutic radionuclides. Given the vastness of the already existing literature, we have selectively highlighted those we felt served as prototypes for conveying our central message. A special focus of this review is the use of external radiation modalities to activate non-radioactive materials that home on to tumors. This is particularly exciting because of the explosion of interest in nuclear particle therapy worldwide with installation of dozens of proton therapy centers and increasing numbers of carbon ion therapy centers for treatment of cancer with greater precision and efficacy.^{2,3} In addition, we highlight multimodal imaging as well as combination therapy for dramatically enhanced image guided therapy, labelled as theranostics,¹³ which may provide distinct advantages in diagnostic/therapeutic sensitivity and specificity, and imaging resolution. Another important feature of this review is describing coupling of image-guided precision radiotherapy options with the possibility of internal activation of nanomaterials. The entire scope of this review is provided in Figure 1. The review ends with a critical assessment of current challenges and our subjective view of future opportunities, with a vision to engage global multidisciplinary research efforts for rapidly advancing this emerging frontier.

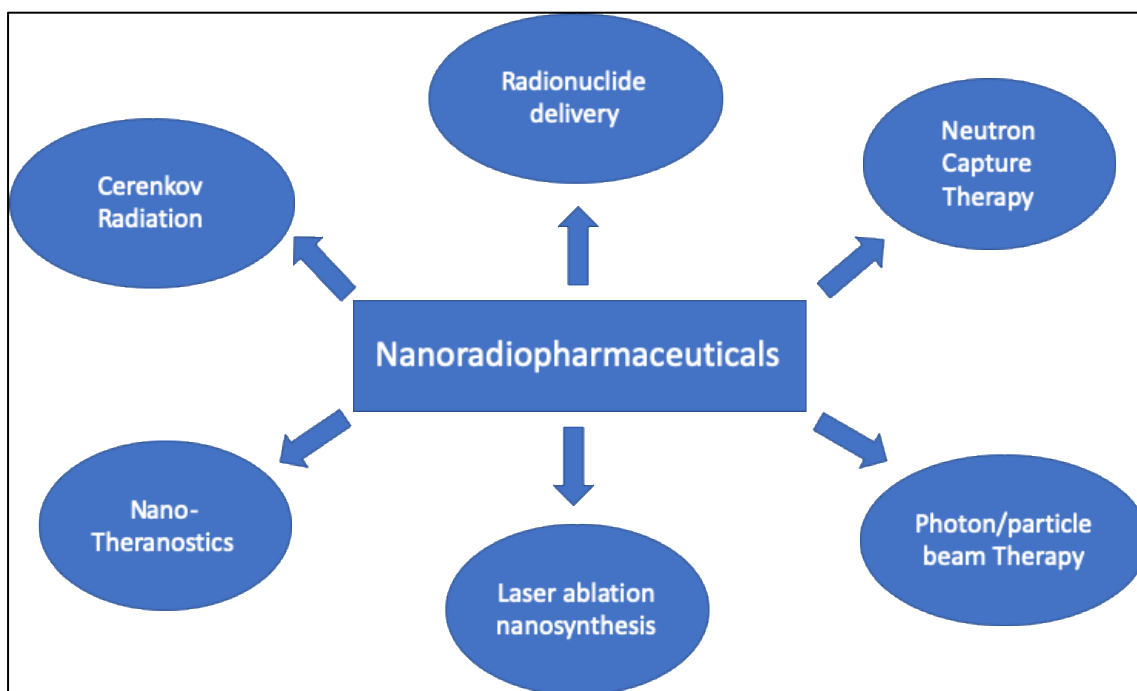


Figure 1: The scope of nanoradiopharmaceuticals covered in this review

2. Conventional radionuclide delivery systems

Currently, there are more than 80 known radionuclides used to produce radiopharmaceuticals in more than 75 nuclear reactors and 200 cyclotrons operated worldwide. Most of them are produced in research reactors.²⁴ Accelerators offer several advantages over nuclear reactors for medical isotope production. “Baby cyclotrons” with energy $E \leq 20$ MeV are convenient for direct placement in medical centers, where they can readily be operated on site for production of ultrashort-lived radionuclides for PET diagnostics.²⁵ Another emerging technology utilizes isotope generators having much smaller sizes. The use of generators permits multiple injections of nuclides in patients to produce long term cumulative effects.²⁶

The requirements for a diagnostic or therapeutic radionuclide include both physical and biochemical characteristics.^{27,28} The physical characteristics of the radionuclides include their half-life, decay mode, and the emission properties.²⁹ Biochemical characteristics include *in vivo* stability and toxicity of radionuclides. Additional biomedical factors to be considered are the type and size of the targeted cancer, density of the target and its heterogeneity.

The type of emission of a specific radiopharmaceutical gives its diagnostic and/or therapeutic character.²¹ The major emissive species and their tissue penetration range are

depicted in Figure 2. The emissions of α , β^- and Auger electrons have variable energy, penetrating range, and linear energy transfer (LET). The α particles can cause irreversible damage to DNA within a core cross-section of 2-3 tracks, whereas the β^- particles require nearly a hundred tracks to cause similar damage.³⁰⁻³² High- and low-energy (between 0.1 and 2.2 MeV) β^- emitters are ideal radioisotopes for the treatment of small to large clusters of tumor cells.³³ The high tissue penetration range (1–10mm) of β^- particles allows them to be used for relatively large tumors. The tissue penetration range of α emitters is about 50–100 μm ; thus α emitters hold great promise as therapeutics for small cancer lesions and micrometastatic cancers. Auger electrons have an energy of <30 keV and very small subcellular path lengths of 2 nm–12 μm .³⁴ Radionuclides that emit Auger electrons are usually effective only when their carrier molecules can penetrate the cell membrane to act intracellularly.³⁴ The ultra-selective localized irradiation of DNA in the cell nucleus through Auger electron-carrying metabolic substrates offers an extremely effective strategy for inducing cell death and breaking resistance to more conventional types of chemo-radiation therapies.³⁵

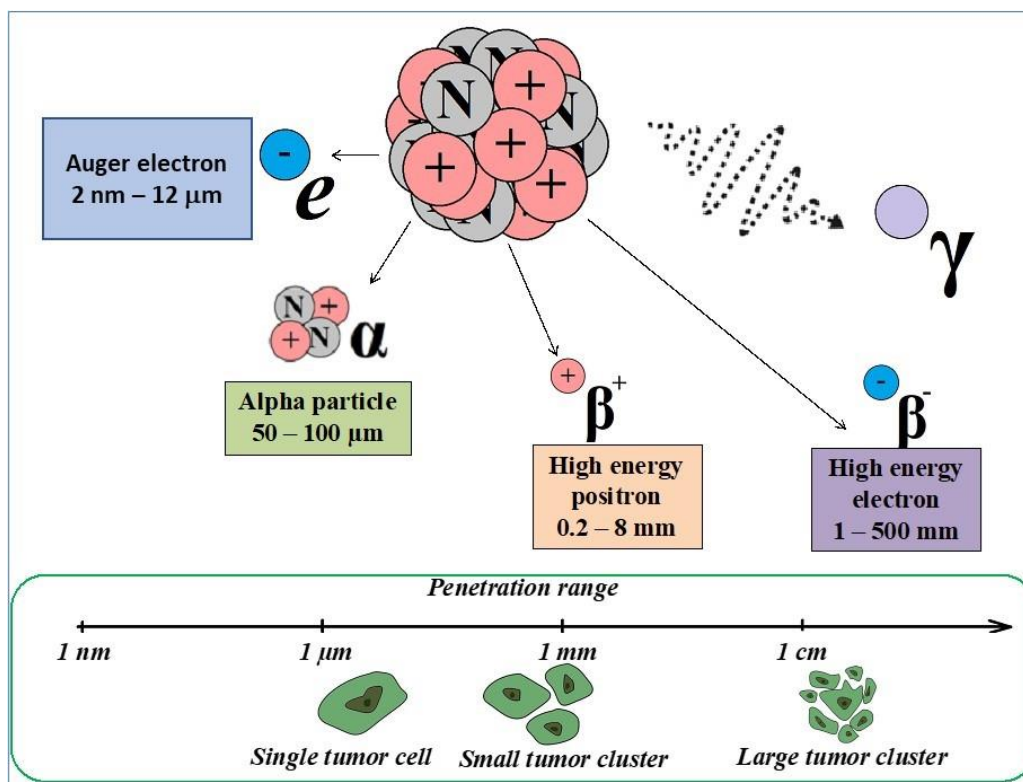


Figure 2: The major emissive species of radionuclides and their penetration range. β^+ and γ -emitters are the ideal radionuclides to produce *in vivo* imaging. Therapeutic radioisotopes emit α or β^- particles, or Auger electrons. These radiations cause cytotoxic DNA damage through numerous mechanisms,

such as generation of reactive oxygen species, single and double stranded DNA breaks, and inhibition of DNA repair mechanisms.³⁰⁻³⁵

A snapshot of the various biomedical applications of radionuclides in diagnostics, therapeutics and theranostics is provided in Figure 3. Gamma-emitters with an energy range between 100 and 370 keV and half-lives of a few minutes to several days are the ideal radionuclides for SPECT imaging.³⁶ Ultrashort-lived high-energy β^+ emitters with half-lives from a few seconds to several hours and annihilation energy of 511 keV are used for PET imaging.¹⁷ β^- emitters account for the largest share of the global therapeutic radionuclide market. The main demand is for preparations based on iodine-131 (^{131}I), samarium-153 (^{153}Sm), rhenium-186 (^{186}Re), yttrium-90 (^{90}Y) and lutetium-177 (^{177}Lu).³⁷ In 90% of cases of targeted radionuclide therapy in the clinic, ^{131}I and ^{90}Y isotopes are used.³⁸ The ^{188}Re radionuclide is one of most promising generator-type therapeutic β^- emitters, with the energy of β^- emission of 1.96 MeV (25.6%) and 2.12 MeV (71.1%), and a half-life of 17 hours.³⁹ In clinical practice, radiopharmaceuticals based on ^{188}Re are used for the treatment of prostate and breast cancers. With the development of radiopharmaceuticals based on the generator-type ^{188}Re , a rapid expansion of its usage can be expected. Recently, several α -emitters (^{225}Ac , ^{213}Bi , ^{223}Ra , etc.) have emerged as promising radionuclides for targeted therapy.⁴⁰ For example, ^{223}Ra is used for the treatment of bone cancer (osteosarcoma).

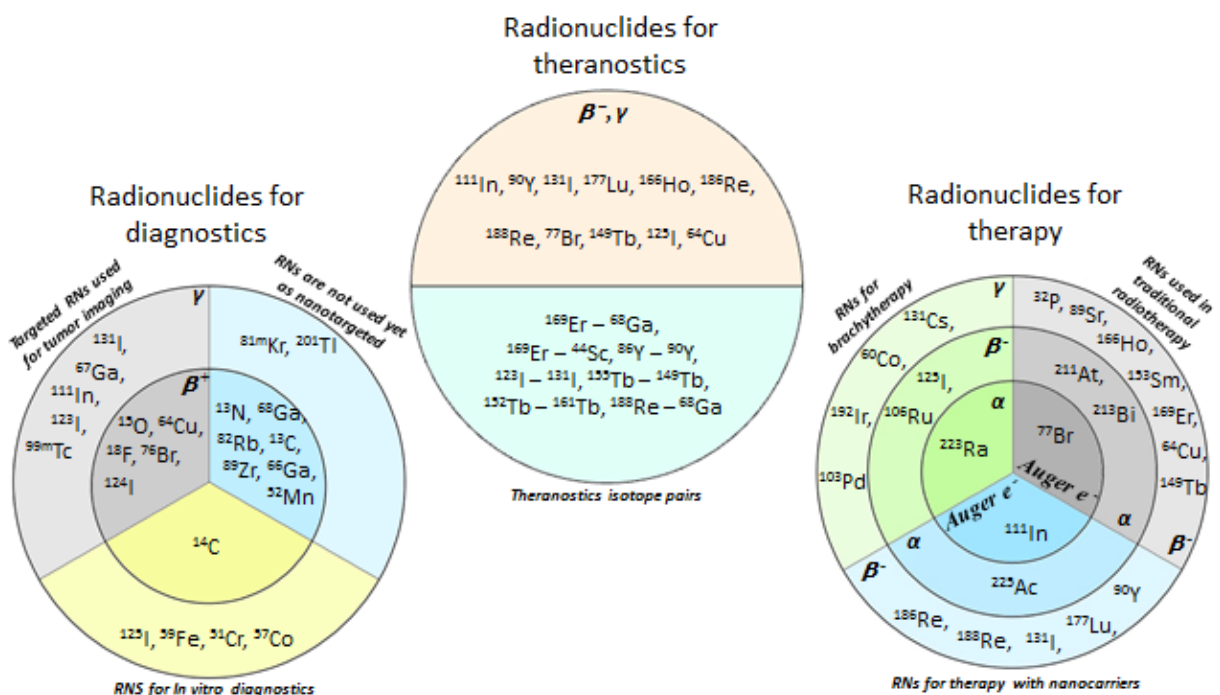


Figure 3: Snapshot of various biomedical applications of radionuclides. Radionuclides for nuclear imaging/diagnostics.^{21,22,36,41-46} The grey color stands for the targeted radionuclides used for tumor imaging, while the blue color stands for the radionuclides used in PET and SPECT diagnostics, but are not used yet as nanotargeted radionuclides; yellow – *in vitro* diagnostics. Radionuclides for therapy:⁴⁷⁻⁵⁸ *Blue color stands for the radionuclides used in therapy with nanocarriers, grey color represents radionuclides used in traditional radiotherapy, and green color denotes brachytherapy radionuclides. Radionuclides for theranostics either use radionuclides that exhibit diagnostic and therapeutic species (examples are highlighted by Orange color), or combine diagnostic and therapeutic radionuclides in one construct (some examples are highlighted by blue).^{37, 59-65}

More than 80% of medical radionuclides produced are used for *imaging-based diagnostics*. SPECT with gamma detection and PET based on positron (β^+) detection are used to produce *in vivo* images.⁶⁶ PET offers high spatial resolution, sensitivity, and accurate signal quantification with the potential for providing accurate metabolic information about tumors.¹⁷ The image resolution and sensitivity advantages of PET are crucial, especially in the case of small lesions.

Radionuclides for therapy must cause significant damage to the tumor cell during radioactive decay or radiation exposure. The half-life should be longer than the time required for preparation of the radiopharmaceutical and its introduction and localization in the tumor, but not significantly larger than that. The ideal half-life range for isotopes used in radionuclide therapy is typically from 6 hours to 7 days.⁶⁷ The isotopes must be produced with utmost chemical purity and be free of trace impurities of accompanying elements and metals and must be stable, both in short-term storage and in contact with biological fluids.

Theranostics achieved by using radionuclides that emit both diagnostic and therapeutic species mainly comprises of radionuclides that in addition to generating therapeutic β^- or α particles, also emit γ radiation for imaging. Gamma radiation should be of low energy (the optimal value is 140 Kev) in order to ensure efficient operation of the gamma camera and minimize negative effects on normal tissues.³⁷ Alternatively, theranostics can be achieved by combining separate diagnostic and therapeutic radionuclides. The examples of promising theranostic radionuclides and theranostics pairs for possible personalized medicine are presented in Fig 3.

Conventionally, target specificity has been attained by tagging radionuclides with antibodies, antibody-fragments, smaller peptides, carbohydrates and other macromolecular biorecognition agents. Monoclonal antibodies (mAbs), many already approved by FDA, are used in diagnostics and therapy due to their distinct pharmacokinetics, such as highly specific binding to the antigens and easy clearance because of relatively small size.⁶⁸ The mAbs can specifically bind target antigens without further modifications. Significant progress has been made in the application of mAb in radioimmuno therapy (RIT).⁶⁹⁻⁷¹

However, antibodies due to their relatively large size have limited penetration in both normal tissues and solid tumors,⁷² and exhibit slow pharmacokinetics as a result of which a major part of the radionuclide decays before the radiopharmaceutical is localized at the intended tumor site. Moreover, the long circulatory half-life of a blood-borne radiolabeled antibody causes prolonged high background activity levels, leading to unwanted non-specific normal organ exposure to radiation. This limitation can be overcome by using antibody fragments. However, due to the ultrasmall size of antibody fragments, it is not always possible to perform effective labeling with radiometals, since the pharmacokinetics of such small fragments is affected by incorporation of chelators.

In contrast, peptides used for tumor targeting are small and show rapid diffusion into (target) tissues resulting in rapid pharmacokinetics, while their fast blood clearance could lead to high tumor-to-background ratios, shortly after administration of the radiopeptide. Another promising targeting approach uses scaffold proteins, which are much smaller and can offer better affinity, low immunogenicity, excellent solubility, and thermodynamic stability.^{73,74} The use of aptamers, which are synthetic, single-stranded oligomers containing ribonucleotides (RNA aptamers) or deoxyribonucleotides (DNA aptamers), presents another promising targeting strategy because of their small size, stability, ease of production, non-immunogenicity, *etc.*⁷⁵

The tumor accumulation of molecular radionuclides with poor pharmacokinetics can be substantially improved when combined with pre-targeted nanoparticles. Herein, nanoparticles modified with specific functionalities are initially administered in tumor-bearing mice, and allowed to accumulate at tumor sites with high precision owing to their inherent target specificity. This is followed by treatment with radiolabelled complementary molecules having avidity to the pre-targeted nanoparticles, which thus home-in at the

nanoparticle-infiltrated tumor sites. In one example, Steen *et al.* pre-targeted CT-26 tumors using trans-cyclooctene (TCO) functionalized nanoparticulate polypeptide-graft-polypeptoid polymers (PeptoBrushes). This was followed up by a second injection of an ^{111}In -labeled tetrazine-based imaging agent with high complementarity to the TCO-modified PeptoBrushes. The resulting *in vivo* imaging data showed clear tumor visualization with high tumor-to-background ratio, which is not possible in case of administration with imaging agent alone (without nanoparticle-based pre-targeting) [<https://doi.org/10.1021/acsnano.9b06905>].

3. Nanocarriers for radionuclides

Nanoparticles have revolutionized the field of medicine owing to several advantages over molecular drug carriers.¹²⁻¹⁴ Their high surface-to-volume ratio, as well as well-defined porosity in some cases, allows high radionuclide loading and thus labelling. They can be synthesized with suitable physico-chemical parameters, including uniform size and shape, which ensures a good transport and pharmacokinetics *in vivo*. Rich surface chemistry of nanoparticles allows them to be properly functionalized (*e.g.*, by polymers such as PEG) to avoid immediate recognition by the immune system as well as to attach targeting moiety to ensure their delivery inside tumor cells, or even within desired subcellular compartments. Moreover, nanoparticle-based formulations show passive tumor-targeting as a result of the EPR effect.^{13,14,76} A biorecognition agent tagged with the nanoparticles provides active targeting to further enable their internalization inside tumor cells, as well as within desired subcellular compartments.^{13,14} Finally, based on their intrinsic properties, certain nanoparticles can provide *per se* additional imaging (*e.g.*, fluorescence, MRI, photoacoustic, *etc.*) or therapeutic (*e.g.*, photothermal, magnetothermal, PDT, *etc.*) functionalities, which could be enabled in parallel with the nuclear medicine modality.^{13,14}

Nanoparticles for biomedical applications were originally developed as simple drug carriers for disease-directed delivery of large dosages of pharmaceuticals. Initially, nanoparticles were tagged with radionuclides for studying their pharmacokinetics, pharmacodynamics, biodistribution, and *in vivo* excretion using non-invasive imaging modalities such as planar gamma scintigraphy, SPECT, and PET. However, over the years, nanoparticulate-carriers of radionuclides have rapidly emerged as exciting radiopharmaceuticals with vastly expanded scope and applications, including targeted,

image-guided therapy and theranostics.¹⁹⁻²⁴ Radionuclides may either be a major constituent of the nanoparticle, or 'encapsulated' within the nanoparticles, or incorporated on their surface by direct conjugation or *via* chelation with a conjugated chelating agent.⁷⁷⁻⁷⁹ Nanoparticle-containing radionuclides can target tumors by both passive and active targeting approaches (Figure 4). Described below are only selected examples of nanoparticles used as radionuclide carriers with their distinct merits.

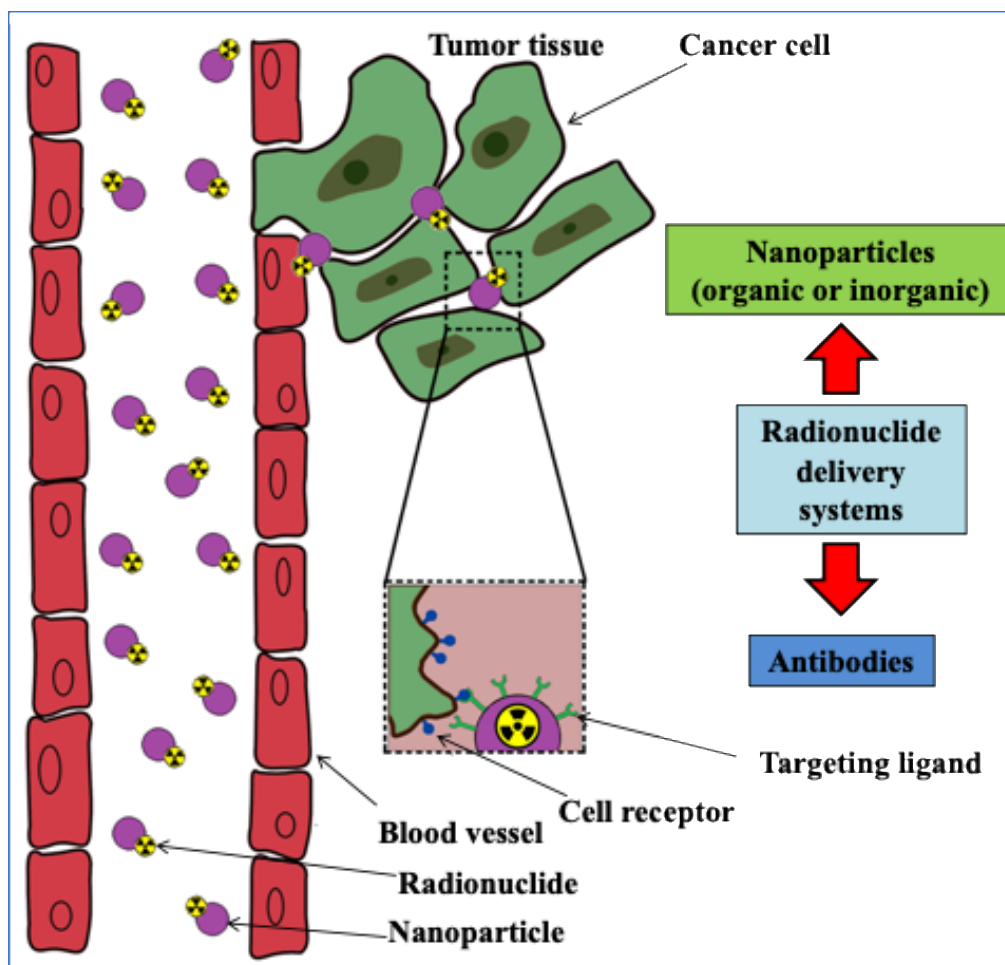


Figure 4: Passive and active targeting of radiopharmaceuticals

'Soft' or 'organic' nanocarriers:

'Soft' nanoparticles are composed mainly of lipids, polymers, proteins, and their combinations. Lipid-based nanocarriers primarily involve liposomes and solid-lipid nanoparticles (SLNs), while polymeric nanocarriers are either composed of natural polymers such as chitosan, or artificial ones such as polyesters, polycaprolactones, PLGA, *etc.* Dendrimers are a special type of hyperbranched artificial polymers which can be prepared

with exquisite size control. Albumins are the most extensively used protein-based nanoparticles in biomedical applications. We shall briefly discuss some of such nanocarriers from the perspective of development of nanoradiopharmaceuticals.

Liposomes are the earliest and most well-known carriers of active agents, owing to their structural resemblance to biological membranes.⁸⁰ They have a hydrophilic interior and hydrophobic bi-layered membrane, which can host both hydrophilic and hydrophobic drugs. They can incorporate radionuclides in their interior 'core', or in their membrane bilayer, or conjugated to their external surface, as depicted in Figure 5.

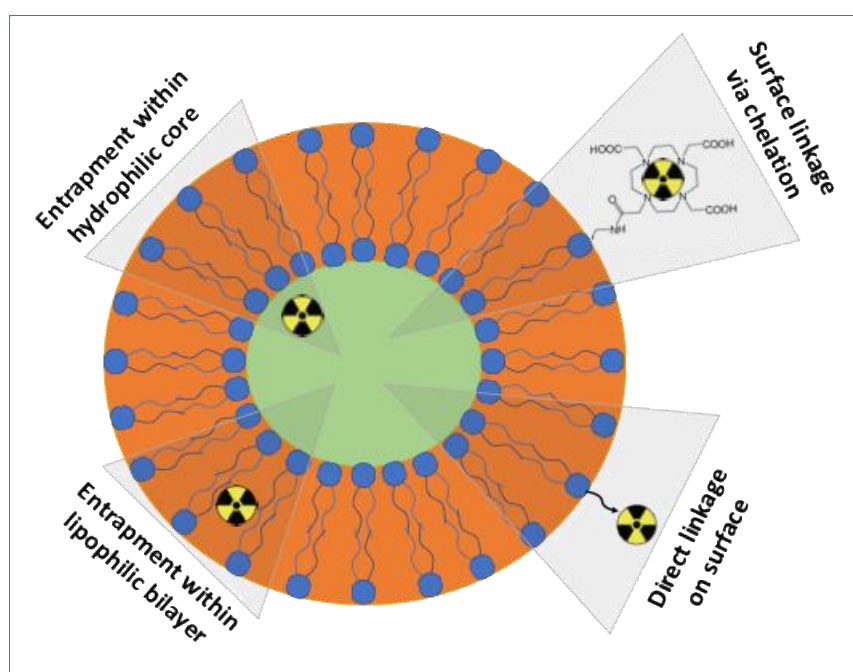


Figure 5: Different strategies for radionuclide incorporation with Liposomal nanocarriers. Liposomes have a hydrophilic interior and hydrophobic bi-layered membrane, which can incorporate radionuclides in their interior 'core', or in their membrane bilayer, or conjugated to their external surface

Radionuclides are mainly incorporated within liposomes *via* complexation with chelators, such as dodecane tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-triacetic acid (NOTA), conjugated on the liposome surface.⁸¹ This surface chelation approach, despite yielding high loading (up to 90%), results in poor *in vivo* stability. The stability of chelated radionuclides can be enhanced, when they are either encapsulated within the hydrophilic

core or entrapped in the lipophilic bilayer of liposomes. Lipophilic chelators such as BMEDA and HMPAO are useful in the bilayer-encapsulation approach.⁸²

Simple radionuclide-containing liposomes have been used for tracking the biodistribution, pharmacokinetics, targeting, and compartmental modeling. SPECT/CT imaging have been used for studying the *in vivo* fate and targeting using liposomes labelled with ^{99m}Tc, ¹¹¹In, ¹⁸⁸Re, *etc.*⁸³⁻⁸⁶ PET imaging was demonstrated using liposomes labelled with ⁶⁴Cu, ¹²⁴I, ¹⁸F, and ⁸⁹Zr.⁸⁷⁻⁹⁰ In a recent demonstration, cyclic RGDfK conjugated liposomes, radiolabelled with ¹¹¹In, were used for targeted delivery and efficient *in vivo* SPECT imaging of α V β 3 integrin expressing human melanoma tumors (Figure 6).⁹¹ In yet another recent development, tumor-targeted delivery of an EGFR-tagged and ¹¹¹In-labelled liposomal formulation was found to be substantially enhanced using ultrasound-induced cavitation following co-delivery of microbubbles.⁹²

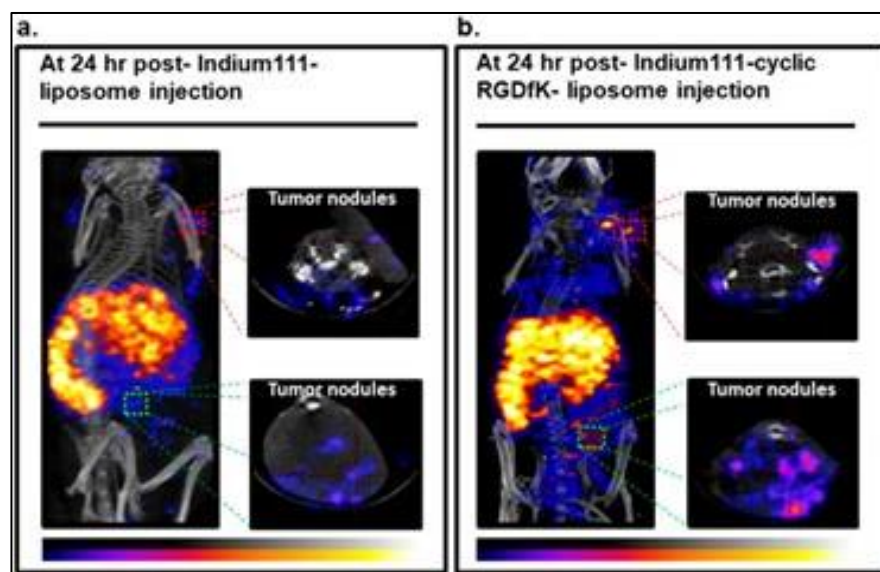


Figure 6: Non-invasive SPECT/CT imaging of nude mice with human melanoma tumors, injected with the (a) non-targeted ¹¹¹In-liposomes, and (b) targeted ¹¹¹In-labeled-cyclic RGDfK-liposomes.

Adapted from Reference [91].

The β^- emitting radionuclide ¹⁸⁸Re has been incorporated with PEGylated liposomes for radiotherapeutic applications. A recently concluded multicenter phase-0 low-radioactivity clinical trial of the ¹⁸⁸Re-liposome in patients with metastatic cancer found that favorable

tumor accumulation and clinically acceptable pharmacokinetics, biodistribution, and dosimetry results could be achieved.⁹³

Solid lipid nanoparticles (SLNs), on the other hand, are mainly composed of lipids (*e.g.* triglycerides), which are solids at room temperature.⁹⁴ Videira *et al.* demonstrated that SLNs labelled with ^{99m}Tc can be used as efficient probes for lymphoscintigraphy in adult male Wistar rats following delivery *via* the inhalation route.⁹⁵ In an example of active-targeted delivery using SLNs, Ucar *et al.* surface-modified paclitaxel loaded with a folate derivative and ^{99m}Tc tricarbonyl complex. *In vivo* studies conducted on female Wistar albino rats revealed a higher uptake of the targeted SLNs in folate receptor positive organs using gamma scintigraphy.⁹⁶ Andreaozzi *et al.* showed that intravenous injection of ⁶⁴Cu labelled SLNs in mice facilitates their biodistribution analysis using PET imaging.⁹⁷

Polymeric nanoparticles include nanoparticles of natural polymers (*e.g.* chitosan), synthetic polymers (*e.g.* PLGA, PLA, polycaprolactone), hyperbranched polymers (*e.g.* dendrimers), polymeric micelles, *etc.* Such polymeric nanoparticles have also been labelled/loaded with radiopharmaceuticals for imaging and theranostic purposes.⁹⁸ These nanocarriers, owing to their synthetic diversity, are ideal systems for developing multifunctional, theranostic nanoplatforms. Sun *et al.* have synthesized a dual therapeutic paclitaxel-encapsulated triblock-copolymeric nanocarrier (diameter about 100 nm), containing farnesyl thiosalicylate (FTS), which inhibits the cancer causing Ras oncogene as the hydrophobic block, poly-oligo(ethylene glycol) methacrylate (POEG) as hydrophilic block, and poly-4-vinylbenzyl azide (PVBA) as middle block. Furthermore, an attached PET imaging probe (⁸⁹Zr or ⁶⁴Cu) showed rapid tumor uptake and slow clearance of this nanoformulation following their intravenous administration in 4T1.2 tumor bearing mice (Figure 7) and revealed effective inhibition of tumor growth.⁹⁹

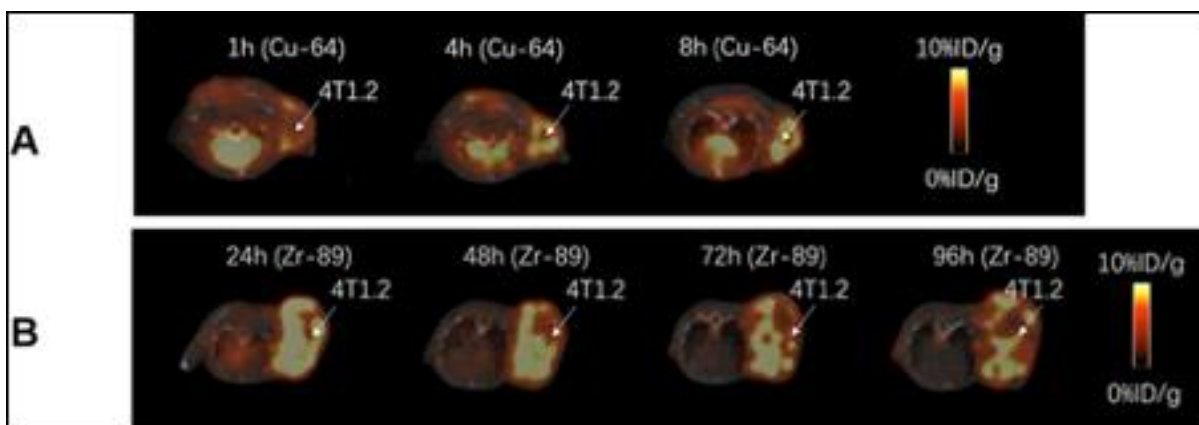


Figure 7: Real-time PET imaging showing time-dependent tumor uptake of radiolabeled PTX/POVF nanomicelles in a mice bearing 4T1.2 tumors. Imaging of (A) early time points (1-8 hours) post injection using Cu-64 labeled nanocarriers, and (B) late time points (24 – 96 hours) using ^{89}Zr labeled nanocarriers. Adapted from reference [99]. Printed with permission from ‘Elsevier’.

Recently, poly(ethylene glycol)-poly(lactic acid) (PEG-PLA) nanoparticles, co-incorporating the chemotherapy drug 5-fluorouracil (5FU) and the therapeutic radionuclide ^{131}I , and functionalized with the tumor-targeting antibody Cetuximab, were fabricated by Wu *et al.*¹⁰⁰ Targeted delivery and combination therapy *via* induction of apoptosis were validated in colorectal cancer cells. In the xenograft mouse model, the targeted dual-therapeutic nanoparticles exhibited prolonged blood circulation and tumor accumulation, as compared to control, monotherapeutic formulations (containing only 5FU or ^{131}I). Combined radio-chemotherapy *in vivo* was also demonstrated with the dual-therapeutic polymeric nanoformulation, as evidenced by tumor-size reduction, along with histological and immunohistochemical analyses.

In yet another recent development, a star copolymer (or polymeric nanostar), with 7-8 centre-cross-linked arms, was synthesized. The multiple arms of the nanostar were modified with several functionalities (Gd^{3+} for MRI, ^{89}Zr for PET imaging, or ^{177}Lu for endoradiotherapy) to fabricate a multifunctional theranostic radiolabelled nanoprobe.¹⁰¹ These multifunctional polymeric nanostars could demonstrate exceptionally high passive targeting to tumors *in vivo*, as confirmed by quantitative MR and PET imaging. Survival studies in tumor-bearing mice showed significantly high survival in mice treated with ^{177}Lu -labelled star polymers, when compared to that in untreated mice.

Albumin nanoparticles loaded with ^{99m}Tc have been used for SPECT/CT imaging applications, such as imaging of tumors,¹⁰² sentinel lymph node,¹⁰³ arterial dispersal,¹⁰⁴ pulmonary delivery,¹⁰⁵ liver functions,¹⁰⁶ *etc.* Ji and coworkers have prepared a tumor-targeted formulation of doxorubicin-loaded and ^{131}I labelled BSA nanoparticles.¹⁰⁷ Recently, Zhang and coworkers developed a hyperbranched semiconducting polymer based theranostic nanoformulation, co-incorporating the radioisotope ^{99m}Tc and the drug methotrexate, for targeted imaging and therapy of rheumatoid arthritis. *In vivo* SPECT/CT imaging demonstrated that upon intravenous injection in a mice model of rheumatoid arthritis, the nanoformulation could navigate across the blood-joint barrier and accumulate in inflamed joints. Furthermore, methotrexate was specifically released at the inflammation site, thus enhancing the therapeutic outcome and reducing off-target toxic effects.¹⁰⁸

The application of smart nanoparticulate systems is a rapidly-emerging dimension for advanced medical imaging and therapy [DOI: [10.1002/aisy.202000087](https://doi.org/10.1002/aisy.202000087)]. For example, the target specific retention of radionuclides can be enhanced using smart nanosystems that involves the formation of self-assembled nanoparticles from smaller, radiolabelled building blocks, in response to specific stimuli that are inherent to the target tissues. Such site-specific enhanced retention of radionuclides leads to substantially improved outcome of nuclear imaging and/or therapy. In a report, Lin *et al.* have synthesized a ^{18}F -labelled low-molecular weight probe that forms hydrophobic dimers in a reducing microenvironment, which in physiological conditions further self-assemble into bigger nanoparticles *via* hydrophobic interactions. Upon administration in mice, *in vivo* PET imaging demonstrated higher signals in only those organs (tumor, liver and kidneys) that contain high levels of the reducing antioxidant glutathione (GSH) [DOI: <https://doi.org/10.1039/C7CC03040A>]. The researchers have further demonstrated the use of a similar strategy for triggering nanoparticulate formation and thus higher retention in drug-treated tumors having enhanced expression of the apoptosis marker caspase-3 [Org. Biomol. Chem., 2020,18, 3512-3521]. Such smart nanosystems can be used for several other applications, such as enhanced penetration in target tissues, modulation of tissue-microenvironment for improved outcome, *etc.*

‘Hard’ or ‘inorganic’ nanocarriers

'Hard' nanoparticles are mainly composed of inorganic components, which have some advantages over their organic counterparts. Specialized synthetic methods (*e.g.* hot-colloidal synthesis, laser-ablation) allow precise control of their physical parameters, such as size, shape, porosity, and crystallinity, which in turn facilitates their reproducible *in vivo* behavior, such as prolonged circulation, desirable biodistribution, targeted delivery, facile excretion, *etc.*^{109,110} From the point of view of radionuclide delivery, their size characteristics make possible a time-delayed clearance to exclude the accumulation of radionuclides in kidneys at their early highly radioactive stage, which could have otherwise caused a series of renal damages.¹² Several such nanoparticles are known to have special physical properties at the nanoscale, such as plasmonic properties of noble metal nanoparticles allowing for tandem photothermal therapy, superparamagnetic property of iron-oxide nanoparticles as MRI contrast agents, *etc.*¹¹⁰⁻¹¹² Most of these nanoparticles are known to be non-immunogenic and biocompatible.

Gold nanoparticles, owing to their plasmonic properties, are used as probes for optical imaging, photoacoustic imaging, photothermal therapy, *etc.*^{13,14} Combining radiopharmaceuticals with gold nanoparticles facilitates the fabrication of multifunctional and theranostic nanoparticles. ⁶⁴Cu labelled gold nanoparticles have been used for dual optical and PET imaging in prostate cancer.¹¹³ Gold nanoparticles labelled with ¹³¹I have been used for targeted tumor SPECT/CT imaging and radionuclide therapy of MMP-2 overexpressing tumors,¹¹⁴ and EGFR-expressing lung tumors.¹¹⁵ ¹⁷⁷Lu labelled gold nanoparticles, conjugated with the cyclic RGD peptide, were used to effectively target and suppress $\alpha\beta3$ -integrin-positive C6 gliomas in athymic mice, with no discernible sign of systemic or organ toxicity.¹¹⁶

Magnetic nanoparticles have magnetic properties that make them attractive probes for magnetic resonance imaging (MRI), magnetic hyperthermia therapy (MHT), magnetic targeting, *etc.*^{13,14} Radiolabelling of magnetic nanoparticles enables a combination of imaging and therapeutic potential, which includes dual modality imaging (MRI+PET, MRI+SPECT), dual therapy (MHT+radiotherapy), and theranostics (MRI+radiotherapy, MHT+PET, MHT+SPECT), *etc.*¹¹² Madru *et al.* prepared PEG coated iron oxide nanoparticles labelled with ^{99m}Tc for combined MR and SPECT imaging of sentinel lymph node and biodistribution in Wistar rats.¹¹⁷ The core size and shell compositions of radiolabelled core-shell nanoparticles containing

magnetite (Fe_3O_4) or cobalt ferrite (CoFe_2O_4) nanocores hybridized with polymeric shells were found to dictate their radiolabeling ability and *in vivo* fate.¹¹⁸ Magnetic melanin nanoparticles (MMNs) labelled with ^{64}Cu were developed as a multimodal, versatile, biomimetic theranostic system for combined PET, photoacoustic (PA) and MR imaging *in vivo* in mouse bearing U87MG tumors [Figure 8], along with image-guided cancer photothermal therapy.¹¹⁹ In a recent demonstration, Ince *et al.* combined magnetic nanoparticles with ^{131}I and the drug Thymoquinone for multimodal theranostic application in lung cancer *in vivo*. While the radioisotope was used for SPECT imaging and radionuclide therapy, the magnetic nanoparticles provided MR imaging and magnetic hyperthermia therapy. In a xenograft model of lung cancer, significant reduction in tumor volume was observed owing to combination therapy, as observed using *in vivo* imaging. Complete tumor elimination could be observed after two weeks of treatment with the nanoformulation.¹²⁰

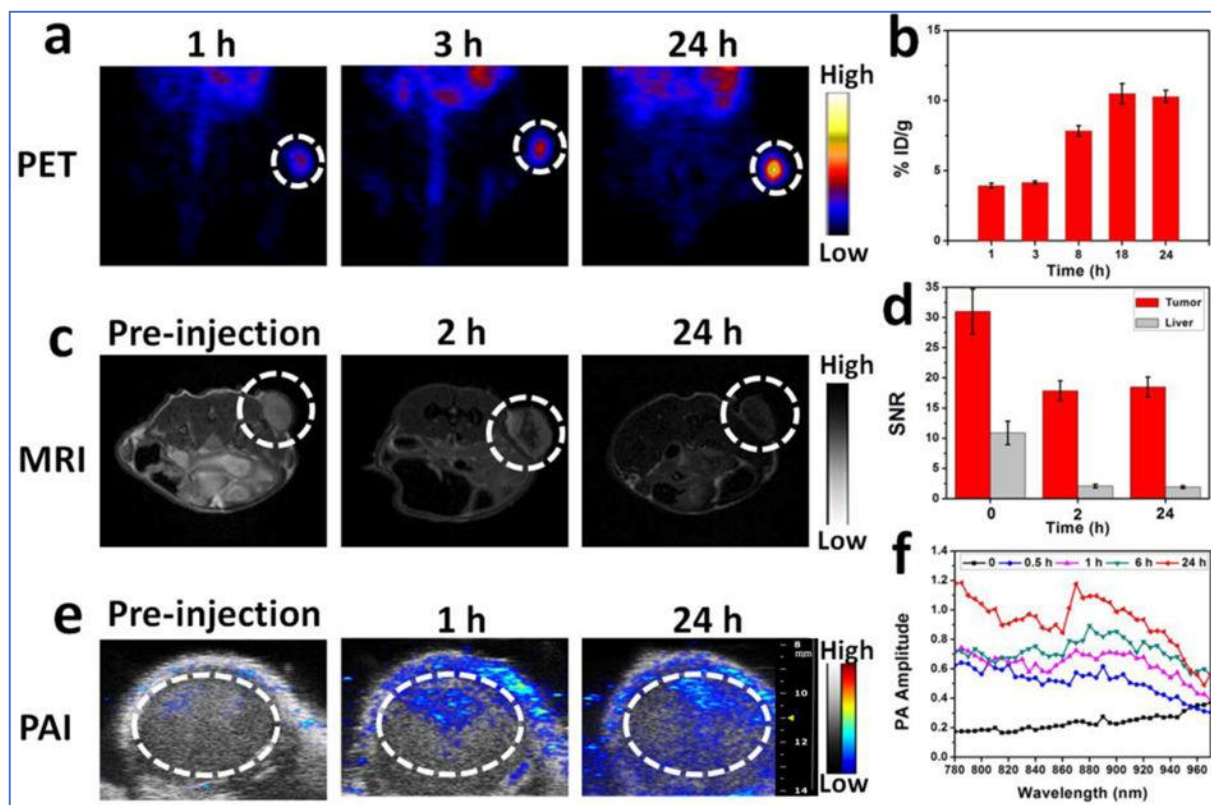


Figure 8: *In vivo* multi-modality PET, MR and PA imaging, in U87MG tumor-bearing mice intravenously injected with ^{64}Cu -MMNs. (a) PET images at different times post-injection, with tumors indicated by white dotted circles. (b) Tumor uptake of ^{64}Cu -MMNs, presented as %ID/g. (c) MR images pre- and post-injection, with tumors indicated by white dotted circles. (d) Quantitative MR signal from U87MG

tumor and liver, pre- and post-injection of ^{64}Cu -MMNs. (e) PA images of pre- and post-injection, with tumors indicated by white dotted circles. (f) PA spectra of U87MG tumor pre- and post-injection of ^{64}Cu -MMNs. Adapted from reference [119].

Carbon-based nanoparticles such as single-wall carbon nanotubes (CNTs) radiolabelled with ^{125}I were used for bio distribution studies in mice using SPECT imaging.¹²¹ In an early report of CNTs for radiotherapy, ultrashort carbon nanotubes (US-tubes, length 20–50 nm) were loaded with the α -emitting radionuclide, ^{211}At , as a mixed halogen ($^{211}\text{AtCl@US-tube}$) for targeted radiotherapy.¹²² Ruggiero *et al.* incorporated radionuclides such as the α -emitter ^{225}Ac or the PET tracer ^{89}Zr with single walled CNTs *via* chelation. These radiolabeled CNTs, tagged with the tumor neovascular-targeting antibody E4G10, were used for both targeted radioimmunotherapy (using ^{225}Ac) and PET imaging (using ^{89}Zr) in a murine xenograft model of human colon adenocarcinoma (LS174T).¹²³ Nanographene oxide (NGO) was radiolabeled with ^{111}In and conjugated with anti-HER2 antibody (trastuzumab) for targeted SPECT imaging of breast cancer, with better pharmacokinetics and clearance from circulation, as compared to radiolabeled antibody only.¹²⁴

Silica-based nanoparticles, have been extensively used for drug delivery studies. We synthesized ^{124}I labelled-ormosil nanoparticles (diameter 20-25 nm), conjugated with a near infra-red (NIR) fluorophore, for biodistribution, clearance and toxicity analysis *in vivo*. Radioactivity counting from harvested organs, revealed higher nanoparticle accumulation in liver, spleen and stomach, than in kidney, heart and lungs, following their intravenous injection in healthy mice, with no indication of toxic side effects.¹²⁵ Miller *et al.* showed multiple incorporation of the positron emitting radionuclide ^{89}Zr within mesoporous silica nanoparticles (MSNs) for PET imaging with enhanced signal strength.¹²⁶ In a demonstration of dynamic multimodal imaging, Cheng *et al.* loaded isolated neural stem cells (NSCs) with ^{111}In -labelled MSNs, which were then administered intracranially as well as systemically in mice bearing orthotopically xenografted glioblastoma. Dynamic *in vivo* SPECT imaging allowed real-time tracking of NSC migration towards glioblastoma. Additionally, the radionuclide-decay was associated with strong fluorescence, which allowed concurrent optical imaging of NSC migration (Figure 9).¹²⁷ Hortelao *et al.* have reported another example of dynamic radioimaging using ^{124}I labelled nanomotors comprised of mesoporous silica-nanoparticles, co-loaded with the enzyme urease and gold nanoparticles. Following direct

administration into the bladder of mice, dynamic PET imaging was used to demonstrate the urea-powered swarming behavior of the radiolabelled nanomotors *in vivo* [doi: 10.1126/scirobotics.abd2823]. In a recent report involving theranostic silica-based nanoformulation, Rainone *et al.* demonstrated the targeted SPECT imaging and therapy of HER2 overexpressing breast cancer *in vivo* using trastuzumab half-chain conjugated, doxorubicin encapsulated and ^{99m}Tc radiolabelled silica nanoparticles. Analysis of treatment efficacy *in vivo* using PET imaging revealed significant reduction in tumor volume using the theranostic silica-based nanoformulation, with higher efficacy than that shown by liposomal Doxorubicin (Caelyx).¹²⁸

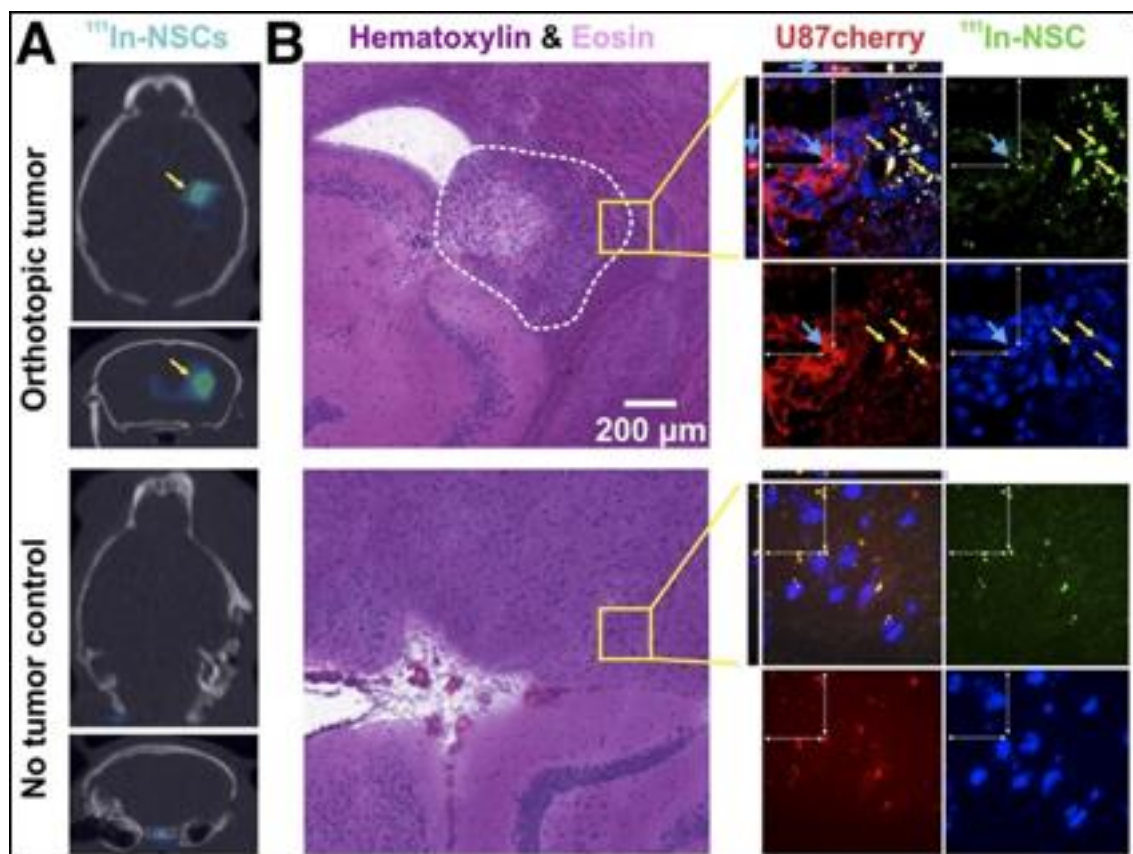


Figure 9: Tracking of NSCs in mouse brain after systemic injection. (A) SPECT imaging of ^{111}In -MSN-NSCs after systemic injection in tumor-bearing (top) and control (bottom) animals. (B) Hematoxylin and eosin staining confirms orthotopic tumor growth (dashed white line) in experimental animals and normal structure of brain tissue in naïve control (lower). Confocal microscopy (yellow boxes) of adjacent tissue areas reveal strong homing behaviors of NSCs labeled with ^{111}In -MSN (green; yellow arrows) near border of tumor mass (red), with occasional indications of intratumoral penetration by

radiolabeled NSCs (blue arrow; orthogonal views). In contrast, naïve control tissue shows only sporadic residual SPECT signal of nonmigratory NSCs. Adapted from reference [127].

Upconversion nanophosphors (UCNPs) are inorganic crystalline matrices such as NaYF_4 , doped with small amounts of specific rare-earth ions that allows the multiphoton non-linear optical phenomenon of energy upconversion, whereby absorption of lower energy (higher wavelength) radiation leads to higher energy (shorter wavelength) emission.^{13,129} UCNPs not only have emerged as attractive optical imaging probes spanning the visible and NIR regions, but also provide a platform for co-incorporation of additional probes, such as radionuclides, for multimodal image-guided therapy applications. Lee *et al.* have used a targeted, multimodal nanoformulation for imaging of tumor angiogenesis using $\text{Er}^{3+}/\text{Yb}^{3+}$ co-doped NaGdF_4 UCNPs radiolabeled with ¹²⁴I. The conjugated RGD peptide allowed tumor targeting in a mouse model bearing U87MG tumor xenografts, visualized using tri-modal imaging (small-animal PET, clinical 3-T T1-weighted MRI, and NIR optical imaging).¹³⁰ In an example of targeted theranostics, an UCNP nanoformulation radiolabelled with the beta-emitter ⁹⁰Y and tagged with the tumor-targeting protein DARPIn, modified with therapeutic exotoxin-A fragment, was fabricated. Synergetic therapy effect due to a combined action of the targeted toxin and radioisotope ⁹⁰Y was observed in mice bearing HER2-positive SK-BR-3 human xenograft tumors, which could also be observed non-invasively using NIR optical imaging.¹³¹

4. *In situ* activation via external trigger

One major shortcoming of radionuclide labelling of molecules or nanoparticles is the lack of external control on the radiation emission properties. This can lead to undesirable radiation damage to non-target regions, along with potentially long-term persistence of radioactivity in the body. To overcome this limitation, over the past few decades external activation of tumor-localized targets has been investigated. Here, external sources of ionizing radiations (*e.g.* x-rays, ion-beams) are focussed on the target tissues thereby providing better spatio-temporal control of therapy. Nanoparticles containing high atomic number elements (*e.g.* Hf, Bi, Gd, *etc.*) have been found to efficiently absorb x-rays and subsequently emit low-energy species such as Auger electrons, thereby, escalating the local radiation dose to produce localized therapy.^{6,132} In addition, nanoparticle can enhance nuclear particle irradiation therapies using charged particles (ion beam), as well as neutrons.^{2,3} These

nanoparticles, after being localized at target sites through carefully-crafted delivery methods *in vivo*, locally escalate the dosage of externally applied radiation and enhance site-specific damage. Such localized radiation dose-escalation promises to treat deep-seated lesions, with minimal collateral damage. Even though such therapies do not involve interaction with nuclear particles, we include these in this review for reasons already described in the introduction.

X-ray radiosensitization:

Radiation therapy (RT) involving externally applied x-rays is routinely used in clinics for the treatment of cancer and some other diseases. Since X-rays have unlimited penetration through soft-tissues, as a result of which a single beam will irradiate all tissues (normal or diseased) in its path, a series of low-intensity beams from various angles are converged on the target site for achieving better target specificity. A recent development in this area involves *in situ* generation of cytotoxic photoelectrons and/or Auger electrons upon sensitizing non-radioactive nanoparticles (radiosensitizers) with x-rays, which leads to higher localized dose-escalation and reduced requirement of administered dose.^{6,132} Radiosensitizers or radiation enhancers consist of primarily non-radioactive high atomic number (high Z) elements (metals), which have high x-ray absorption cross-section.¹³²

Gold nanoparticles are potent radiosensitizers owing to their high photoelectric absorption coefficient, along with optical properties for facilitating additional modalities. The effective dose enhancement of x-ray activated therapy in tumor-bearing mice treated with gold nanoparticles was first reported by Hainfeld *et al.*¹³³ Since then, several reports have emerged highlighting targeted radiation therapy enhancement, combination therapy, theranostics, *etc.*, in cultured cells and tumor-bearing animals treated with gold nanoparticles. A roadmap to clinical use of gold nanoparticles for radiation sensitization has been published by a number of groups now, underscoring the popularity of gold as a radiosensitizer and the need for meticulous documentation of the optimal operating parameters for maximal radiosensitization.¹³⁴ Antosh *et al.* prepared ultrasmall (1.4 nm) gold nanoparticles, conjugated with the tumor-targeting pH Low-Insertion Peptide (pHLIP). *In vivo* studies in tumor-bearing mice revealed efficient tumor targeted delivery of the gold-pHLIP nanosensitizers, and significant reduction in tumor volume at a low administered dosage (10 mg/kg) of the nanosensitizers exposed to 20 Gy of radiation. Biodistribution measurements

also demonstrated the enhanced tumor-retention of the targeted nanoparticles.¹³⁵ Recently, Ding *et al.* demonstrated advanced tumor targeting of ultrasmall gold nanoparticles (Au NPs) by conjugating a multifunctional peptide Tat-R-EK, consisting of the cell-penetrating peptide Tat, a tumor-microenvironment responsive cathepsin B cleavable linker, and a zwitterionic antifouling block. Once targeted within the tumor microenvironment, the locally overexpressed cathepsin B cleaves the peptide linker to expose the cell penetrating Tat peptide on the Au NP surface, leading to enhanced internalization within the tumor cells. This multi-targeted nanoformulation led to enhanced tumor cell uptake *in vitro* and *in vivo*, resulting in superior x-ray sensitized toxicity, along with rapid renal clearance in mice bearing orthotopic LM3 liver tumors.¹³⁶

In addition to acting as x-ray radioenhancers, gold-nanostructures also can carry additional therapeutic agents that can sensitize cancer cells to therapy. We have demonstrated that silencing of the sphingosine kinase (SphK1) gene using specific short-interfering RNA (siRNA) conjugated with gold nanorods can sensitize head-and-neck squamous cell carcinoma (HNSCC) cells to radiation therapy. Here, the GNRs perform the dual function of carrying the siRNA against SphK1 gene to target cells, and further radiosensitization upon x-ray exposure of the targeted cells. In a mouse model of subcutaneously implanted HNSCC cells, a significant reduction in tumor volume was observed upon sequential GNR-mediated SphK1 gene silencing and subsequent low-dose (1.0 Gy twice a week) x-ray irradiation.¹³⁷ Recently, Yang *et al.* showed that incorporation of an siRNA targeting the hypoxia-inducible factor-1 α (HIF-1 α) protein with zwitterionic polyamidoamine dendrimer-entrapped gold nanoparticles can lead to both endogenous and exogenous sensitization of radiation therapy in the treatment of both primary and metastatic tumors in mice.¹³⁸

Bismuth is another high-Z metal that has shown promise in CT contrast enhancement and radiosensitization. Researchers at Jiangsu University demonstrated tumor-targeted CT imaging and radiosensitization using hyaluronic acid (HA)-functionalized bismuth oxide nanoparticles (HA-Bi₂O₃ NPs).¹³⁹ Deng *et al.* synthesized biodegradable, red-blood cell (RBC) coated and folate-conjugated bismuth (F-RBC bismuth) nanoparticles. Favorable blood circulation and tumor-targeting efficiency were rendered by RBC and folate, respectively. In a mouse model of breast cancer, significant tumor suppression and improved survival ratio

were observed upon treatment with F-RBC bismuth nanoparticles and x-ray radiation.¹⁴⁰ We have recently shown that laser ablation can be used to produce stable pure elemental Bi nanoparticles which provide the highest local concentration of Bi atoms for significantly enhanced CT contrast and radiotherapy at a much lower x-ray dosage.¹⁴¹

Gadolinium oxide/fluoride nanoparticles can combine their MR contrast enhancement capability with radiosensitization potential for image-guided therapy. Rajaei *et al.* have combined the benefits of bismuth and gadolinium by synthesizing multifunctional, theranostic bismuth gadolinium oxide nanoparticles (BiGdO₃), for combined radiosensitized therapy, computed tomography (CT) and magnetic resonance imaging (MRI). *In vivo* studies using BALB/c mice bearing 4T1 breast tumors revealed potent radiosensitizing effect of the PEG-coated BiGdO₃ nanoparticles.¹⁴² Nanobiotix, Inc., France (<https://www.nanobiotix.com/>) has developed hafnium oxide nanoparticles with an average diameter of 50 nm (NBTXR3) as a potent radiosensitizer for locally advanced soft tissue sarcomas (STS).^{143,144}

Radiosensitization can also be induced by certain polymer-based nanoparticles, with the added provision of concurrent delivery of chemotherapeutic drugs. For example, Yin *et al.* have prepared amphiphilic block-copolymer consisting of poly(ethylene glycol)-block-poly(L-glutamic acid) (PEG-b-PLG) and the small-molecule radiosensitizer metronidazole (MN). The obtained MN-grafted PEG-b-PLG (PEG-b-P(LG-g-MN)) nanoassemblies were further loaded with the anticancer drug doxorubicin. Upon irradiation with electron beams, these polymeric nanoassemblies showed a high sensitization enhancement ratio (SER) of 2.18. Upon combination with doxorubicin delivery in mice bearing 4T1 tumors, robust chemoradiosensitized ablation of bulky hypoxic solid tumors (~ 500 mm³) could be obtained at a low radiation dose (4 Gy) [DOI: 10.1016/j.biomaterials.2018.08.014]. Synergistic chemo and radiosensitization for the treatment of cancer by combining activable prodrugs with radiation is an emerging concept. Recently, Geng *et al.* have demonstrated the site-specific uncaging of chemotherapy drugs or imaging probes *via* localized radiation treatment of corresponding azide-caged prodrugs. Such target-specific liberation of prodrugs with external radiosensitization provides potent combination of chemo- and radiation therapy at tumor sites, while avoiding chemotherapy-associated systemic toxicity [Nature Chemistry, 13, 805–810].

Ion beam particle therapy

Another significant development is the use of charged particles, such as protons, ions, *etc.* as irradiation sources. Unlike x-ray photons, which are uncharged, charged particles are characterized by a 'Bragg Peak', whereby the maximum radiation dose (peak) occurs at a particular depth which relies on the incident beam energy. Thus, by tuning the beam energy, the tissue depth of maximum dose can be made to coincide with the tumor volume, with reduced effect on the skin and normal tissues. High-Z metal nanoparticles can absorb ion beams and locally generate secondary electrons (from small angle scattering of outer electrons), and x-rays, leading to further dose enhancement. Therefore, ion beam therapy of solid tumor pre-loaded with nanoparticles benefits from the dual effects of (a) maximum dose deposition at the tumor volume ('Bragg Peak'), and (b) localized dose enhancement by nanoparticle-ion beam interaction.^{3,145}

Ever since Kim *et al.* reported radiation dose enhancement and cytotoxicity following bombardment of cells loaded with metallic (gold or iron) nanoparticles with 45 MeV protons (H^+) *in vitro*, several metal-based nanoparticles have emerged as potent radiosensitizers in proton therapy.¹⁴⁶ It is widely believed that proton irradiation leads to ejection of low-energy electrons from outer atomic orbitals which can produce DNA damage and oxidative stress for tumor destruction. Increased ROS production *in vitro* suggested local generation of electrons and x-rays as the plausible mechanism of the radiosensitized dose-enhancement.¹⁴⁷ In a recent development, Kang *et al.* have employed superparamagnetic iron oxide nanoparticles (SPIONs), conjugated with folate and paclitaxel, for targeted dual therapeutic (chemo- and proton therapy) application. The nanoparticles were targeted to folate-positive C6 brain cancer cells *in vitro*. Following proton beam irradiation, the highest radiosensitization enhancement factor was observed for the dual-therapeutic nanoparticles, as compared to other controls (paclitaxel only, SPION only). This data shows the possibility of combining other therapies with proton therapy using a single nanoparticle system.¹⁴⁸

In addition to experimental evidence, a number of simulation studies were also done to investigate the radiosensitization of proton beams using metallic nanoparticles. Ahmad *et al.* investigated the impact of proton energy and nanoparticle concentration on the radiosensitization behavior of gold, silver and platinum nanoparticles, using tissue phantom

experiments and *in silico* Monte Carlo simulations. Both observations revealed significant longitudinal shift (2.2-4.7 mm) of the distal edge of the Bragg peak with gold nanoparticles. Of the three metals, platinum caused the greatest concentration-dependent shift. At the same time, dose-enhancement (6-21%) with varying nanoparticle concentrations and proton energies were observed.¹⁴⁹

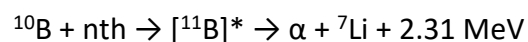
Carbon ion beam therapy is also gaining popularity in clinics for nano-radiosensitized therapies. Nanoparticle radiosensitization in this case also works on the ejection of electrons. By studying DNA damage, Lacombe and coworkers reported that platinum nanoparticles irradiated with carbon-ion beams facilitate potent DNA damage. Auto-amplified electronic cascades inside the nanoparticles were found to form a key mechanism in radiosensitization close to the nanoparticles.¹⁵⁰ Later on, Kaur *et al.* demonstrated that the cytotoxicity and relative biological effectiveness (RBE) values of carbon-ion irradiated human cervix cancer (HeLa) cells was enhanced when cells were pre-treated with glucose-capped gold nanoparticles. The radiation dose required for killing 90% of cells was reduced by 30% following nanoparticle treatment.¹⁵¹ Li and coworkers studied hydroxyl-ion production as a plausible mechanism for gold-nanoparticle-mediated radiosensitization of carbon ion therapy. Enhancement in hydroxyl radical production could be directly correlated with the increased lethality of gold nanoparticle-sensitized carbon-ions.^{152,153}

Porcel *et al.* have shown that gadolinium-chelating polysiloxane nanoparticles (AGiuX), which were already shown to sensitize photon beam (x-rays) therapy, can also sensitize carbon ion radiation therapy and amplify cell death. The excellent MRI contrast enhancement properties of chelated gadolinium also allows these nanoparticles to serve as theranostic agents for image-guided therapy.¹⁵⁴ Another gadolinium-based radiosensitized theranostic nanoformulation was reported by Li *et al.*, who demonstrated enhanced cytotoxic effect on non-small cell lung cancer (NSCLC) cells treated with gadolinium oxide nanocrystals and fast carbon ions. A concentration-dependent increase in the production of hydroxyl radicals and reactive oxygen species in treated cells was observed, leading to cell death *via* several mechanisms, such as DNA damage, cell cycle arrest, apoptosis, and cytostatic autophagy.¹⁵⁵

Neutron capture therapy

Neutron capture therapy (NCT), which is conceptually different from x-ray or ion-beam therapies, forms another prime example of externally-applied radiative dose-escalation therapeutic strategy. Here, a non-radioactive isotope is converted to a radioactive one following bombardment with epithermal neutrons (energy higher than thermal neutrons, *i.e.* 0.025eV) from either a nuclear reactor or an accelerator. The radioisotope then undergoes nuclear reaction by capturing the incident neutron in the nucleus to emit localized toxic radiation.² If this procedure is carried out around cancer cells, it leads to potent therapy of cancer by *in situ* action of the emitted radiation. So far, primarily compounds based on Boron-10 (¹⁰B) and Gadolinium-157 (¹⁵⁷Gd) have been used as the target isotopes for NCT owing to their high neutron capture cross sections (3,837 and 254,000 barns, respectively).¹⁵⁶ While ¹⁰B has been used in both experimental and clinical NCTs (termed as boron neutron capture therapy, or BNCT), ¹⁵⁷Gd has only been used in experimental applications.¹⁵⁷

The Boron-10 isotope which makes up approximately 20% of natural elemental boron undergoes the following instantaneous nuclear fission.^{2,156}:



Both the α particles and the lithium nuclei produce closely spaced ionizations in the immediate vicinity of the reaction, with a range of 5–9 μm , which is approximately the diameter of the target cell. The lethality of the capture reaction is limited to ¹⁰B-infiltrated cells. Therefore, for effective NCT, it is critical to deliver the ¹⁰B-containing probe inside the target cells, prior to irradiation with neutron beam (Figure 10A).¹⁵⁶

On the other hand, gamma rays, internal conversion and Auger electrons are products of the ¹⁵⁷Gd (n, γ)¹⁵⁸Gd capture reaction:¹⁵⁷



Here, the effective path length of the ionizing radiations is even shorter (in the range of 5-40 nm). Therefore, for effective NCT, the ¹⁵⁷Gd-containing probe is needed to be delivered not only inside the target cells, but also within sub-cellular organelles such as the mitochondria, or even better inside DNA strands of such organelles (Figure 10B).¹⁵⁶

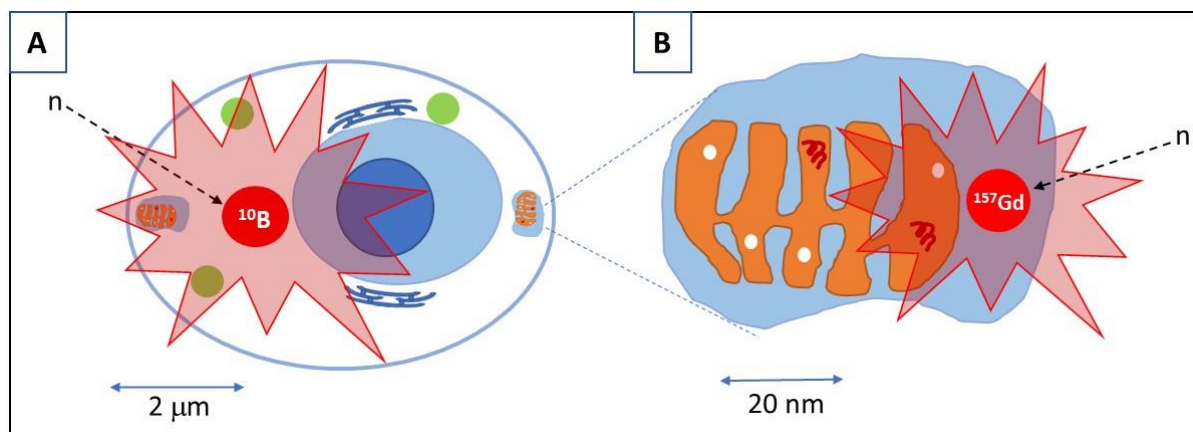


Figure 10. While intercellular localization is required for effective NCT using ^{10}B (A), localization within subcellular organelles is necessary for effective NCT using ^{157}Gd (B).

Gadolinium compounds, such as Gd-DTPA (gadopentetate dimeglumine Magnevist®), have been used routinely as contrast agents for magnetic resonance imaging (MRI) of brain and some other tumors.¹⁵⁸ However, the use of Gd-based agents has not yet been reported for NCT in clinics.

Two boron-containing molecules, namely sodium borocaptate (BSH) and L-p-boronophenylalanine (L-BPA), are already used in clinics for the treatment of high grade gliomas and some other tumors.¹⁵⁷ However, both tumor localization and retention can be significantly improved by the use of nanoparticles, which target tumor sites with higher efficiency than molecular probes.¹⁵⁹ Nanoparticles can not only deliver NCT drugs to target site with higher efficiency, but can also themselves serve as NCT agents. Recently, Tamanoi *et al.* attached L-BPA on the surface of biodegradable periodic mesoporous organosilica (BPMO) nanoparticles. The NCT potential of these nanoparticles were validated following injection in a chicken egg tumor model and subsequent irradiation with neutron beam. Higher inhibition of tumor growth with the nanoparticles, as compared to that using the free drug, were observed (Figure 11).¹⁶⁰ On the other hand, we have developed a liposomal formulation for targeted BCNT by encapsulating elemental boron nanoparticles within a phospholipid-

based liposome construct, which was surface functionalized with the NIR fluorescent dye Cy5 and tumor-avid biomolecule folic acid (FA). *In vitro* uptake in folate-receptor positive cells was demonstrated by fluorescence microscopy. Sufficient intracellular concentration of boron, suitable for BNCT, was confirmed by Inductively Coupled Plasma Mass Spectrometry.¹⁶¹

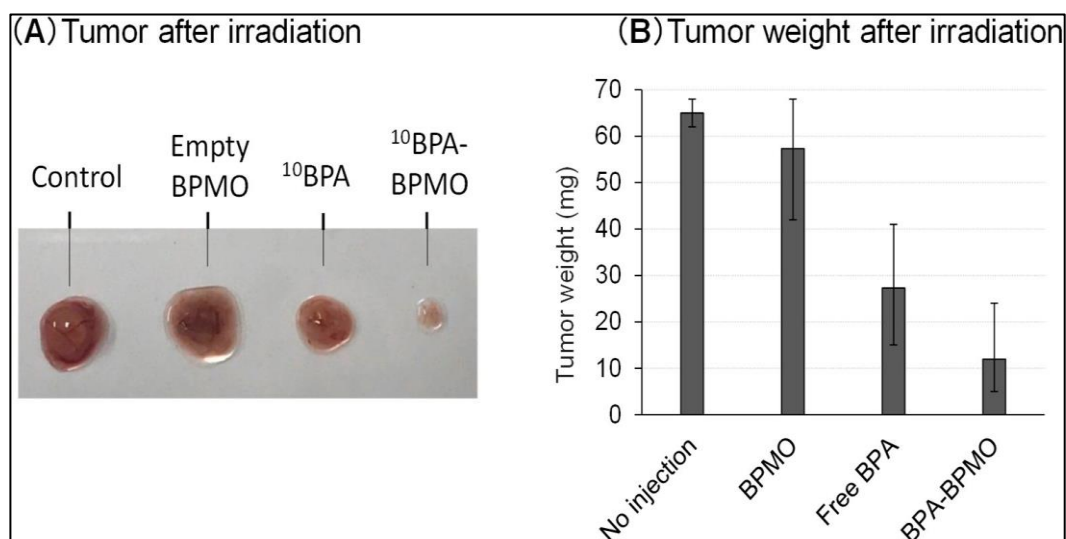


Figure 11. Tumor size after BNCT experiments. (A) Pictures of tumors three days after neutron irradiation, and (B) weight of excised tumors after treatment. Adapted from reference [160].

Boron-containing nanocarriers usually have long circulation time *in vivo*, which leads to high tumor accumulation. Unfortunately, prolonged circulation also leads to high accumulation in non-target organs, such as liver and spleen, which poses a major drawback. Using smaller boron-containing nanoparticles with lesser circulation time, the uptake in non-target organs can be avoided. Yet, in such cases, the tumor accumulation can be enhanced using a pre-targeting approach. In one example, Feiner *et al.* have pre-targeted HER-2 positive tumors using targeting antibody Trastuzumab, modified with trans-cyclooctane (TCO). This was followed up by administration of tetrazine-functionalized smaller boron-containing carbon nanodots, which homed-in at the tumor sites with high precision owing to the specific TCO-tetrazine interaction, while avoiding off-target accumulation [10.1039/d0tb01880e].

As mentioned previously, the use of integrated nanoplatforms facilitate multimodal imaging, combination therapy, theranostics, etc. In an example, Pulagam *et al.* have functionalized ⁶⁴Cu-labelled gold nanorods (AuNRs) with the boron-rich water-soluble complex cobalt bis(dicarbollide) ([3,3'-Co(1,2-C₂B₉H₁₁)₂]⁻). These ⁶⁴Cu-labeled

multifunctionalized AuNRs were robustly uptaken by gastrointestinal tumor xenografts in mice following systemic administration, without any noticeable side effect. PET-imaging mediated tumor detection, followed by both light-activated photothermal therapy (PTT) and neutron-irradiated BNCT of the tumor could be achieved, thus demonstrating image-guided combination therapy using the single nanopatform *in vivo* [DOI: 10.1021/acsami.0c17575].

Gadolinium NCT nanoformulations have been reported with nanoparticles containing chelated gadolinium ions, entrapped gadolinium compounds, as well as gadolinium as an intrinsic part of the nanoparticles. Tokumitsu *et al.* compared the NCT abilities of the commercial gadopentetate (complex of gadolinium and DTPA) solutions to that of gadopentetic acid–chitosan complex nanoparticles. Intratumoral injection of these formulations in mice bearing subcutaneous B16F10 melanoma tumors, followed by irradiation with thermal neutrons, resulted in significant suppression of tumor growth in the nanoparticle-administered group as compared to that in the gadopentetate solution-administered group.¹⁶² Dewi *et al.* incorporated Gd-DTPA, a common MRI contrast agent, on the surface of calcium phosphate nanoparticles. Mice bearing colon carcinoma Col-26 tumors were injected with the nanoparticles, followed by irradiation with average neutron fluence of 1.8×10^{12} n/cm². A substantial reduction in the tumor burden was observed in the nanoparticle-injected animals, as compared to non-treated animals. The tumor-regression results were supported by histopathological analysis on tumor slices post-mortem.¹⁶³

Beyond boron and gadolinium, some other elements have shown potential in NCT, although their biological activity is yet to be reported. The non-radioactive Samarium-152 (¹⁵²Sm), doped in boron nitride nanotubes (BNNTs), was shown to be activated by thermal neutrons to generate the radioactive, beta-emitting ¹⁵³Sm.¹⁶⁴ We have reported that ¹⁵²Sm-enriched samarium oxide nanoparticles (NPs), synthesized by laser ablation described below, could be converted into a highly efficient therapeutic β^- emitter (¹⁵³Sm) for nuclear therapy under neutron beam irradiation.¹⁶⁵

5. Recent approaches and concepts

Examples of some recently emerging approaches and concepts that can bring substantial advancements in nuclear nanomedicine are listed below.

Laser ablation for synthesis of nanomaterials for nuclear nanomedicine

For efficient *in vivo* pharmacokinetics, biodistribution, target specificity, clearance, biocompatibility, and reproducibility, nanoparticles should ideally have uniform size, shape, surface properties, and be devoid of unwanted contaminants. Conventional chemical synthesis cannot satisfy many of these requirements as it involves complex reactants with strictly defined quasi-equilibrium system parameters, as well as toxic by-products.¹⁶⁶⁻¹⁶⁷ Pulsed laser ablation in liquids, which profits from a natural generation of nanoclusters under the action of laser radiation on a target, followed by their release into the liquid to form nanoparticle solutions, is free of these limitations opening up access to the synthesis of a variety of appealing nanomaterials for nuclear nanomedicine.^{168,169} Laser ablation can be performed under ultrapure conditions (deionized water, *etc.*) to exclude any toxic contamination and be adapted for the fabrication of virtually any type of nanomaterial, including isotope-enriched nanoparticles such as ¹⁰B for NCT, whose chemical synthesis is unacceptably expensive due to a high cost of starting materials. Furthermore, dealing with non-equilibrium nanosynthesis conditions, laser ablation gives access to formation of complex alloys and core-shells, allowing a combination of different functionalities (nuclear, plasmonic, magnetic, *etc.*) in one construct. This approach is particularly efficient in the case of ultrashort laser ablation,¹⁶⁸⁻¹⁷⁰ which requires a very small pulse energy to ablate a material unity and thus can form nearly monodispersed nanoparticles. This approach can be easily scaled up using multi-beam laser systems, which allow one to synthesize nanoparticles with a rate of 4 gh⁻¹ and higher.^{171,172} Furthermore, if the productivity exceeds a breakeven value of 550 mg·hour⁻¹, laser-based synthesis becomes more economical than traditional chemical synthesis routes¹⁷², which promises the less costly synthesis of many important materials for nuclear nanomedicine.

Profiting from the flexibility and cleanness of laser-ablative synthesis, a variety of nanomaterials having a great potential for nuclear medicine have been fabricated, including single element Si,¹⁷⁰ Au,^{168,169,173} Pt,¹⁷⁴ Bi nanoparticles,¹⁷⁵ high Z-elements-containing nanostructures of gadolinium oxide,¹⁷⁶ tantalum oxide,¹⁷⁷ hafnium oxide,¹⁷⁸ yttrium oxide,¹⁷⁹ samarium oxide,¹⁶⁵ *etc.*, complex alloys/core-shells, including AuFe,¹⁸⁰ PtAu,¹⁸¹ BiAu,¹⁸² Au-Si core shells,¹⁸³ or Gd₅(Si_{1-x}Ge_x)₄ nanocomposites.¹⁸⁴ Depending on an intended application, these nanomaterials could be employed as sensitizers of therapies, contrast agents in imaging or carriers of radionuclides. In addition, they can be used as therapy agents after their

radiative activation, as it was proposed in our recent study. Here, laser-synthesized ^{152}Sm -enriched samarium oxide nanoparticles could be converted into a highly efficient therapeutic ^{153}Sm beta-emitter for nuclear therapy under neutron beam irradiation.¹⁶⁵

Several studies have already explored the use of laser-synthesised nanomaterials in nuclear medicine tasks. Torrisi *et al.* explored laser-produced Au nanoparticles as x-ray contrast agents for diagnostic imaging and confirmed their efficiency and safety in the treatment of breast-calcified tumors.¹⁸⁵ We demonstrated the possibility of fast (less than 30 minutes) conjugation of PEG-coated Si nanostructures with radioactive ^{188}Re , which is substantially shorter than the ^{188}Re half-life of 17 hours.¹⁶ Upon intravenous administration in a small animal model, the conjugates circulated freely in the blood stream to reach several organs, with high accumulation in target tumors. The observed biodistribution was dramatically different from that of a ^{188}Re salt, which mostly accumulates in the thyroid gland. The nanoparticle-based delivery also ensured excellent retention of ^{188}Re in the tumor, again in contrast to the salt, thus maximizing the therapeutic effect. Ultimately, the radionuclide nanocarrier exhibited complete time-delayed bioelimination. Tests of rat survival showed excellent therapeutic effect, with 72% of treated rats surviving, while none of the untreated (control) animals survived (Figure 12).¹⁶ In a separate work, we studied conjugation of Si nanoparticles with radioactive ^{68}Ga for PRT imaging.^{186,187} Recently, Torresan *et al.* prepared iron-boron nanoparticles by laser-assisted synthesis for MRI-guided BCNT.¹⁸⁸

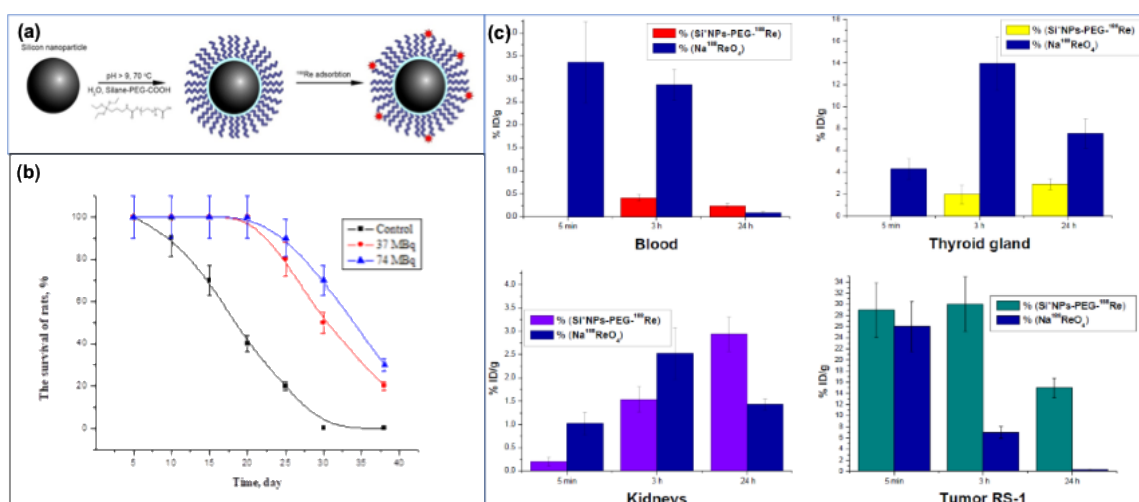


Figure 12. (a) Schematic of functionalization procedure describing the coating of laser-synthesized Si nanoparticles by PEG and their subsequent decoration by radioactive ^{188}Re atoms. (b) Survival curves for the rats administered intratumorally with PEGylated silicon nanoparticle- ^{188}Re conjugate having

different doses of radioactivity (37 and 74 MBq). Control group were injected with physiological solutions. (c) Biodistribution of ^{188}Re radionuclide under intratumoral injection of PEGylated silicon nanoparticle- ^{188}Re conjugate in Wistar rats implanted with liver cholangioma. Different colors show relative amounts of radioactivity in different organs 5 min, 1 hour, 3 hours, 24 hours and 48 hours of the administration. The blue color shows the relative amount of radioactivity in organs for control mice group administered with ^{188}Re in the free state (sodium perrhenate $\text{Na}^{188}\text{ReO}_4$ molecules). Adapted from reference [16]. Printed with permission from 'Springer Nature', under 'Creative Commons Attribution 4.0 International License'.

Other nuclear and combination therapies:

Recent evidence suggests that radiation can be contextually linked not only to adaptive immune system activation, but also to innate immune system activation as well.¹⁸⁹ This is postulated to be the driver for high linear energy transfer radiation leading to more immune activation with the putative link being the formation of micronuclei during mitosis of cells with unrepaired DNA damage, and their defective nuclear laminar envelopes allowing access to cytosolic oligonucleotide sensor proteins.^{190,191}

In terms of nanoparticles that can trigger such a response, a number of approaches have been advanced in recent years.¹⁹² An abstract from the Nanobiotix group reports that patients with soft tissue sarcomas treated with hafnium oxide nanoparticles (NBTRX3) and radiation, have increased expression levels of cytokines, interleukins and immune checkpoints, and of several T cell lymphocyte activation markers compared to those treated with radiation alone.¹⁹³ In a preclinical analysis of colorectal cancer cells treated with hafnium oxide nanoparticles and radiation, there was greater cell death, DNA double strand breaks, micronuclei formation and cGAS-STING pathway activation, than when cells were treated with radiotherapy alone.¹⁹⁴ Recently, the group demonstrated abscopal therapeutic effect in the same colorectal cancer mouse model using combination effect of NBTRX3 and radiation therapy (Figure 13).¹⁹⁵ The group postulates that this immune activation could synergize with immunotherapy for even greater tumor eradication efficacy not just at the irradiated site but also at distant sites of metastatic disease.

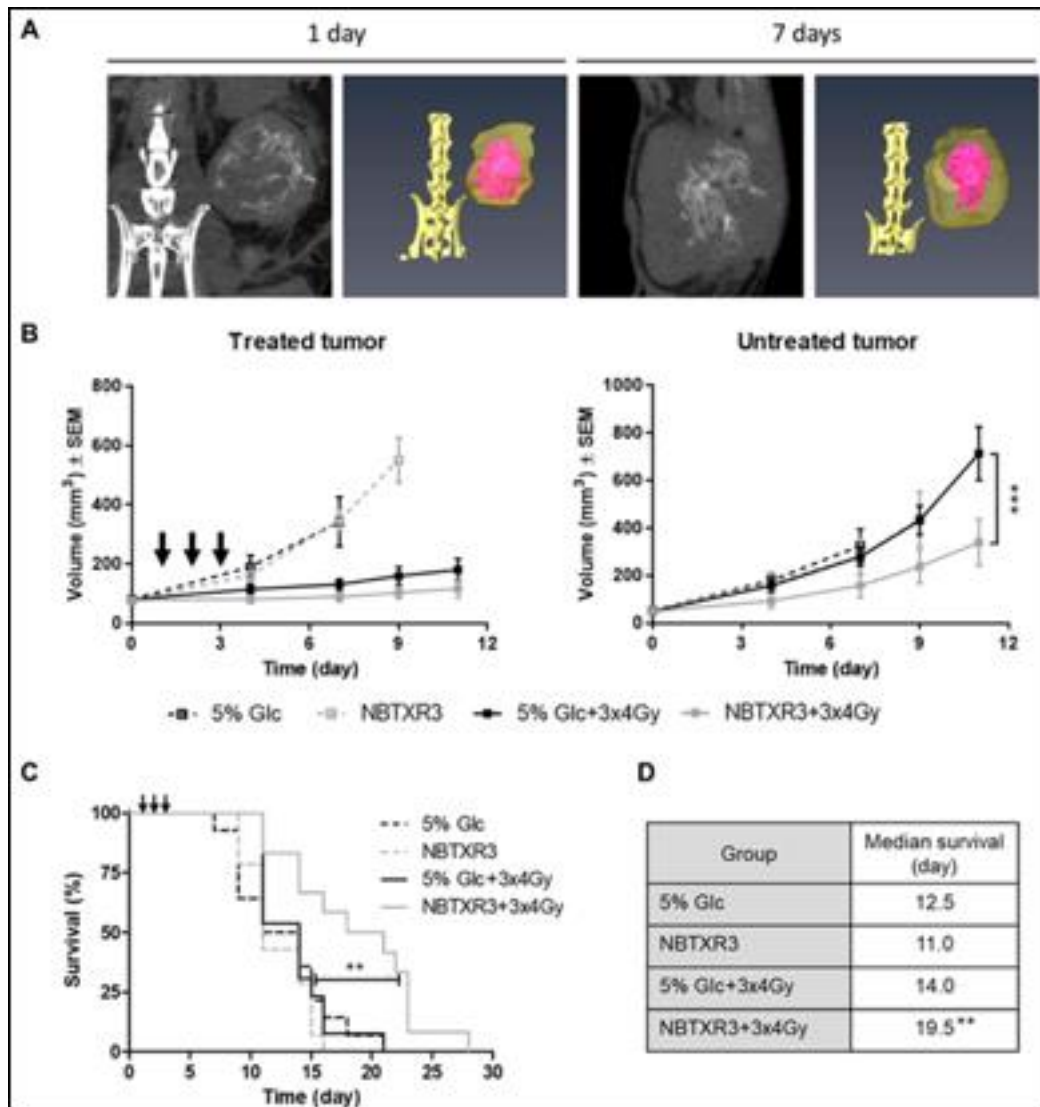


Figure 13: Distribution and persistence of NBTXR3 after intratumoral injection was imaging by μ CT performed 1 day (left panel) and 7 days (right panel) after NBTXR3 injection. Pink, NBTXR3; brown, tumor (A). For abscopal experiments (B) tumor growth curves of treated (left panel) and distant untreated (right panel) tumors (C) survival curves and (D) median survival were obtained from two independent experiments (n=2), with 12–15 mice per group. Arrows indicate radiotherapy sessions (4 Gy). Statistical analysis: two-way ANOVA test (B) or Log-rank (Mantel-Cox) test (C). **p<0.01; ***p<0.001 Adapted from reference [195]. Printed with permission from ‘Dove Press’.

In another example of radiation with nanoparticles activating an immune response, poly(lactic-co-glycolic) acid (PLGA) particles with a core of water-soluble catalase and a shell loaded with a toll-like-receptor-7 agonist, imiquimod, targeted an immunosuppressive tumor microenvironment that is hypoxic and radiation resistant. Here, the catalase overcame the detrimental effect of tumor hypoxia and radiation therapy induced immunogenic cell death,

which synergized with both the imiquimod and concurrent treatment with cytotoxic T-lymphocyte associated protein 4 (CTLA-4) checkpoint blockade. Combination therapy not only decreased the size of the irradiated tumor, but also reduced metastases *via* the induction of an abscopal response. This systemic immune response was cancer-specific and durable since it protected mice from a tumor rechallenge.¹⁹⁶

Theranostics using Cerenkov radiation

Cerenkov radiation (CR) or Cerenkov luminescence refers to the continuous-wave blue light emission arising from the interaction between a charged particle travelling faster than light, and a dielectric medium (with refractive index > 1). Several radionuclides, particularly those emitting beta-particles (electrons or positrons), are known to produce Cerenkov luminescence in water and biological tissues.¹⁹⁷ Owing to poor depth penetration by blue emission, Cerenkov luminescence itself is insufficient to provide any biologically relevant optical signal from deep tissues. However, the blue Cerenkov emission can facilitate deep-tissue excitation of several useful optical probes having high absorption in the blue region, through the process of Cerenkov Radiation Energy Transfer (CRET). Such optical probes include red/NIR emitting fluorophores, quantum dots, photosensitizers, *etc.* The energy transfer process leads to several exciting biomedical applications *via* the interfacing of radiation therapy and biophotonics.^{198,199}

In one early demonstration of CRET, Dothager *et al.* combined positron emitting isotopes, ^{64}Cu and ^{18}F , with large Stokes-shift quantum dots, Qtracker705 which could efficiently absorb CR and produce highly red-shifted photoluminescence emission in the NIR region (emission maxima at 705 nm). A high CRET ratio was observed (8.8 ± 1.1 *in vitro*, and 3.5 ± 0.3 *in vivo* in mice with subcutaneous pseudotumors), thus demonstrating the potential of a combination of positron emitting probes and NIR QDs in deep-tissue imaging for tumor diagnosis. Simultaneous PET imaging is also possible to complement optical imaging.²⁰⁰

Phototherapies, such as photodynamic therapy (PDT), relies on localized generation of cytotoxic reactive oxygen species (ROS) following light-activation of molecular or nanoparticulate photosensitizers (PSs). However, the absorption maxima of most potent photosensitizers lie towards the shorter wavelength (blue) region of the spectrum. Externally applied blue (low-wavelength) light, owing to poor tissue penetration, is therefore unable to trigger such photoactivated therapies in deep tissues and disseminated lesions. However, *in*

situ internally-generated blue Cerenkov luminescence can facilitate deep-tissue excitation of photosensitizers such as porphyrins, chlorins, *etc.*, showing a strong absorption band (Soret band) in the blue region, co-incorporated with positron emitting radionuclides.^{201,202}

Cai and coworkers showed extensive PDT mediated tumor damage in tumor-bearing mice treated using Cerenkov luminescence of the radionuclide zirconium-89 (^{89}Zr , $t_{1/2} = 78.4$ h) to activate a well-known photosensitizer (chlorin e6, Ce6) in hollow mesoporous silica nanoparticles (HMSNs) loaded with Ce6 and oxophilic ^{89}Zr ($[^{89}\text{Zr}]\text{HMSN-Ce6}$) This study highlighted the use of an internal luminescence source to achieve deep-seated tumor therapy. Concurrently, live-animal PET imaging can also be combined to facilitate the monitoring of therapy progression in real-time.²⁰¹ The researchers then further modified this formulation as a 'core-satellite' nanoconstruct by assembling copper sulfide (CuS) nanoparticles on the surface of the HMSNs, entrapped with porphyrin molecules and radiolabeled with ^{89}Zr . The inclusion of CuS nanoparticles which nonradiatively produces local heat upon optical excitation, enabled the additional capability of photothermal therapy. These hybrid nanotheranostic particles enabled simultaneous PET and Cerenkov luminescence imaging with PDT and PTT, for applications in image-guided therapy. Synergistic CuS-mediated PTT and porphyrin-mediated PDT led to complete tumor elimination, no visible recurrence, and minimal side effects.²⁰³ Recently, Wang *et al.* incorporated the photosensitizer zinc tetra(4-carboxyphenoxy) phthalocyaninate (ZnPc4), radiolabelled with ^{131}I , within Cr^{3+} -doped zinc gallate (ZGC) nanoparticles for combined radiation therapy and Cerenkov radiation-induced PDT. Robust tumor elimination *in vitro* and *in vivo* were observed using this dual therapeutic approach (Figure 14).²⁰⁴

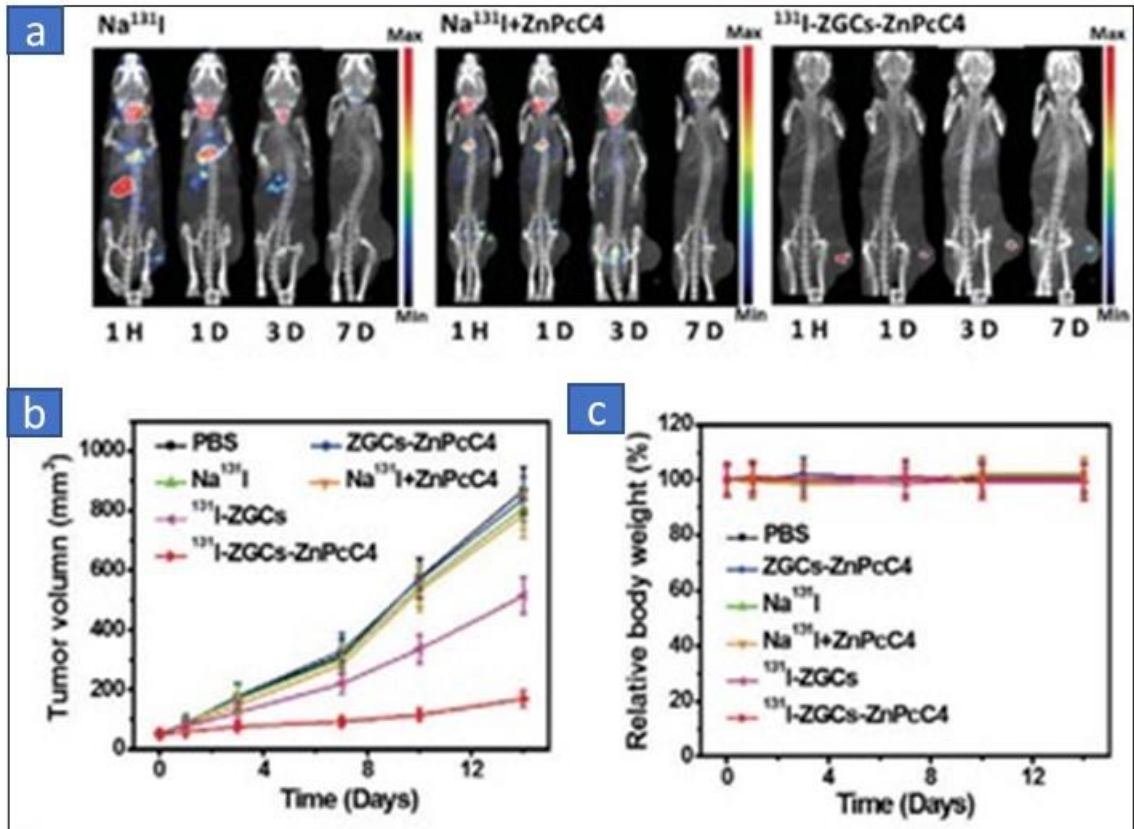


Figure 14: (a) Representative SPECT/CT images of 4T1 tumor bearing mice intratumoral injected with 100 μCi Na^{131}I , 100 μCi Na^{131}I + 20 μg ZnPcC4, and ^{131}I -ZGCs-ZnPcC4 (100 μCi , 200 μg) at various time points. (b) Tumor growth curves, and (c) body weight curves of 4T1 tumor bearing mice following various treatments. Adapted from reference [204].

6. Nanoradiopharmaceuticals in clinics

Until recently, molecular delivery vehicles such as peptides, antibodies and antibody fragments and their therapeutic radionuclide payloads have been primarily used in the treatment of non-Hodgkin's lymphoma *via* targeting the CD20 epitope on B cells with ^{90}Y (Zevalin) or ^{131}I (Bexxar) labeled monoclonal antibodies. A lack of robust overall survival data and logistical/financial challenges with their administration by medical oncologists have resulted in a limited clinical adoption of these compounds. More recently, however, FDA clearance of ^{223}Ra -chloride (Alpharadin/Xofigo) for treatment of bone metastases from hormone-refractory prostate cancer, Lutetium-labeled somatostatin analog (^{177}Lu -dotatate, Lutathera) for treatment of somatostatin receptor-positive gastroenteropancreatic neuroendocrine tumors, iobenguane ^{131}I (^{131}I -MIBG) for the treatment of unresectable, locally advanced or metastatic pheochromocytoma or paraganglioma, and Lutetium-labeled

prostate specific membrane antigen targeting peptide (^{177}Lu -PSMA) for the treatment of metastatic or treatment-resistant prostate cancer, have re-energized the field. Serendipitously, this renewed interest in radiopharmaceuticals coincided with a burgeoning interest in nanomedicine allowing for possible intersections of these two paradigms. The preceding sections have highlighted the preclinical promise of nanoradiopharmaceuticals as cancer diagnostic or therapeutic agents. While these sections provide a glimpse of what could come in the future, only a few have advanced to clinical trials and none are currently FDA approved in the U.S. As outlined in the preceding sections, some potential advantages of nanoradiopharmaceuticals include the ability to tune the polyvalency of ligands on the surface of nanoparticles for optimal receptor binding and efficient molecular targeting, the ability to couple imaging and therapy in a single construct for multi-modal theranostics, the ability to titrate nanoparticle size to modify whole-body and intra-organ biodistribution, and the option of remote on-demand triggering of intratumoral ionization events without systemic exposure of the patient to radiation or the operator to radiation. However, a global view on the use of nanomaterials and nanotechnologies in all possible nuclear medicine modalities is still lacking, that complicates the assessment of clinical potential of nanoradiopharmaceuticals.

Nevertheless, beyond the use of nanoparticles containing radioisotopes for imaging and therapy, in the realm of clinical use of nanoparticles for radiotherapy, a few applications have already advanced to clinic. As noted earlier, Nanobiotix (<https://www.nanobiotix.com/>) has synthesized hafnium oxide nanoparticles that are directly injected into tumors. When the treated tumor was subjected to external irradiation, it triggered secondary electron showers that amplify the effect of radiation alone and has shown promise in the treatment of soft tissue sarcomas. Nanobiotix has initiated a broad phase 3 clinical trial in collaboration with clinics such as MD Anderson in the U.S. for the application of their Hf nanoradiopharmaceuticals for treatment of head and neck cancer. NH TheraGuix (<http://nhtheraguix.com/>) has synthesized gadolinium nanoparticles that are intravenously administered to serve as both MRI agents) and therapeutic agents *via* a similar release of secondary electrons upon irradiation. Early results in the treatment of brain metastases with escalating doses of the nanoparticle, suggest that the particles could serve as a potent radiation sensitizer. Similarly, boronated compounds are used for BNCT in the clinic in Japan,

Finland, Argentina, Taiwan and China. One combination of an accelerator-based neutron source and boronophenylalanine synthesized by Stella Pharma (<https://stella-pharma.co.jp/en>) was granted Japanese FDA approval recently. Lastly, ^{90}Y microspheres, albeit not nanoparticles in a strict sense due to their micrometer size, are routinely used in the treatment of hepatocellular carcinoma *via* intraarterial administration.²⁰⁵ Collectively, these inroads into clinical radiation therapy practice made by nanoparticles, set a precedence and define a roadmap for future innovations in nanoradiopharmaceuticals.

7. Current Challenges and Future perspectives of Nanoradiopharmaceuticals

It is amply evident that the convergence of nanotechnology with nuclear medicine has advanced a plethora of cancer imaging and therapy paradigms. A few challenges intrinsic to nanotechnology are those of nanoparticle uniformity, biocompatibility, biodistribution, and pharmacokinetics. The US National Nanotechnology Initiative (NNI) recognized these attributes and equally exciting challenges of nanoparticle use in medicine, and created the Nanotechnology Characterization Laboratory in Frederick, MD (<https://ncl.cancer.gov/>) as a central facility for testing nanomaterials for standardization, physicochemical characterization, biocompatibility, *etc.*, and outlined lessons learned over the course of analyzing nanoparticles from numerous suppliers (academia, industry, and government).²⁰⁶ Quite naturally, similar challenges await the forays of nanoradiopharmaceuticals into clinical realms.

Outlined below are our subjective opinions on some of the important challenges for rapid advancement and clinical acceptance of nanoradiopharmaceuticals, and some thoughts on possible approaches to overcome them.

- A. Targeted delivery of nanoradiopharmaceuticals: Coupling a targeting moiety to a nanoradiopharmaceutical can be challenging, especially if the radionuclide has a short life time. However, using recent advances in nanochemistry, the surface of the nanoparticles can be functionalized with linking groups which couple within minutes to the complementary linking group on the targeting peptide or antibody. The use of active targeting strategies with nanoformulations enables high radionuclide loading and avoid immediate accumulation in kidneys, which are difficult with conventional targeting molecules alone, while the targeting group brings the nanoradiopharmaceutical complex to the tumor site. Additionally

nanoradiopharmaceuticals enable passive tumor targeting based on the EPR effect, which leads to their enhanced accumulation in the tumor area.⁷⁶ However, the heterogeneity of the EPR effect across various organisms (*e.g.* mice *versus* humans) and tumor types pose a challenge, especially in the clinical translation of nanoformulations. Active targeting approaches that drive nanoformulations to tumor tissues/cells are more promising.^{13,14} Other emerging approaches, such as co-delivery of nanoradiopharmaceuticals with microbubbles followed by ultrasound-induced cavitation, can enhance their target specificity to tumor sites.

- B. Easy and safe bioelimination of nanoradioformulations: Large transport vehicles carrying radionuclides can maximize the loading capacity and avoid immediate accumulation in the kidneys; however, they also may complicate clearance from the organism causing toxicity issues, as observed in the case of silica nanoparticles.²⁰⁷ To overcome this problem, one can use biodegradable nanostructures such as Si nanoparticles which biodegrade into orthosilicic acid Si(OH)_4 , which are naturally excreted from the body with the urine. On the other hand, for radiation- or particle beam-activated nanoparticles, the size may not be so important and can be selected depending on the clearance and toxicity prospects of each nanomaterial (for example, it can be selected to be within the glomerular filtration range).
- C. Dosimetry: Accurate dosimetry during diagnostic use helps with treatment planning and during therapeutic use helps with prediction of biological and clinical outcomes. By using isotope pairs or similar isotopes for imaging and therapy (*e.g.* ^{123}I and ^{131}I), one can eliminate the uncertainty that arises from using a surrogate (say $^{99\text{m}}\text{Tc}$ -labeled macroalbumin aggregates for glass/resin ^{90}Y microspheres). Use of greater sensitivity techniques (say, PET over SPECT) also improves diagnostic accuracy. Notably, however, most of the dosimetry information is currently based on a whole-organ level rather than at a microscopic level. The latter may be especially critical for nanoradiopharmaceuticals because of their greater likelihood of achieving non-homogeneous dose distributions within tumors. It is worthwhile to document the heterogeneity and use it as an input parameter in Monte Carlo models. Furthermore, radiobiological models need to be fitted to actual clinical data so that future treatment planning based on these models is accurate.

- D. Decay product selection: Whenever radioisotopes are used in clinical practice, there is little ability to alter the decay products and/or kinetics; these follow first principles of physics and chemistry. However, there may be an opportunity to modulate what the target structure (the tumor) or non-target tissue (adjacent normal tissue) sees. For instance, core-shell nanoparticles with a core comprised of a radiopharmaceutical and a shell that selectively blocks a specific component of the emission spectrum may have clinical utility. For example, gadolinium NCT could be enhanced by reducing the deep penetrance of gamma emissions that cause normal tissue dose accumulation. In another incarnation, the Cerenkov radiation from a radiopharmaceutical in the core of a nanoparticle can be coupled to a photosensitizer in the shell of the nanoparticle to increase the yield of oxygen free radicals and thereby augment anti-tumor activity. This approach not only exploits an otherwise stray radiation emission from the radiopharmaceutical but also confines the additive/synergistic and cumulative effect to the irradiation zone.
- E. Stability: Whenever radiopharmaceuticals are coupled to dyes or nanoparticles *via* chelating agents, there is always a possibility that they may be dislodged from the chelating agent in biological tissues. This challenge persists with nanoradiopharmaceutical formulations, unless the radiopharmaceutical is entrapped within a core-shell nanostructure or there is no chelating agent used (say, with endohedral metallofullerenes). Otherwise, one still has to grapple with the known toxicity of unbound metal ions like Gd^{3+} . Strategies to address this, somewhat beyond the scope of this review, include use of deferiprone, deferoxamine, deferasirox that chelate free Gd^{3+} ions or N-acetylcysteine to reduce oxidative stress caused by these ions in end organs like the brain and kidney. However, invoking mitigation strategies requires recognition of high levels of free unbound metal ions (in serum or urine), which is not the current practice.
- F. In-situ generation of radioactivity: A suitable nanoformulation should contain the maximum concentration of the isotope harboring the highest particle (*e.g.* neutron) capture cross-section. This can be ensured by isotopic enrichment of the nanoparticles. An example is boron where the ^{10}B isotope (only 20% in natural abundance) has the higher neutron capture cross-section, as compared to the more abundant ^{11}B and thus ^{10}B isotopically enriched nanoparticles will perform the NCT

at a relatively lower dosage of radiation. However, the cost of such selective isotope enrichment may be very high. In such a case, a method like laser ablation, as discussed in this review, is of great value, as it requires a relatively small amount of the sample to fabricate nanoparticles of virtually any promising material.

- G. Nuclear particle production technology at Clinical sites: A limiting factor also is the availability of the nuclear production facility to produce the radionuclide and nuclear particles such as neutrons for NCT at the clinical site. A number of accelerator-based solutions have been proposed where acceleration of protons at a high flux onto a lithium/beryllium target can yield epithermal neutrons of sufficient fluence to treat patients in a clinical facility. Another direction is to use deuterium-deuterium (DD) and deuterium-tritium (DT) reactions to create neutrons, and position these neutron sources in a circular array around a patient, such that the cumulative flux at the tumor is high enough to complete treatment in a realistic time frame (<http://en.fukushima-sic.co.jp/products.html>).
- H. Proton Capture therapy: Proton boron fusion therapy capitalizes on the high proton capture cross-section of the ^{11}B nucleus to convert it to a metastable hyperexcited ^{12}C nucleus that disintegrates into an alpha particle and a beryllium nucleus, which then creates two alpha particles for a cumulative yield of 3 alpha particles per reaction. Some recent reports have indicated that with an appropriate energy, protons can be captured in ^{11}B -containing liquid solutions.²⁰⁸ A counterargument has been that there are some neutrons created along the path of the proton beam and these thermal neutrons interact with the 20% ^{10}B fraction of natural boron to initiate an *in situ* localized BNCT reaction along the proton beam path. Without controlled experiments with isotopically pure compounds, it is difficult to know which of these phenomena manifests. The employment of ^{11}B enriched nanoparticles can further enhance proton capture technology and view this area very promising.
- I. Synergistically integrating combination therapy: A very promising direction in nano-based nuclear therapy is combination with other therapies such as chemotherapy, photodynamic therapy, photothermal therapy, gene therapy and immuno therapy. A particularly appealing concept is synergistic enhancement produced by combination therapy. An unanswered question is whether to introduce spatial and temporal coupling of any two treatment paradigms or to decouple and administer

sequentially, concurrently or contemporaneously. Decoupling offers more flexibility of control on dose, duration, sequencing and location of action. However, in some scenarios, *in situ* delivery using integration of dual functionalities within a single nanoconstruct may be advantageous.

- J. Recent methods of producing suitable nanoplateforms: Introducing different methods of producing nanoformulations with laboratory safety, and working with small samples and cost effectiveness can also be valuable in advancing this field. A suitable method described in this review is laser ablation, which makes possible the fabrication of a variety of promising ultrapure formulations, from nanocarriers of radionuclides to nanoparticles for radiation or particle beam activation (Bi, B, *etc.*). Furthermore, profiting from essentially non-equilibrium growth this method also offers the possibility to produce nanoformulations (alloys, core-shells, mixed crystalline phases) enabling several functionalities for cancer imaging and therapy (nuclear, magnetic, *etc.*) in one entity. Another method, introduced earlier for a PDT drug nanoformulation is reprecipitation, where a solution of the active component is mixed with a nonsolvent to precipitate nanoparticles containing the active components.²⁰⁹

In summary, as highlighted in the preceding sections, nanoparticles may either embed radionuclides as their diagnostic or therapeutic cargo such that they are inherently radioactive and emit radiation from within or be non-radioactive, or home specifically to tumors whereupon they are extrinsically activated by radiation (X-rays, heavy ions, or neutrons) to generate *in situ* radiation on-demand. Other paradigms that have seamless clinical translational potential include laser ablation, which allows straightforward and scalable synthesis of high-purity excipient-free biodegradable materials; coupling radionuclide Cerenkov radiation with photosensitizers; and combination with immunotherapy. Collectively, these advances and exciting attributes of nanoradiopharmaceuticals may provide distinct advantages in diagnostic/therapeutic sensitivity and specificity, imaging resolution, and scalability of turnkey platforms for complementary multimodal imaging or combination therapy. Coupling image-guided precision radiotherapy options with the possibility of internal activation of nanomaterials could herald an era of exciting technological and therapeutic advances.

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Vocabulary

Nanoradiopharmaceuticals: A broad definition encompassing nanoparticles that are either composed of or tagged with radioisotopes, or can engender ionizing radiation upon irradiation with electromagnetic radiation or particle-beams, for diagnostic and/or therapeutic purposes.

Multimodal nanoparticles: Nanoparticles containing several types of active agents capable of performing multiple, usually medically relevant, tasks.

Theranostics: A single probe that can concurrently or sequentially perform at least one diagnostic and one therapeutic function.

Radiation therapy: A clinically practiced therapeutic paradigm that involves ablation of tumor cells with ionizing radiation emanating from non-radioactive nanoparticles with high atomic numbers exposed to x-ray radiation.

Ion-beam therapy: An emerging therapeutic paradigm that involves ablation of tumor cells with ionizing radiation emanating from certain non-radioactive nanoparticles exposed to ion-beams.

Neutron capture Therapy: A therapeutic paradigm that involves ablation of tumor cells with ionizing radiation emanating from ^{10}B or ^{157}Gd containing non-radioactive probes exposed to neutron beams.

Laser ablation nanoparticle synthesis: A top-down method of synthesizing monodispersed and ultrapure nanoparticles involving exposure of corresponding bulk materials with high-energy laser beams.

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