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## **Roadmap for metal nanoparticles in radiation therapy: current status, translational challenges, and future directions**

Jan Schuemann, Alexander Bagley, Ross Berbeco, Kyle Bromma, Karl Butterworth, Hilary Byrne, B Devika Chithrani, Sang Hyun Cho, Jason Cook, Vincent Favaudon, et al.

### ► To cite this version:

Jan Schuemann, Alexander Bagley, Ross Berbeco, Kyle Bromma, Karl Butterworth, et al.. Roadmap for metal nanoparticles in radiation therapy: current status, translational challenges, and future directions. *Physics in Medicine and Biology*, 2020, 65 (21), pp.21RM02. 10.1088/1361-6560/ab9159 . hal-02992974

**HAL Id: hal-02992974**

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Submitted on 30 Jan 2024

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## Roadmap for metal nanoparticles in radiation therapy: current status, translational challenges, and future directions

Schuemann, J., Bagley, A., Berbeco, R., Bromma, K., Butterworth, K. T., Byrne, H., Chithrani, D. B., Cho, S. H., Cook, J. R., Favaudon, V., Gholami, Y. H., Gargioni, E., Hainfeld, J. F., Hespeels, F., Heuskin, A-C., Ibeh, U. M., Kuncic, Z., Kunjachan, S., Lacombe, S., ... Krishnan, S. (2020). Roadmap for metal nanoparticles in radiation therapy: current status, translational challenges, and future directions. *Physics in Medicine and Biology*. Advance online publication. <https://doi.org/10.1088/1361-6560/ab9159>

### Published in:

Physics in Medicine and Biology

### Document Version:

Peer reviewed version

### Queen's University Belfast - Research Portal:

[Link to publication record in Queen's University Belfast Research Portal](#)

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# Roadmap for metal nanoparticles in radiation therapy: current status, translational challenges, and future directions

To cite this article before publication: Jan Schuemann *et al* 2020 *Phys. Med. Biol.* in press <https://doi.org/10.1088/1361-6560/ab9159>

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# Roadmap for Metal Nanoparticles in Radiation Therapy: Current Status, Translational Challenges, and Future Directions.

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## Abstract:

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21 This roadmap outlines the potential roles of metallic nanoparticles (MNPs) in the field of radiation  
22 therapy. MNPs made up of a wide range of materials (from Titanium, Z=22, to Bismuth, Z=83) and a  
23 similarly wide spectrum of potential clinical applications, including diagnostic, therapeutic (radiation  
24 dose enhancers, hyperthermia inducers, drug delivery vehicles, vaccine adjuvants, photosensitizers,  
25 enhancers of immunotherapy) and theranostic (combining both diagnostic and therapeutic), are being  
26 fabricated and evaluated. This roadmap covers contributions from experts in these topics summarizing  
27 their view of the current status and challenges, as well as expected advancements in technology to  
28 address these challenges.  
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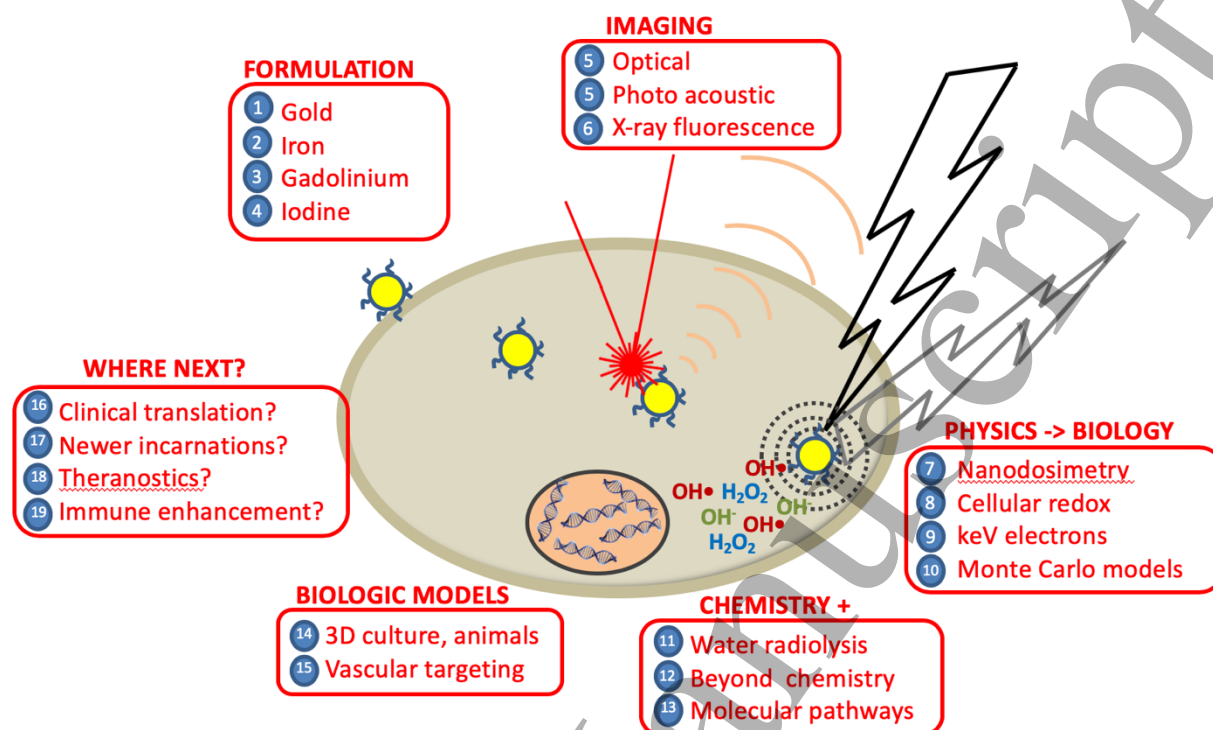


Figure 1: Graphic illustration of how each contribution in this collection connects to the study of (metallic) nanoparticles and their interaction with cells and radiation.

## Introduction:

MNPs are characterized by their small size (typically <100nm), large surface area to volume ratio, and high interaction cross-section with incident ionizing or non-ionizing radiation. The high radiation interaction cross-section is well known but modeling, mechanistic underpinning, and extensive characterization of consequences of physical, chemical and biological interplay in mediating anti-tumor effects remains inadequately understood. A variety of MNPs are also quite readily endowed with biological functionalities such as stealth coatings that help them evade capture and clearance by circulating and tissue-resident macrophages. MNPs can further be decorated with tumor targeting ligands that facilitate tumor homing or coupled with photosensitizers or other biological mediators of tissue damage. To penetrate fortified stromal barriers erected by tumors including vasculature, fibrous/connective tissue, and immune evasion strategies, MNPs can further be armed with multiple ligands specific for each barrier. Not surprisingly, this interface between nanomaterial design, radiation interaction, and tumor biology is a fertile ground for active research by multiple disciplines, often with non-intersecting boundaries.

This special issue roadmap is an attempt to collate and weave together the extant knowledge in the arena of nanoparticle research. By including experts from diagnostic, therapeutic and theranostic

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3 applications of MNPs, we endeavor to provide a broad overview in the field, benchmarking what is  
4 known today, which gaps in knowledge and technology will be addressed in the near future and  
5 forecasting what the future horizons hold in store.  
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Accepted Manuscript



# Gold Nanoparticles for Imaging and Therapy

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Nanoprobes, Yaphank, NY, USA

## Status

**Imaging:** The extensive historical wealth of chemistry of gold nanoparticles (GNPs) with their high density, high atomic number, variable size, and facile attachment of a variety of ligands make them ideal as imaging agents. Once passivated to be biologically tolerated, they can be injected intravenously or locally, or embedded in other materials and devices. Imaging can be by X-ray absorption (e.g., CT or microCT), but newer devices rely on X-ray fluorescence(Manohar *et al* 2016); gold can also be radioactive. The nanoparticle aspect gives them more flexibility and control by adjusting size, shape, and adding one or more targeting ligands(Panahi *et al* 2017). This allows control over blood half-life, clearance, and specific uptake. Results are showing excellent vasculature imaging as well as tumor imaging(Hainfeld *et al* 2006) and other targets such as stroke clots(Kim *et al* 2017). Cells (e.g., macrophages) have been loaded with GNPs and shown to target vulnerable plaque(Chhour *et al* 2016). Gold nanoparticles have been the favorite choice for electron microscope studies for cell and molecular labeling (e.g., with immunogold). GNPs have been combined with fluorescent molecules to provide dual labeling(Takizawa and Robinson 2003). Some GNPs are fluorescent. GNPs are also useful for photoacoustic imaging and as SERS probes. Silver and gold enhancement, which catalytically deposits silver or gold on the GNPs grows their size to a level detectable by the light microscope for histology or other studies(Hayat 1995). This metallic growth also increases the sensitivity. Larger GNPs (~30nm) can be seen in low (pM) concentration by the naked eye and are commonly used in lateral flow devices, such as the pregnancy test kit, or strips to detect infectious diseases. In short, GNPs have been and are a tremendously useful platform for imaging.

**Therapy:** One group showed cells grown on a gold monolayer were killed 160 times more than those grown on plastic after X-ray irradiation(Regulla *et al* 1998). X-rays are absorbed by gold atoms, knocking out electrons that create ionizations and free radicals, so more dose is deposited locally around the gold. It was shown that gold nanoparticles delivered to tumors (in test animals) and irradiated resulted in much greater tumor destruction than radiation alone(Hainfeld *et al* 2004). A number of groups have contributed extensive theoretical modeling to better understand and predict these effects<sup>10</sup>(Her *et al* 2017). Gold better absorbs kV energy X-rays (<400 keV) than MV (>4 MV) X-rays

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3 so the photoelectric effect and radioenhancement have generally been shown to be best in the kV  
4 range(Butterworth *et al* 2012). However, since clinical irradiations are done with LINACS in the MV  
5 range, some studies focused on that range and have also shown significant dose enhancement. GNPs  
6 also enhance proton therapy.  
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10 Gold nanoshells and nanorods absorb near infrared radiation (NIR, ~800 nm) enabling heating of  
11 tumors or lesions and their subsequent ablation (photothermal therapy) (Ahmad *et al* 2016). NIR is  
12 reduced by a factor of 10 in 2 cm of tissue which limits this to superficial lesions. However, a clinical trial  
13 is underway using an array of light pipes to irradiate and heat deeper prostate cancers (clinicaltrials.gov  
14 ID NCT02680535).  
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### 20 **Current and future challenges**

21 For ex-vivo or animal imaging at the macroscopic, light, and electron microscope levels, new  
22 targeting moieties and reactive groups will further add functionality and enable new applications. The  
23 challenge will be to synthesize these functionalized nanoparticles and yet retain appropriate stability.  
24 For example, putting a novel targeting moiety on the GNP surface may induce unwanted aggregation.  
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28 In vivo imaging and therapy with GNPs has been achieved for small animals. However,  
29 translation to human use has lagged and largely not been done due to several problems: 1) poor  
30 clearance, 2) skin discoloration at high levels, 3) possibly high cost (although reasonable if benefit is  
31 great). CT imaging has high resolution but low sensitivity so relatively large amounts of GNPs need to be  
32 injected, raising the issue of toxicity. For therapy, only a small amount of what is injected iv ends up in  
33 the tumor, again requiring relatively large amounts to be injected. Local injections mostly avoid this  
34 problem but do not adequately address most clinical cases where there are multiple lesions, some  
35 microscopic, and a direct injection may not fully cover all cells, leaving some undertreated with resulting  
36 recurrence(Smilowitz *et al* 2018). Clearance is also a problem. Less than 5 nm particles can exit rapidly  
37 via the kidneys. However, this fast clearance does not give enough time to maximally load tumors or  
38 other targets. IV antibodies take ~24 hours and multi-passes to maximally load targets. These particles  
39 then clear via the hepatobiliary route, but unfortunately, macrophages take up the GNPs throughout the  
40 body and because gold is insoluble and not digestible it just stays in cells. Liver clearance has been  
41 reported to be only 9% in 6 months(Sadauskas *et al* 2009). This whole body retention will be a problem  
42 for FDA approval. Because GNPs are highly colored, cells in the skin (probably mostly macrophages)  
43 take them up and skin discoloration is virtually permanent. Gold is also costly. Unless small amounts  
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3 can be utilized for in vivo purposes, these factors present significant challenges and have impeded  
4 progress toward the clinical translation of GNPs(Cui *et al* 2017, Schuemann *et al* 2016).

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6 Additionally, as mentioned, GNP X-ray dose enhancement is better with kV photons than  
7 megavoltage, but MV instruments are the ones currently used clinically due to better penetration to  
8 deep tumors.  
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### 11 12 13 **Advances in science and technology to meet challenges**

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15 For tumor and other targeted imaging and therapy of GNPs in humans, one solution is to use  
16 small acceptable amounts of gold. Imaging using fluorescent photons is more sensitive than CT, and  
17 development of this equipment is underway. Heating of superficial or deeper tissue with light pipes and  
18 NIR appears to work well with small amounts of gold nanorods or nanoshells injected iv. For better  
19 clearance <6 nm particles in a biodegradable matrix have been developed to extend blood half-life, then  
20 later break down and be cleared through the kidneys. However, during the long blood residence they  
21 will be taken up by macrophages and may have difficulty exiting the lysosomes, even when the polymer  
22 is digested. Because of the ease of chemically functionalizing GNPs with thiols that bind to their surface  
23 the problems of passivating them to avoid toxicity and linking targeting molecules (e.g., peptides and  
24 antibodies) does not appear to be a problem for GNPs as it can be for other types of NPs. The amount  
25 of gold needed for X-ray dose enhancement (at kV) is calculated to be rather high (~2% Au by weight)  
26 for a significant effect. However, some experiments have shown good dose enhancement with far less  
27 gold and using MV photons(Wolfe *et al* 2015). Usually a poorly understood biological effect is invoked  
28 to explain such unexpected results, but indicates that some GNPs might be acceptable for clinical use.  
29 Other high atomic number nanoparticles (e.g., Gd, Ho, Ti, I) may provide favorable alternatives.  
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40 Concerning the kV vs. MV controversy, kV methods are being revisited: 1) Grid, microbeam, and  
41 minibeam. Spatially segmented beams (intervening non irradiated zones) were found to eliminate the  
42 high skin entrance dose of kV X-rays(Dilmanian *et al* 2019); 2) Stereotactic kV irradiation also avoids  
43 entrance dose; 3) CT machines use bow-tie filters that upon rotation produce isodose in the subject,  
44 again avoiding skin entrance dose and dose fall-off(Schultz *et al* 2011). kV machines are much less  
45 expensive, portable, and require less shielding, and could make nanoparticle dose enhancement cancer  
46 treatments available to developing countries.  
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### 52 53 **Concluding remarks**

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GNPs have incredibly useful properties and flexibility making them one of the best choices for imaging all the way from EM to animal imaging, using various methods including electrons, ultrasound, protons and X-rays. For therapy a number of forms have been developed for heating, drug release, and dose enhancement.

I have been making and using GNPs for over 40 years. A focus has been their dose enhancement for improved cancer therapy. They are among the best radioenhancers ever found. However, the problems with clinical translation have driven me to develop iodine nanoparticles that have better clearance, are colorless (no skin discoloration) and are less expensive (Hainfeld *et al* 2019, 2018). It is hoped that these, GNPs, or other dose enhancing NPs will soon be useful for patients.

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# Magnetic Nanoparticles for MRI and Radiotherapy

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## Status

In addition to demonstrating therapeutic gain with nanoparticle-enhanced radiotherapy, it is also advantageous to incorporate imaging to aid in diagnosis, monitor treatment response or to track nanoparticle uptake. Many options exist for leveraging existing diagnostic procedures (CT contrast, magnetic resonance imaging (MRI) contrast, positron emission tomography (PET) tracers). MRI stands out due to providing soft tissue contrast and use of non-ionizing radiation. Nanoparticles enhancing MRI contrast can therefore show detailed organ-specific uptake and can exploit differential tissue uptake in disease diagnosis without exposing patients to extra dose.

Current clinically-used MRI contrast agents contain gadolinium (Gd) which, with seven unpaired electrons, confers strong longitudinal (T1) contrast. Chelated Gd nano-structures can provide diagnostic-quality MRI contrast. However, MRI contrast can also be achieved with Fe<sup>3+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup> and super-paramagnetic iron oxide nanoparticles (SPIONs).

SPIONs are well known for their MRI contrast-enhancing capability, due primarily to their ability to shorten the transverse (T2) relaxation time of water protons ([Weinstein et al. 2010](#)). The current status of SPION-enhanced contrast MRI is evolving rapidly as concern grows over long-term toxicity of conventional Gd-based contrast agents ([Fornell 2018](#)).

In radiotherapy, enhancement by SPIONs has been investigated in simulation studies ([McMahon et al. 2016](#)) and demonstrated *in vivo* using synchrotron x-rays ([Choi et al. 2012](#)) and proton beams ([Kim et al. 2012](#)). Remarkably, none of these studies explored the potential to leverage the MRI contrast-enhancing properties of SPIONs alongside their radio-enhancement properties. Such studies are needed, given the increasing role of MRI in treatment planning and the urgent need for high-precision measurement of the Bragg peak in particle therapy.

## Current and future challenges

In addition to addressing the challenges in clinical translation, a novel nanotheranostic for radiotherapy enhancement must be forward-looking, taking advantage of and enhancing emerging technologies. The combination of imaging with therapy is an overarching theme of these emerging techniques, with utilisation of MRI in particular expected to increase in future. Many nanoparticles for

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3 radio-enhancement are currently under development or have been reported on over the last 20 years,  
4 but none have yet been successfully translated for clinical use ([Kuncic and Lacombe 2018](#); [Schuemann](#)  
5 [et al. 2016](#)).

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8 Three recent innovations invite the development of magnetic nanoparticles for MRI and  
9 radiotherapy which can rise to this challenge:

#### 10 11 12 1. *The MRI-linac*

13 MRI-linac technology enables image-guided radiotherapy with precise targeting of soft-tissue  
14 structures ([Liney et al. 2018](#)). Best practice implementation and workflows exploiting the advantages  
15 of time-of-treatment MR imaging are still being explored ([Liney et al. 2019](#)). This provides an  
16 opportunity for magnetic nanoparticle enhancement to be incorporated as part of standardised  
17 treatment as that is developed. Along with visualisation of anatomical changes during treatment, the  
18 MRI-linac offers the tantalizing prospect of observing the effects and perhaps effectiveness of  
19 radiation treatment inter- and intra-fraction. This poses a challenge for nanoparticle treatments to  
20 become adaptive, identifying and responding to individual patient response during a course of  
21 treatment.

#### 22 23 24 2. *Particle therapy*

25 In particle therapy, a key current challenge to be met is integration of image-guidance techniques,  
26 similar to that successfully used in conventional MV-beam radiotherapy. MRI offers arguably the best  
27 option, especially with pencil beam scanning, where a relatively straightforward technique can be  
28 applied to correctly locate the Bragg peak for each pencil beam ([Oborn et al. 2017](#)). A future challenge  
29 is leveraging SPIONs for enhancing both MRI contrast and radiotherapeutic efficacy in particle therapy  
30 ([Liu et al. 2018](#)). Feasibility studies are required to explore the potential of this concept.

#### 31 32 33 3. *Targeted internal radiotherapy*

34 To date, the effects of radio-enhancement in many *in vivo*, *in vitro* and *in silico* studies have mainly  
35 been considered in the context of radiation delivered by an external beam (i.e. external beam  
36 radiotherapy or brachytherapy) ([Schuemann et al. 2016](#)). Targeted internal radionuclide therapy is an  
37 alternative treatment approach to achieve more localized radiotherapy by delivering short range  
38 radiation via internalization of radioisotope in a tumour ([Zhang et al. 2010](#)). Leveraging an appropriate  
39 nanoparticle delivery vehicle gives access to MRI-based tracking and diagnostics ([Gholami et al. 2015](#)).

### 40 41 42 **Advances in science and technology to meet challenges**

#### 43 44 45 1. *Gd-based nanoparticles for MRI-linac application*

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3 Radio-enhancing nanoparticles with inbuilt MRI contrast are well within current capabilities, and  
4 perfectly suited to take advantage of the recent development of MRI-linacs. Utilising conventional Gd  
5 contrast, the AGuIX<sup>®</sup> nanoparticle has been designed for radio-enhancement and MRI contrast. This  
6 nanotheranostic has undergone extensive pre-clinical evaluation and has progressed to phase I clinical  
7 trial using conventional separate MRI and radiotherapy treatment ([Verry et al. 2019](#)). Its applicability  
8 to combined MRI-linac systems is obvious, where time-of-treatment imaging gives information on  
9 nanoparticle uptake at each treatment fraction.

## 15 2. SPIONs for MRI-guided particle therapy

16 To demonstrate the feasibility of SPION radio-enhancement in particle therapy, advances are needed  
17 in both simulation and experimental approaches. In simulations, more studies are required to advance  
18 our knowledge of the physico-chemical stage of radiation damage and to correctly quantify the radio-  
19 chemistry processes in the immediate vicinity of activated SPIONs ([Rudek et al. 2019](#)). Technological  
20 advances are also needed in both software and hardware to enable MRI-guidance in particle therapy  
21 ([Oborn et al. 2017](#)), which will allow new experiments to be designed to evaluate radiotherapeutic  
22 gain by SPIONs.

## 28 3. Radio-labelled SPIONs for targeted internal radiotherapy and PET/MRI

29 Nanoparticles can be labelled with various radioisotopes for use in both internal radionuclide therapy  
30 and diagnostic imaging ([Boros et al. 2015](#); [Yuan et al. 2018](#)). SPIONs are the best candidates for a  
31 radiolabelled nano-theranostic platform (Figure 1) due to their favourable physical and chemical  
32 properties for chelate-free radiolabelling ([Yuan et al. 2018](#)), their MRI contrast properties, and their  
33 excellent biocompatibility ([lv et al. 2015](#)).

34 The six main types of radiation clinically used for internal radionuclide therapy and emission  
35 tomography are:  $\beta^-$ ,  $\beta^+$ ,  $\alpha$  particles, Auger electrons, conversion electrons, and  $\gamma$ -rays ([Ersahin et al.](#)  
36 [2011](#)). As the energy of the emitted particles is typically in the kilo-electronvolt (keV) range for Auger  
37 electrons,  $\beta^-$ ,  $\beta^+$  particles, the probability of interaction with high-Z nanoparticles can be significantly  
38 higher than that for an external photon beam, which is typically in the mega-electronvolt (MeV)  
39 energy range. The interaction probability is also increased by the close proximity of the radiation  
40 source to the nanoparticles. Thus, radio-enhancement by nanoparticles should be more significant for  
41 internal radionuclide therapy than for external beam radiotherapy ([Gholami et al. 2019](#)).

42 In addition, theranostic radioisotopes such as  $^{67,64}\text{Cu}$  ( $\beta^-$ ,  $\beta^+$ ,  $\gamma$ ),  $^{90}\text{Y}$  ( $\beta^-$ ,  $\beta^+$ ) and  $^{177}\text{Lu}$  ( $\beta^-$ ,  $\gamma$ )  
43 decay with multiple radiation species enabling them to deliver simultaneous *in vivo* dose  
44 quantification by utilizing nuclear imaging (e.g. PET).

45 The inherent MRI contrast afforded by radio-labelled SPIONs in addition to PET imaging and  
46 delivery of a therapeutic radiation dose delivers a compelling theranostic platform.



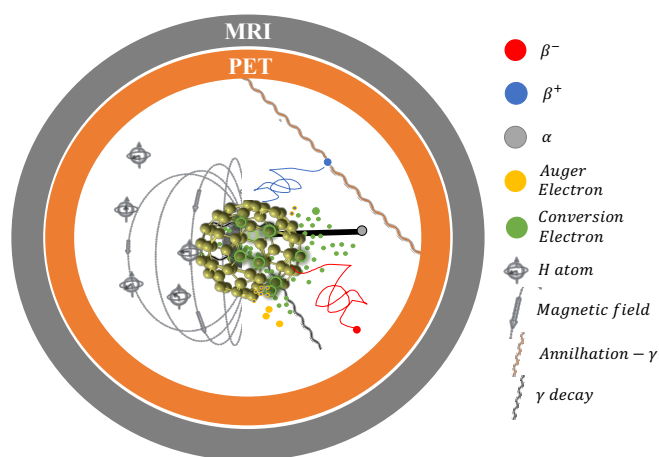


Figure 1. Schematic diagram of a radiolabelled SPION platform for internal radiotherapy and multimodal imaging.

### Concluding remarks

Radiotherapy enhancement can be achieved with nanoparticles incorporating MRI contrast, exploiting some of the latest and most exciting innovations in the radiotherapy space: MRI-linacs; image-guided particle therapy; and radionuclide theranostics. SPION-enhanced MRI-guided particle therapy, in particular, presents an exciting concept that integrates several advanced technologies that up until now have been studied disparately. Feasibility studies are needed to demonstrate the potential benefits of this new paradigm.

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# Gadolinium Nanoparticles for Imaging and Therapy in Oncology

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## Gadolinium as molecular agent.

Gadolinium is the most frequently used lanthanide in the clinic essentially as positive contrast agent for magnetic resonance imaging (MRI) since the 80s. A molecular agent (Motexafin gadolinium) has been used in combination with radiotherapy for treatment of glioma, non-small cell lung cancer and brain metastases.<sup>1</sup> Despite good tolerance of the treatment and encouraging preclinical results, motexafin gadolinium did not succeed in increasing significantly overall survival.

## Metal based nanoparticles for radiotherapy.

Most of the studies performed on radiosensitization used nanoparticles taking advantage of gathering high Z elements in a single object. The potential of this strategy for enhancing the efficacy of radiotherapy has been shown even at very small concentrations of NPs in tumors (ppm range) associated with enhancement factors varying from 10 to 100% using different elements.<sup>2,3</sup> McMahon *et al.* have proposed a nanoscale dose deposition mechanism that leads to important formation of radical species in direct vicinity of the nanoparticles.<sup>4</sup> Despite intense research, only two nanoparticles have been accepted for clinical trials as radiosensitizers for the moment: (i) NBTXR3 (hafnium oxide NPs) administered locally<sup>5</sup> and AGuIX (polysiloxane based NPs surrounded by gadolinium chelates) administered intravenously.<sup>6</sup>

## Why develop ultrasmall nanoparticles for radiosensitization ?

Development of ultrasmall NPs with a hydrodynamic diameters of less than 5 nm for biomedical applications is relatively recent due to different reasons: (i) NPs have been initially developed for drug delivery needing large cargo for chemotherapeutic agents, (ii) the enhanced permeability and retention effect was described to be limited to larger NPs (HD > 10 nm; mass > 40 kDa)<sup>7</sup> and is limited to uptake around 1% of the administered dose,<sup>8</sup> (iii) larger NPs are characterized by higher interaction with X-Rays and (iv) display longer circulation time in the bloodstream, (v) difficulties to gather imaging and therapeutic functionalities in smallest nano-objects.

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3 However, advantages of ultrasmall hybrid nanoparticles over regular nanoparticles may be key points  
4 for their further rapid translation into the clinic: (i) large nanoparticles are often severely accumulated  
5 in organs like liver and spleen<sup>9</sup> while ultrasmall NPs are rapidly eliminated by the kidneys after  
6 intravenous administration,<sup>10</sup> (ii) prolonged accumulation in organs for large nanoparticles can be  
7 associated with toxicity due to presence of metals, (iii) smaller nanoparticles are associated with  
8 better penetration in tumors,<sup>11</sup> (iv) the size of NPs has also tremendous impact on interaction with X-  
9 Rays, indeed for larger nanoparticles most of ionizing events like secondary electrons occur in the bulk  
10 of the NPs drastically reducing deposited dose around the NPs.<sup>12</sup>

### 17 **Why use gadolinium-based nanoparticles for image guided radiotherapy?**

18 Our team has shown gadolinium to be of interest for radiosensitization by developing AGuIX NPs.  
19 These NPs act as an efficient radiosensitizer under different types of irradiations (photons from keV  
20 to MeV, protons or carbons), are efficiently uptaken in tumors by the EPR effect after intravenous  
21 administration in animals or in patients with retention for more than a week (particularly adapted for  
22 fractionated radiotherapy) and are eliminated effectively by the renal clearance.<sup>8</sup> Gadolinium is a  
23 choice element for its MRI contrast agent properties to better delineate the tumor before  
24 radiotherapy which is a key factor for modern radiotherapy. Indeed, the new generation of  
25 radiotherapeutic techniques has led to the possibility to deliver very precisely increased dose in the  
26 tumor.<sup>13</sup> In these conditions, the delivery of the dose is challenging due to uncertainties of imaging,  
27 requiring precise delineation of tumors to avoid unwanted irradiation of surrounding tissues especially  
28 for tumors located close to organs at risk. The use of MRI is increasing considerably for radiotherapy  
29 planning due to its excellent contrast in soft tissues in comparison with CT (computed tomography)  
30 and the absence of ionizing radiation especially for pediatric cancer.<sup>14</sup>

31 In this perspective, the development of MRI/LINAC is a real opportunity that synergizes very well with  
32 gadolinium based compounds. Two apparatus are now on the market and display magnetic fields of  
33 0.35 T (ViewRay) and 1.5 T (Elekta),<sup>15</sup> which are relatively low in comparison to new MRI apparatus  
34 resulting in lower signal-to-noise ratios and resolution. These apparatus will highly benefit from  
35 positive contrast agents, especially nanoparticles like AGuIX that present two to three times higher  
36 longitudinal relaxivities in comparison to commercial molecular agents. In our opinion, the association  
37 of MRI-LINAC with gadolinium based nanoparticles creates synergies and can become the new  
38 standard of care for different cancer indications needing better targeting.

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55 **Conflict of interest**  
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3 FL and OT have to disclose the patent WO2011/135101. OT has to disclose the patent  
4 WO2009/053644. These patents protect the AGuIX<sup>®</sup> NPs described in this publication. FL and OT are  
5 employees from NH TherAguix and possess shares of this company.  
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# Iodine Nanoparticles

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## Status

Radiotherapy (RT) is used in about 70% of all cancer treatments. High atomic number (Z) radiosensitization is an intriguing strategy for improving RT effectiveness and could therefore benefit many patients. Tremendous efforts have explored and advanced implementation, including theoretical analyses, chemical syntheses of novel high-Z nanoparticles, in vitro and in vivo experimental studies, even some with patients. However, progression to routine clinical success is still yet to be achieved.

## Current and Future Challenges

For optimal use the requirements should include: 1) use of kV X-rays (Cho, 2005, Pignol and Lechtman, 2012,), 2) low toxicity, 3) achieving >0.5% of the heavy atom concentration (by weight) in the tumor (Loughery et al., 2019), and 4) use of IV rather than intratumoral injection (Smilowitz et al., 2018). However, not meeting some of these requirements may still result in some patient benefit. Highly loading tumors by IV delivery (requirements 2&3) is challenging since only a fraction of the injected dose will home to the tumor, even with targeting. This means that the material must be non-toxic at very high levels (grams/kg administration), a failure of most nanoparticles. Also, maximal IV tumor loading takes many circulatory passes and typically takes at least a day. The nanoparticle must therefore be larger than the kidney filter (~5nm) and have a long blood half-life, also avoiding rapid liver/spleen depletion. Very few nanoparticles have met these requirements. For example, gold nanoparticles satisfy many of the above mentioned requirements, but have very poor whole body clearance (<10% liver clearance in 6 months, Sadauskas et al., 2009) and permanently discolor the skin at the high levels needed (Hainfeld et al., 2018).

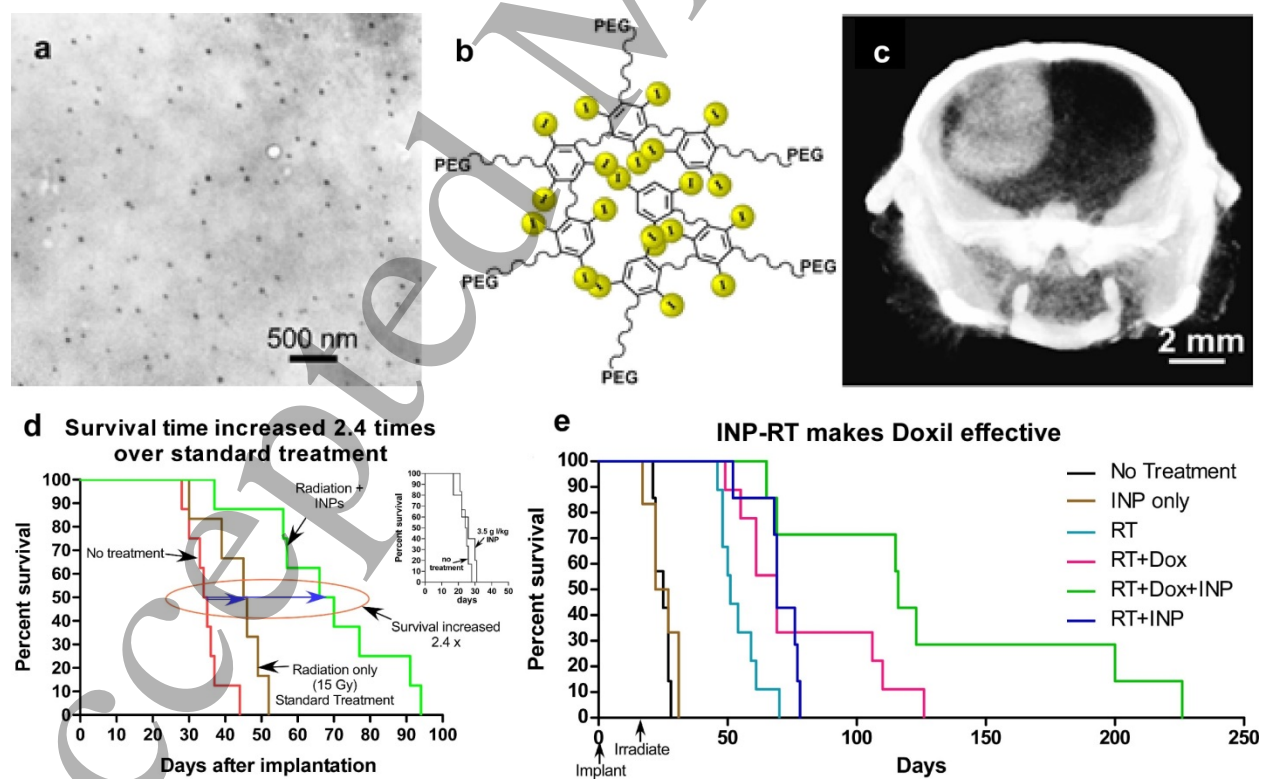
## Advances in Science and Technology to meet the Challenges

Iodine is a candidate heavy element and the successful development of non-toxic X-ray contrast media has led to radiosensitization clinical trials, originally by Norman and collaborators (Rose et al., 1999) using a modified CT, and later by Adam and collaborators (Adam et al., 2016) using a synchrotron (tumor concentration reaching 0.3%). A deficiency with this approach is the rapid kidney clearance

which compromises tumor loading and tumor to non-tumor ratios. Our solution was the synthesis of iodine nanoparticles (INPs, Hainfeld et al. 2018) which are non-toxic at 7 g iodine/kg, 20 nm in size, and show a blood half-life of 40 hours (2.7 days). The INPs (Fig. 1) are colorless and do not discolor skin and show slow but steady whole body clearance (>90% over 15 months). These INPs were found to specifically load orthotopic glioma brain tumors in mice to 0.6% and showed a doubling or more median life extension after kV irradiation compared to irradiation without the INPs. Improvements may be expected with active targeting and treatment of brain metastases where we have observed much higher levels of INP loading. We also found that INP-enhanced RT of advanced orthotopic human gliomas in mice synergizes with DOXIL chemotherapy (Hainfeld et al., 2019, Fig. 1e) raising the possibility that INP-RT may benefit other combination therapies including chemo-, immune- and oncolytic virus therapies.

### Concluding Remarks

Iodine nanoparticles therefore appear to overcome problems with many other high atomic number nanoparticles and should help advance high-Z nanoparticle radiosensitization to clinical use and patient benefit.





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3 Figure 1. Iodine nanoparticles: (a): Electron micrograph; (b): schematic; (c): accumulation in U87 glioma  
4 in mouse (microCT), (d): therapeutic efficacy, and e) INP-RT greatly increases Doxil effectiveness. Taken  
5 from Hainfeld et al., 2018, 2019.  
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Accepted Manuscript

# Optical and Photoacoustic Imaging of GNPs

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Keywords: gold, nanoparticles, imaging, optical, photoacoustics

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## Status

The use of GNPs in biomedical optical and photoacoustic imaging was facilitated by few key properties. First, gold has been known to be non-toxic and biocompatible since antiquity. Second, current methods allow production of GNPs in various sizes and shapes which enables tuning of their physico-chemical properties. Third, GNPs exhibit strong surface plasmon resonances in visible and near-infrared (NIR) regions that are ideally suited for imaging of biological specimen. Lastly, the surface of GNPs can be easily modified to introduce various functionalizations including targeting moieties or different coatings that can alter biological interactions.

We reported one of the first studies demonstrating optical imaging using GNPs conjugated with anti-EGFR antibodies for specific labeling of cancer cells in cultures and biopsies from cancer patients (Sokolov et al., 2003). This approach is based on the ability of GNPs to strongly scatter light due to excitation of surface plasmon resonances, which are dependent on size and shape of GNPs, and also physical proximity between the individual particles. We showed that molecular specific interactions of immunotargeted GNPs with cells can lead to formation of closely spaced GNP assemblies that are associated with a strong red shift in GNPs scattering spectra due to the plasmon resonance coupling (Sokolov et al., 2003, Aaron et al., 2009). This phenomenon was used for optical imaging of cancer cells with high contrast in cell cultures, tissue phantoms and animal models (Aaron et al., 2007, Aaron et al., 2009). A number of elegant studies used the effect of plasmon resonance coupling to image interactions and trafficking of biological molecules with sub-diffractive spatial resolution including dynamic behavior of integrin molecules on a cellular cytoplasmic membrane (Rong et al., 2008), transport of cell membrane receptors to endosomes (Aaron et al., 2009), and monitoring of caspase-

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3 activity in live cells (Jun et al., 2009). In parallel with the imaging studies, a theoretical framework  
4 was introduced to estimate distances between coupled gold nanoparticles that provided the  
5 foundation for the concepts of “plasmonic ruler” and “plasmon coupling microscopy” (Wu and  
6 Reinhard, 2014) as new tools in biological imaging.  
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10 GNP s also exhibit a strong two-photon luminescence (TPL) providing another dimension in  
11 biological applications that was first demonstrated in imaging of a single gold nanorod (GNR) in a  
12 mouse ear blood flow (Wang et al., 2005). Subsequently, TPL with GNPs was applied for molecular  
13 imaging of cancer cells (Durr et al., 2007). GNPs with NIR extinction spectra are ideally suited for TPL  
14 imaging *in vivo* due to increased tissue penetration in the NIR . Further, GNPs exhibit a very short, in  
15 order of tens of picoseconds, fluorescence lifetime under two-photon excitation as compared to  
16 nanoseconds for organic fluorophores that can enable high contrast in fluorescence lifetime imaging  
17 microscopy (FLIM) of cells and tissue (Zhang et al., 2010).  
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21 The absorbance property of GNPs was used to enable sensitive molecular imaging in optical  
22 coherence tomography (OCT) (Lapierre-Landry et al., 2017). In this method a modulated heating laser  
23 is used to induce highly localized oscillating changes in the refractive index of media in immediate  
24 vicinity of a GNP. These oscillations are detected with high sensitivity by OCT and allow detection of  
25 GNPs with high contrast (Lapierre-Landry et al., 2017).  
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29 Introduction of photoacoustics (PA), a hybrid modality with optical excitation and ultrasound  
30 detection, provided an imaging depth of several centimeters in tissue . PA signal is generated by a  
31 thermoelastic expansion of media around light-absorbing molecules or nanoparticles. GNPs found  
32 their application in PA imaging (PAI) due to their exceptionally high absorbance that is several orders  
33 of magnitude higher than that of organic dyes. Initial studies demonstrated the use of gold nanoshells  
34 coated with polyethylene glycol (PEG) for visualization of the vasculature in a rat brain (Wang et al.,  
35 2004). Subsequently, we showed that plasmon resonance coupling can also be used in PA for highly  
36 sensitive detection of labeled cancer cells using antibody targeted GNPs (Mallidi et al., 2007). Later,  
37 we used plasmon resonance coupling to achieve an unprecedented sensitivity of just few tens of  
38 cancer cells in lymph node micrometastases in animal models of head and neck cancer (Luke et al.,  
39 2014). PAI with GNPs also showed significant promise in cell tracking applications including  
40 mesenchymal stem cells (Donnelly et al., 2018). Further, GNPs were used in multimodal strategies for  
41 tumor detection using PAI and margin delineation by Surface-Enhanced Raman Scattering (SERS)  
42 (Kircher et al., 2012).  
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### Current and future challenges

Most of NIR absorbing GNPs showed unfavorable pharmacokinetics (PK) with a low accumulation at a target site, e.g. tumor, following *i.v.* administration. This behavior could be associated with a relatively big size, typically >60 nm, and instability of GNP coating in blood.

Generally, particles with size <5 nm are quickly excreted via kidneys, usually within several hours, whereas an increase in size is associated with a higher propensity of uptake by the reticuloendothelial system (RES) and an increased accumulation in liver and spleen where GNPs can reside for months while slowly being excreted via the hepatobiliary route (Poon et al., 2019).

Coating of GNPs with PEG or other biocompatible polymers is the most common approach to reduce opsonization and subsequent uptake of GNPs by RES. The efficiency of this protection is dependent on the density of PEG molecules on the GNP surface. However, PEGylation does not eliminate non-specific uptake by RES as polymer molecules can be displaced by physiological concentrations of cysteine and cystine (Larson et al., 2012).

High levels of accumulations and a long residence time of NIR-absorbing GNPs in liver and spleen in combination with non-biodegradable nature of gold brings up concerns about potential long term-toxicity. Most studies report no significant adverse effects of GNPs ranging in size from 12 to 85 nm. Comprehensive evaluations that take into account the whole nanoparticle construct including the coating and the geometry (e.g., size and shape) of the gold core most likely will be required for each GNP that is considered for a clinical translation.

Another common problem of NIR-absorbing GNPs is their decreased photostability due to thermal degradation caused by laser irradiation with a high peak intensity that leads to a decrease of the photoacoustic signal (Chen et al., 2010).

### Advances in science and technology to meet challenges

The thermal instability of GNPs can be addressed by a combination of advanced GNP synthesis and surface modification techniques. Specific examples include silica coating of GNRs (Chen et al., 2010) as well as synthesis of miniature GNRs (Chen et al., 2019).

To address challenges of clinical translation of GNPs, we introduced the concept of biodegradable plasmonic nanoparticles that consist of 5 nm GNPs assembled into sub-100 nm nanoclusters using biodegradable polymers (Tam et al., 2010). In a biological media the nanoclusters can fully dissociate over time into primary 5 nm particles which are favorable for a rapid clearance from the body.

Delivery of GNPs to tumor can be improved by developing better coatings. We showed that inclusion of a small alkyl chain between the gold surface and the outer PEG layer can greatly improve

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3 stability of GNPs and diminish their uptake by macrophages up to 5 days (Larson et al., 2012). Another  
4 approach is based on the use of erythrocyte membranes as a GNP coating that was shown to decrease  
5 GNP uptake by macrophages (Gao et al., 2013).  
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### 8 9 **Concluding remarks**

10 Intricate dependence of plasmon resonances of GNPs on their geometry, interparticle distance and  
11 environment has led to innovative directions in development of biosensors, bioanalytical assays and  
12 cell imaging tools. There is also continuous flow of exciting reports of preclinical imaging and  
13 therapeutic developments involving GNPs. Future adoption of GNPs in the clinic will rely on  
14 optimization of GNPs pharmacokinetics *in vivo* and a better mechanistic understanding of GNPs  
15 toxicity and clearance. Applications of GNPs for intravital imaging, cell tracking, and therapy will likely  
16 undergo further exploration with emphasis on multimodal imaging and theranostic approaches.  
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### 24 **Acknowledgements**

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26 The authors acknowledge support from Cancer Prevention and Research Institute of Texas (CPRIT  
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# In Vivo Imaging of Metallic Nanoparticles Using Benchtop X-ray Fluorescence Computed Tomography Techniques

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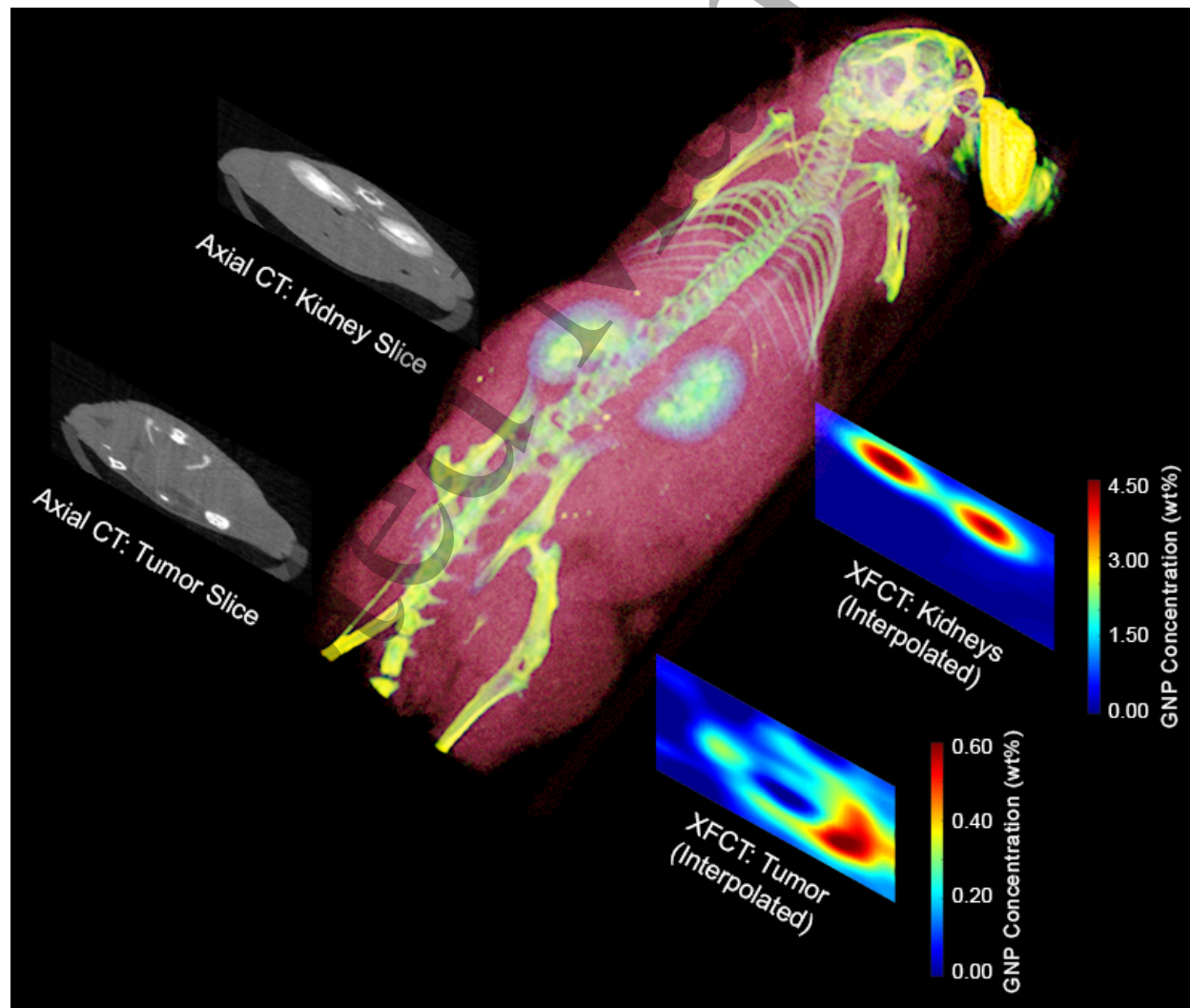
Author contact: Sang Hyun Cho (scho@mdanderson.org)

## **Status**

Over the last decade, x-ray fluorescence (XRF) computed tomography (XFCT), traditionally a synchrotron x-ray imaging modality, has also been implemented with ordinary polychromatic x-ray sources (*viz.*, “benchtop XFCT”), aiming to enable routine XFCT imaging of biological objects (e.g., small animals) under typical biomedical laboratory settings. In particular, it was the absence of a proper quantitative imaging tool for small animal studies involving high atomic number ( $Z > 50$ ) metallic nanoparticles, such as gold nanoparticles (GNPs), which prompted the initial efforts on benchtop XFCT development with small animal-sized phantoms (Cheong *et al.*, 2010; Jones *et al.*, 2012). The feasibility of extending benchtop XFCT beyond GNP imaging has been demonstrated through similar phantom imaging studies using high-Z probes such as platinum, gadolinium and iodine (within the context of multiplexed imaging) (Kuang *et al.*, 2013a; Kuang *et al.*, 2013b), as well as low-Z molybdenum NPs (Hertz *et al.*, 2014).

To date, a clear pathway towards the fulfillment of the aforementioned goal of benchtop XFCT imaging has been well established, for example by the first successful animal imaging study

(albeit performed postmortem) using an experimental benchtop XFCT system and GNPs (Manohar *et al.*, 2016). More recently, another postmortem animal imaging study has demonstrated the feasibility of benchtop XFCT imaging of mice injected with molybdenum NPs (Larsson *et al.*, 2018). Additionally, very recent publications have reported benchtop XFCT and XRF imaging/mapping of live mice injected with gadolinium NPs (Zhang *et al.*, 2019) and GNPs (Jung *et al.*, 2020), respectively. Despite these reports and other on-going (unpublished) efforts, *in vivo* imaging with benchtop XFCT is still in its infancy. In particular, the feasibility of performing whole-body imaging of live animals using benchtop XFCT techniques, while meeting the key requirements for routine applications (to be described below), has yet to be demonstrated.



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3 **Figure 1.** Illustration of the possibility of quantitative multimodal imaging of GNP distributions in an animal  
4 using benchtop XFCT and conventional CT together. 3D volume rendering of the mouse from postmortem  
5 CT data along with smoothed XFCT images and corresponding axial CT images of the kidney and tumor  
6 slices (Reprinted with permission, Manohar *et al.* 2016).  
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11 According to a recent experimental study (Manohar *et al.*, 2018), a practical benchtop XFCT  
12 system with transmission CT capability can be built for whole-body imaging of small animals (e.g.,  
13 ~3-cm diameter mice) containing GNPs at relatively low concentrations on the order of several  
14 hundred parts-per-million (ppm) (e.g., 0.3 mg of GNPs per gram tissue). In general, such a  
15 benchtop XFCT system would be considered acceptable for *in vivo* imaging of small animals, if it  
16 could satisfy the realistic constraints of x-ray dose and scan time (e.g., less than ~40 cGy and 1  
17 hour per imaging session, respectively) for *in vivo* imaging.  
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### 28 **Current and future challenges**

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30 Compared with synchrotron XFCT, benchtop XFCT is typically performed with relatively higher  
31 energy but less intense polychromatic x-ray sources, resulting in less efficient XRF photon  
32 production and detection. This aspect directly affects the material detection limit (or system  
33 sensitivity) at a given x-ray dose as well as the overall scan time (or data acquisition time) of a  
34 benchtop XFCT system. The consequences from this aspect are more pronounced for benchtop  
35 XFCT performed with relatively high x-ray tube voltages (e.g., > 100 kVp or average energy of  
36 ~80 keV), due to increased scatter of higher energy photons that complicates the XRF signal  
37 extraction from the scatter background. Thus, there is a strong motivation to adopt  
38 monochromatic/quasi-monochromatic x-ray sources, if available on a benchtop setting, to  
39 overcome the inherent difficulties of benchtop XFCT. For example, published computational  
40 studies (Manohar *et al.*, 2014; Ahmad *et al.*, 2014) suggested more than one order of magnitude  
41 increase in the XRF signal per a given x-ray dose or the sensitivity, with the use of such x-ray  
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3 sources (vs. polychromatic x-ray sources) for excitation of gold K-shell XRF photons from a GNP-  
4 containing small animal-sized phantom.  
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9 Another critical technical challenge arises from the current difficulty in parallel acquisition (using  
10 array or pixelated detectors) of XRF signals, which would be essential to accomplish *in vivo*  
11 imaging of small animals using a benchtop XFCT system under the given constraints of x-ray  
12 dose and scan time. Additionally, XFCT requires fully spectroscopic data with sufficient energy  
13 resolution (e.g., < 1 keV energy bin), in order to achieve highly sensitive quantitative imaging of  
14 both known and unknown elements present within the imaging objects. For benchtop XFCT, this  
15 requirement has been met reasonably by adopting a commercial, energy-resolving,  
16 thermoelectrically-cooled, single-crystal cadmium telluride (CdTe) detector (Cheong *et al.*, 2010).  
17 In fact, custom-array detectors (or multiple detectors working in parallel) using this detector can  
18 easily be adopted for benchtop XFCT, lowering the scan time and x-ray dose inversely  
19 proportional to the number of detectors (Jones *et al.*, 2012). Nonetheless, there is a practical  
20 limitation for this approach in terms of the number of detectors that can be deployed into benchtop  
21 XFCT setups built on smaller footprints for the purpose of small animal imaging. Thus, despite a  
22 few known/expected difficulties (e.g., inferior energy resolution/charge sharing effect), pixelated  
23 detectors may be found more advantageous for parallel XRF signal acquisition than arrays of  
24 single crystal detectors.  
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45 The remaining technological challenges for benchtop XFCT reside mainly with optimization of  
46 various hardware components and operating parameters. While not specifically  
47 discussed/referenced in this article, numerous investigations have contributed to research  
48 advances in these aspects. Additionally, there are a number of issues that are uniquely associated  
49 with *in vivo* imaging but have not been seriously investigated thus far. Such issues include  
50 application-specific optimization of metallic NPs (in terms of material/size of NPs as well as  
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3 conjugation of targeting moieties), multimodal (e.g., CT+XFCT) and/or multiplexed imaging, and  
4 effects of NP pharmacodynamics/pharmacokinetics on benchtop XFCT scanning and image  
5 reconstruction.  
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### 10 11 **Advances in science and technology to meet challenges**

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13 Currently, laser-driven x-ray sources producing sufficiently intense monochromatic x-rays, based  
14 on inverse Compton scattering, are available on a relatively small footprint and have been  
15 considered for various applications including x-ray CT (Achterhold *et al.*, 2013). Also, other special  
16 x-ray sources that offer quasi-monochromatic spectra based on intense XRF lines (von Busch *et*  
17 *al.*, 2005; Hertz *et al.*, 2014) have been used for benchtop XFCT. Although attractive in terms of  
18 their x-ray spectra, these sources are associated with some limitations (e.g., x-ray energy and  
19 flux/output, size, cost, etc.) similar to those of synchrotron XFCT, which may need to be carefully  
20 considered if benchtop XFCT is intended primarily for preclinical imaging. Meanwhile, as practical  
21 alternatives, carefully designed metal filters, especially in conjunction with high-power (e.g., > 1  
22 kW) x-ray sources, or crystal-based approaches utilizing the Bragg diffraction (e.g., the use of  
23 highly oriented pyrolytic graphite), can also be pursued to tailor the polychromatic x-ray spectrum  
24 for benchtop XFCT imaging of high-Z probes including GNPs (Manohar *et al.*, 2014).  
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41 While readily available, typical pixelated photon-counting detectors provide only a limited number  
42 (~4-5) of rather broad energy bins/windows (in a few keV intervals) and, consequently, may not  
43 meet the detector specifications for ideal benchtop XFCT imaging. Nevertheless, there have been  
44 attempts to adopt some of them for benchtop XFCT (Yoon *et al.*, 2016; Ren *et al.*, 2016; Li *et al.*,  
45 2017), providing some insight into the technical feasibility and reconfirming some benefits of  
46 benchtop XFCT previously demonstrated (e.g. better sensitivity than transmission CT) (Bazalova  
47 *et al.*, 2012; Manohar *et al.*, 2016). Overall, these initial attempts have commonly revealed  
48 somewhat expected consequences from the insufficient detector energy resolution, most notably  
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3 the difficulty in achieving highly sensitive benchtop XFCT. In theory, such consequences may be  
4 avoided or at least mitigated by adopting fully-spectroscopic pixelated detectors providing higher  
5 energy resolution than conventional photon-counting detectors (Seller *et al.*, 2011). Nonetheless,  
6 it remains to be seen whether or not this expectation can be realized under the typical operating  
7 conditions for the whole-body imaging with benchtop XFCT.  
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16 Considering the research advances on the two key issues discussed above (as well as other  
17 issues mentioned earlier but not specifically discussed here), it will soon be possible to produce  
18 a benchtop XFCT system (with simultaneous or sequential CT capability) that can allow for whole-  
19 body imaging of live animals while meeting realistic scan time/x-ray dose constraints. A fully-  
20 optimized benchtop XFCT system (with an ordinary polychromatic x-ray source) will likely enable  
21 several fold improvement in the sensitivity and scan time (e.g., detection limit of ~100 ppm of gold  
22 and less than ~20 minutes of scan time, in the case of GNP imaging), compared to the currently  
23 available systems.  
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### 32 33 34 **Concluding remarks**

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37 Benchtop XFCT is now nearing to become a practical preclinical imaging modality. Further  
38 improvement beyond the currently anticipated technical specifications would still be possible,  
39 depending on future research advances.  
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### 45 **Acknowledgments**

46  
47 This work was supported by the US National Institutes of Health under the award number  
48 R01EB020658. The author acknowledges the former and current lab members as well as  
49 research collaborators for their invaluable contributions to the development of benchtop XFCT  
50 techniques and systems.  
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# Micro- and Nanodosimetry of MNPs

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## Status

In irradiated biological cells containing metallic nanoparticles (MNPs), the presence of the MNPs leads to a local enhancement of the radiation interaction probability. Generally, the atoms in the MNP have a higher atomic number ( $Z$ ) than the atoms constituting biomolecules and water. Hence, they have higher interaction cross sections. Furthermore, radiation interaction with the MNPs not only leads to the production of additional secondary electrons but also to the occurrence of Auger cascades. Auger electrons with energies in the range of few keV deposit most of their energy in the proximity of the MNPs. This implies, in principle, that steep energy deposition gradients may occur as well as short-range changes of the radiation quality (particle track structure) on the nanometer scale (McMahon *et al* 2011).

So far, the investigation of these physical radiation effects of MNPs has only been possible by numerical simulations using Monte Carlo codes. Until recently, many common Monte Carlo codes did not allow track structure calculation. Therefore, most published studies have focused on the calculation of the enhancement of the absorbed dose around the MNP, where results may differ by several orders of magnitude among the published studies even for similar simulated setups (Li *et al* 2020). Only few simulation studies have investigated other quantities like the radiation damage due to low-energy electrons which lead to clustered ionizations at the nanometric scale (Gargioni *et al* 2016, Dressel *et al* 2017, 2019).

## Current and future challenges

Simulations of microdosimetric and nanodosimetric effects of MNPs face the same computational challenges as the host of simulations are aimed at determining the local enhancement of absorbed dose. The most important of these challenges is related to the small dimensions of the nanoparticles which imply a small probability for individual particle tracks or trajectories to intersect with the nanoparticle volume. Therefore, realistic simulations of physical radiation effects require the CPU-intense simulation of large numbers of primary particle histories or the application of suitable variance reduction techniques. However, seemingly

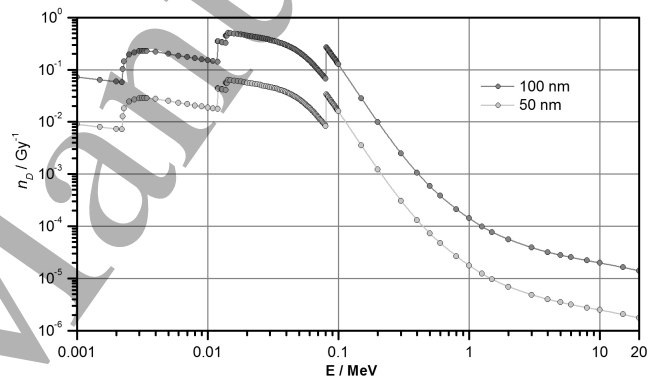


Figure 1. Photon energy dependence of the number of photon interactions per absorbed dose in spherical gold nanoparticles of 50 nm and 100 nm diameter.

1 straightforward solutions such as reducing the incident field size may lead to simulation scenarios where the  
2 results are strongly biased by a lack of secondary particle equilibrium (examples can be found in (Li *et al* 2020,  
3 Dressel *et al* 2017)). For simulations of dose enhancement by MNPs in photon fields, the lack of contribution of  
4 energy depositions from secondary particles that would be produced in an extended radiation field by photon  
5 interactions outside the confined region of the simulated primary photon field, can be estimated from literature  
6 data of photon interaction cross sections (Rabus *et al* 2019). For track structure simulations, a corresponding  
7 correction approach may also exist but has not yet been identified. The multiscale nature of the problem means  
8 that there is a need to consider energy deposition within micro/nano-scopic dimensions across macroscopic  
9 length scales (Zygmanski and Sajo 2016, Martinov and Thomson 2016).

10 Another computational challenge for micro- and nanodosimetry of MNPs is related to the fact that in addition  
11 to the stochastics of the electron tracks emerging from the MNPs, an additional stochastic factor comes into  
12 play which is related to the probability that an MNP in a biological cell interacts with the incident radiation.  
13 Figure 1 shows the photon energy dependence of the probability per dose that a single gold MNP of 100 nm  
14 and 50 nm diameter will experience a photon interaction. (This probability scales with the third power of the  
15 MNP diameter.) It is quite evident that for realistic values of absorbed dose the probability is generally  
16 significantly lower than unity such that the effect of the Poisson statistics of the photon interactions with the  
17 MNPs needs to be considered.

18 For microdosimetric computations this may not be an issue if the considered targets (cell nucleus or  
19 chromosome domains) are large enough to contain a large number of MNPs. For smaller target sizes in the  
20 nanometer range and for nanodosimetry, on the other hand, there may be a need to focus on conditional  
21 specific energy distributions and cluster size distributions for the vicinity of particular MNPs (that experienced  
22 a photon interaction).

23 In addition to these methodological issues, there is also the general challenge of computational micro- and  
24 nanodosimetry related to the different cross section models used by different codes and to potential additional  
25 interaction channels, such as collective excitations within the MNP (Verkhovtsev *et al* 2014). Different cross  
26 section models have been shown to lead to drastic differences particularly between results for nanodosimetric  
27 quantities (Villagrasa *et al* 2019). Additional quantum mechanical effects such as the Heisenberg uncertainty  
28 principle further challenge the accuracy at short length scales and very low energies (Thomson and Kawrakow  
29 2011, Liljequist and Nikjoo 2014). A proper assessment of the uncertainties of such simulation results probably  
30 cannot be only based on comparison of computational approaches. It will rather be necessary to develop and  
31 perform benchmark experiments that test the predictions of the codes. A more long-term challenge that goes  
32 even further would be the development of experimental approaches measuring nanodosimetric cluster size  
33 distributions in the presence of MNPs directly.

### 34 **Advances in science and technology to meet the challenges**

35 Several groups around the world are working on improving the cross-section data used in simulation codes for  
36 applications involving nanoparticles (Wälzlein *et al* 2014). Some of the augmented data sets include effects like

changes in the surface plasmons that were predicted theoretically (Verkhovtsev *et al* 2014). For the case of interactions of MNP with protons, experimental validation of the cross sections by comparison to direct measurements of the yield of emitted electrons has already been performed (Hespeels *et al* 2019). For photon irradiation, similar studies are in progress. Such experiments require a careful consideration of the stochastic aspects of the radiation interaction with MNPs.

Direct measurement of the influence of MNPs on microdosimetric spectra have been proposed using avalanche-confined tissue-equivalent proportional counters that are capable of simulating nanometric site sizes (Mazzucconi *et al* 2020). The idea is to build such detectors with an exchangeable cylindrical outer electrode and to use electrodes coated with MNP on the inner surface or incorporating MNPs in the material of the electrodes.

Figure 2 shows illustrations of two other approaches to directly measure the enhancement of radiation effects by gold nanoparticles on DNA-based detector structures. The first approach is based on the so-called DNA-dosimeter concept where DNA strands are used as resistive elements in nano-circuits and strand breaks are detected by changes in the electrical conductivity (Heimbach *et al* 2017). In contrast to this early work, DNA scaffolds would be used to add MNPs in defined positions from the DNA conductors to study the change in yield of radiation-induced strand breaks in the presence of MNP in functional dependence on their distance from the target.

The second approach illustrated in the lower panel of Figure 2 would use the DNA origami folding technique to produce a base from which oligonucleotides are protruding that are terminated by large molecules, such as biotin. These biotin molecules can be detected by atomic force microscopy (AFM). Here, radiation damage in form of DNA strand breaks would be detected (in a destructive manner) by the disappearance of object spots in the AFM images.

The influence of MNPs would be studied in the same way as before, i.e. by comparing test structures without nanoparticles with structures where MNPs are incorporated at defined positions. The advantage of the second approach would be that measurements can in principle also be performed in a liquid-water environment to also study the influence of MNPs on the production of free radical species.

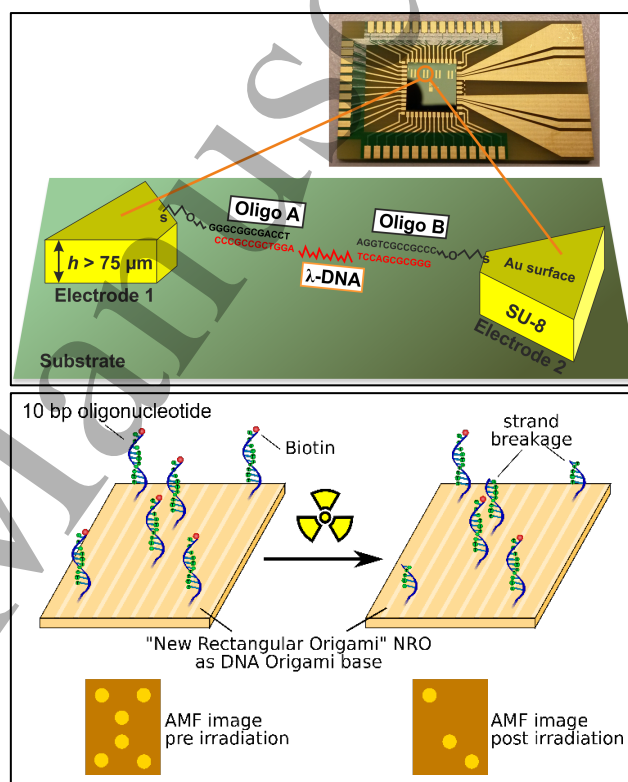


Figure 2. Illustrations of two proposed experimental approaches for measuring the nanodosimetric effects of gold MNPs on DNA-based structures. For details see text.

### Concluding remarks

The envisioned experiments described above will not only be useful for validating simulations of the physical microdosimetric and nanodosimetric effects of MNPs but also, in the long run, such experiments may be further developed to provide a comprehensive set of benchmark data for the complete simulation chain, including the physical, physico-chemical, and chemical stages. This would allow fixing some of the simulation parameters such as, for instance, the contribution of molecular excitation by very low energy electrons to direct strand breaks or the probability of breaking the backbone chain by reactions with hydroxyl radicals. In this way, they may contribute to a synoptic picture of the radiation effects of MNPs that encompasses their physical, chemical and physico-chemical contribution to the radiobiological enhancement of irradiation on the micrometer and nanometer scale.

### Acknowledgments

We acknowledge Woon Yong Baek from PTB and Jussi Toppari from Jyväskylä University (Finland) for contributing their ideas for measurement of radiation effects of nanoparticles using DNA origami structures, Valeria Conte from INFN (Italy) for sharing her ideas regarding microdosimetry of nanoparticles, and Heike Nittmann for preparing the respective illustrations.

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# Can Physics Explain the Radiation Enhancement Effects of MNPs ?

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## Status

When localized in the vicinity of tumor cells, high Z metallic nanoparticles are thought to enhance energy deposition upon radiation treatment. One has first to distinguish between radiation types. keV photons often used in radiobiology studies cause the strongest effect: in this energy range, most of the interactions with the surrounding media occur via the photoelectric effect (PE) which is proportional to  $Z^3/E^3$ . Accordingly, impinging photons trigger more interactions in metal material than in the surrounding water. The effect is further reinforced through the deexcitation cascade: the created vacancy will be filled by outer shell electrons, leading to the emission of characteristic X-rays or low energy Auger electrons. The combination of photoelectrons, Auger electrons and to a lesser extent characteristic X-rays is believed to add extra dose outside MNPs [1]. The effect is spread at the nanometer level (as the range of Auger electrons is limited to <100 nm) and extends micrometers away from MNP due to the other secondaries which typically have energies close to the primary photons. For instance, the gold K-edge at 80.7 keV adds a boost in the PE coefficient if the primaries energy is chosen accordingly. However, in the MeV range, the mass absorption coefficient for water and gold are similar. The probability to enhance the deposited dose is then reduced and limited to the cascade effect upon gold ionization. The combination of photon radiation and MNP thus results in local radiation hardening and pushes the modality toward a high linear energy transfer (LET) type.

The PE mechanism explained above is often put forward to justify the observed enhancement after X-ray irradiation. Indeed, the ratio of the mass attenuation coefficient of gold to water can reach 160 near the gold K-edge [2]. However, a number of experimental studies reported significant radiosensitization effects when gold nanoparticles (GNPs) were used at concentrations as low as 0.003 % of gold per mass of tissue (weight percent, WP) [3]. Most simulation studies report predictions using typical 0.1 to 1 WP, an overrated amount when considering the possible transition to human medicine. According to this amount, it is possible to derive a more realistic mass attenuation coefficient for a homogeneous mixture of water and gold.

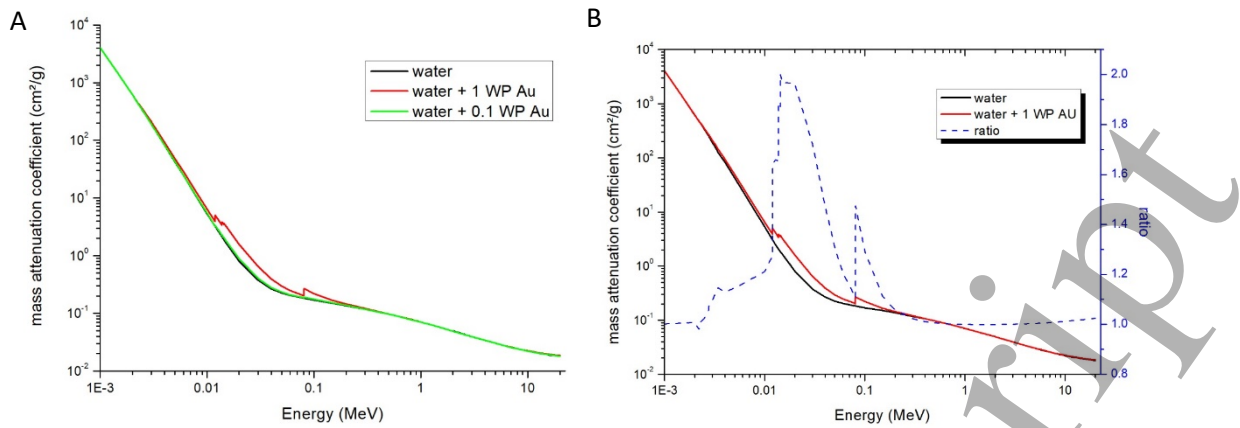


Figure 1 (A) Mass attenuation coefficient for water (black), water with 1% gold mass (red) and water with 0.1% gold mass (green) (B) Mass attenuation coefficient for water (black), water with 1% gold mass (red) and corresponding ratio according to energy (blue)

Figure 1A displays the mass attenuation coefficients with 1 and 0.1% gold mass. 0.1 WP is not sufficient to produce a significant change in the mass attenuation coefficient. However, the K and L edges of gold are discernible if the concentration is increased to 1 WP. The corresponding ratio is presented in Figure 1B, with a strong peak in the keV range as expected, although with a maximum twofold increase.

In addition to limits in the gold amount uptake in cells, it has to be noted that the probability for a GNP to incur PE will not only depend on the gold mass attenuation coefficient but also on its size, with the smallest being the worst case. Lin et al. simulated the number of electrons produced through photon interaction with a directly targeted 50 nm GNP and reported values in the 10<sup>-5</sup>-10<sup>-4</sup> range per incoming photon at best [4]. A similar finding was highlighted in a simulation study of McMahon et al. [5]. Over 99% of GNP present in a cell do not contribute to the dose enhancement effect, even if the few incurring ionizations produce dramatic spikes in dose.

In the past decade, proton therapy centers flourished around the world and the question of dose enhancement thanks to GNP was raised again. The radioenhancement mechanism provided by GNPs originate from mere gold ionizations (with associated cascade), the stopping power in gold being greater by a factor from 2 to 10 compared to water. However, the qualities of generated electrons differ according to the energy spectra of incoming protons. The maximum energy transferred to an electron at rest is given by  $Q_{max} = \frac{2\gamma^2 m V^2}{1 + \frac{2\gamma m}{M} + \frac{m^2}{M^2}}$ , with  $M$  and  $V$  the mass and the velocity of the proton, respectively,  $m$  the mass of the electron and  $\gamma$  the relativistic factor. In patients, most created electrons have energies in the range of hundreds of keV near the proximal end of the tumor while rare

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3 electrons reach a few keV at the distal end. The result is again radiation hardening but in a very  
4 localised fashion around GNPs, giving rise to local high dose peaks.  
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7 Given their higher LET and ionization density, the number of projectiles required to achieved a  
8 therapeutic dose of 2 Gy is quite small (about 200 for 25 keV/ $\mu\text{m}$  protons, depending on cell size) and  
9 unlikely to hit nanoparticles at all [6]. Although higher energy protons are more numerous for a 2 Gy  
10 dose, this low interaction probability is not increased, given the lower stopping power. Indeed, for  
11 protons in the 1-250 MeV range the secondary electron emission due to GNP was found to be  
12 insignificant compared to the keV photon modality [7]. Similarly, Lin et al. reported very low  
13 interactions probabilities per Gy for SOBP protons ( $\sim 10^{-8}$  to  $10^{-4}$  for the 2-50 nm GNP range) [8, 9].  
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### 21 **Current and future challenges**

22 In general, biologically observed enhancement values are higher than predicted [10].  
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25 However, Monte Carlo codes used to compute these predictions often rely on incomplete Auger  
26 cascades. For instance, the deexcitation module after proton ionization in Geant4 is only available up  
27 to the M shell. Vacancies created in outer shells are thus not followed and the potential increase in  
28 dose is not taken into account. This is not a major issue for photon irradiation simulations. However,  
29 medium and high LET protons are unable to ionize inner shells as they deliver a few keV at most and  
30 most ionizations will take place in the outer shells. Moreover, Auger relaxation is the most probable  
31 in this case, as its probability increases with increasing shell number (fluorescence yield = 0.0245 for  
32 the M shell in gold). It is thus very likely that proton simulations performed with Geant4 so far  
33 underestimated the yield of Auger electrons for high Z targets.  
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40 In addition, some codes, like Geant4, rely on condensed history models. These models are more  
41 adapted for macroscopic volumes and electron tracking for energies above a few hundred keV. Geant4  
42 models G4BetheBlochModel and G4BraggModel do not take into account the generation of electrons  
43 below the mean ionization potential of the medium (790 eV for gold) which leads to an  
44 underestimation of low energy electron yield. This underestimation has been recently highlighted by  
45 comparison with experimental results for proton impact on gold nanoparticles. To reproduce low  
46 energy electron emission spectra from a gold target, a single interaction approach, like the one used  
47 in TRAX, NOREC and Geant4-DNA, should be preferred [11, 12].  
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### **Advances in science and technology to meet challenges**

The reliability of track structure codes depends on the implemented interaction cross section sets which are not easily accessible for low energy projectiles. Validation for the TRAX cross sections has been performed indirectly by reproducing experimental data of backscattered electron emission from gold film and GNPs [13]. Since the electron energy distribution is anisotropic, data are still needed in the forward direction where a larger amount of high energy electrons is expected.

As pointed out in numerous experimental works, the spatial distribution of GNPs in cells is not homogenous as assumed in many simulation studies. Aggregation of GNPs is often observed and could decrease radioenhancement by trapping produced electrons hence adding a buffering effect. Accurate representations such as GNP packing in vesicles distributed in cytoplasm should be preferred [14]. In addition, there is a crucial need for the modelling of more realistic geometries at the cell level, with a detailed description of components such as organelles or DNA structure. TOPAS-nBio was recently released to help the understanding of radiobiological processes at the cell and sub-cellular levels, also including a large set of chemical reactions [15]. The inclusion of the cytosol scavenging properties would represent another step forward.

Current findings also raise questions concerning the radiosensitization mechanism which do not seem to be solely driven by physical effects. The great disparity between physical dose enhancement predictions and the experimentally observed radiosensitization effects suggests a biological component to the radiosensitization mechanism. Increasing evidences supporting this concept are piling up highlighting the key role played by the oxidative stress in the radioenhancement effect. In recent articles, Daems et al. and Penninckx et al. demonstrated that gold nanoparticles have the ability to inhibit thioredoxin reductase (TrxR) and glutathione reductase, regulators of redox reactions, in cancer and normal cells [16-18]. TrxR inhibition induces a cell weakening effect prior to radiation exposure, which if timed wisely can lead to a radiosensitization effect. In the context of personalized medicine, genomic assays for mRNA TXNRD1 expression in tumor biopsies can thus drive the application of GNP radiosensitization in the clinic.

### **Concluding remarks**

The preceding discussion points out that although there is a moderate radioenhancing effect demonstrated for keV photons, additional dose deposited when using MeV photons or protons is limited. The development of single interaction Monte Carlo simulations and accurate geometries should shed light on this matter. However, the biological component seems preponderant in the MNP

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3 action mechanism. More studies are needed to assess the impact of MNP on biological pathways in  
4 the absence of radiation, such as regulation of genes involved in DNA repair, slowing down of cell  
5 proliferation or disruption of the cellular redox balance. The scientific community should thus  
6 reconsider the original theory of physical enhancement and encompass the role of MNPs on decreased  
7 detoxification potential of cells.  
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# Role of Low Energy Electrons in Local MNP Radiation Enhancement

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## Status

It is well known that electrons are emitted from the impact of energetic particles or photons on a metal surface. A similar behavior is expected from a metal nanoparticle (MNP) [1,2]. For photons, the mass-energy absorption coefficient is larger by roughly two orders of magnitude in the 10-80 keV range, compared to that of biological tissue [3]. The yield of emitted electrons, which extends from a few eV to energies close to the primary photon energy [2], is proportionally increased in magnitude. However, the electron energy distribution depends not only on the material, but also on the size and shape of the MNP. Electrons emitted with energies above the ionization potential of the medium produce a further generation of lower energy electrons. For initial charged particles, the secondary electron distribution lies essentially in the low-energy (0-30 eV) range due to their production via the excitation of plasmons in the MNP [4]. According to recent calculations, the number of these low energy electrons (LEEs, < 30 eV) produced by high-energy protons interacting with a gold NP (GNPs) would be about an order of magnitude higher than that from an equivalent water volume and not very dependent on the primary particle energy [4]. In contrast, the high electron-emission efficiency of X-ray-irradiated MNPs is restricted to initial energies below 300 keV [3].

Considering the range of electrons in water [5] and the spherical geometrical factor, most of the LEE density lies within submicrometer distances from the surface of a MNP irradiated with fast charged particles or 10-80 keV photons [1, 2]. These LEEs are the most numerous reactive species created around the MNP and carry a large portion of the energy absorbed by the metal. They strongly react with the surrounding medium, mainly via a resonant interaction, which leads to their temporary capture by molecules or moieties of large biomolecules. This capture leads to the formation of molecular transient anions (TA) that decay by reemitting the captured electron (i.e., autoionization) or by dissociating (i.e., dissociative electron attachment) [6]. Since autoionization can leave the target

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3 molecule in a dissociative electronic state, both channels can break bonds and damage biomolecules  
4 [6]. However, in complex molecules, such as DNA, electron transfer between basic units increases the  
5 complexity of the damage mechanism [7].  
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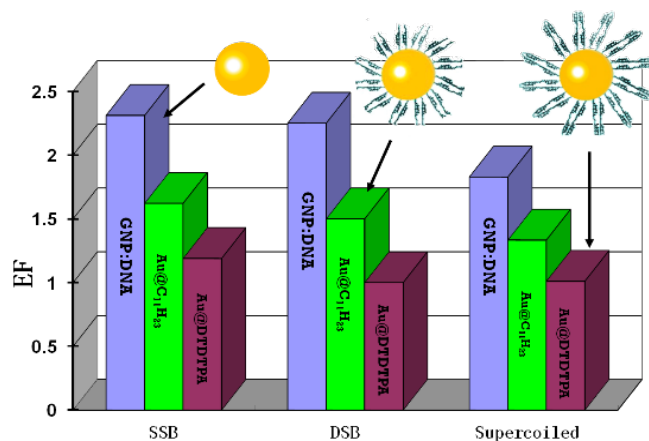
8 During the last two decades, there have been numerous experimental and theoretical studies on  
9 the interaction of LEEs with gaseous or complex condensed biomolecules, ranging from amino acids,  
10 peptides, DNA bases, sugar and phosphate groups, to oligonucleotides of short DNA strands and  
11 bacterial DNA [6-10]. The condensed-phase targets usually consisted of self-assembled monolayers  
12 and multi- or sub-monolayer films of the biomolecule deposited on a conductive substrate. The type  
13 of LEE-induced damages in DNA, analyzed by various techniques, included single and double strand  
14 breaks, base damage and cleavage, and clustered lesions, consisting of single strand breaks and base  
15 damages [6,7,10]. From yields vs energy curves, it was generally concluded that the decay of TAs into  
16 destructive channels played a major role in inducing these lesions [6-9].  
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23 Fig. 1 shows damage enhancement factors (EFs) resulting from irradiation of DNA-GNP complexes  
24 with 60-keV electrons. In these experiments, 5-nm diameter GNPs were bound in ratio 1:1 to plasmid  
25 DNA [10]. For production of a potentially lethal lesion, such as a DSB, bare GNPs produce an EF of  
26 about 2.3, as shown by the left column (blue) of the middle group in Fig. 1. With GNPs coated with  
27 ligands of 2.5 to 4 nm, the corresponding EFs for DSBs are reduced to 1.5 (green column) and 1.0 (red),  
28 respectively. This result corroborates theoretical predictions on the electron distributions from fast  
29 charged particles [4], as it implies that energetic electrons interacting with MNPs generate essentially  
30 LEEs of extremely short ranges (<10 nm). When a similar experiment is performed with 200-keV X-ray  
31 irradiation of 5-30 nm diameter PEG-coated GNPs, longer ligands (~11 nm) are needed to obtain only  
32 a 60% reduction in EF [11], as expected from the larger thermalization range of the X-ray  
33 photoelectrons [2,4,5].  
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#### 43 **Current and future challenges:**

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45 Despite the numerous publications that have appeared in the last decade on MNPs, we still don't  
46 have any experimental results showing the actual distribution of electrons emitted from isolated  
47 MNPs of different sizes, interacting with various types of high-energy ionizing radiation. The present  
48 electron distributions rely on theoretical and model calculations [1,2, 4]. Many parameters need to be  
49 included and refined in these calculations, so that, both at the experimental and theoretical level, the  
50 generation of accurate electron distributions in biological media remains a challenge.  
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54 The efficient use of MNPs in Radiotherapy is limited by the nanoscale range of most emitted  
55 electrons and their secondaries, unless the MNPs can be targeted to vital cellular molecules, such as  
56 DNA. Otherwise, the MNPs only serve to increase the energy deposited and radicals randomly created  
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**Figure 1.** EFs for the formation of SSBs, DSBs and loss of initial DNA configuration induced by 60 keV electrons in GNP-DNA complexes with ratio 1:1. In each group of three histograms, the EFs correspond to enhancement factors for a specific DNA damage, when GNPs of 5 nm diameter bound to DNA are bare or coated with C<sub>11</sub>H<sub>23</sub> or DTDTPA (i.e., dithiolated diethylene triaminepentaacetic) ligands.

in the cytoplasm or outside the cell. Moreover, to take advantage of the high electron-emission coefficient of 10-80 keV photons, conventional X-ray Radiotherapy must be limited to superficial tumors.

### Advances in science and technology to meet the challenges

Electron-energy distributions emitted from irradiated gaseous MNPs could be measured using high-resolution electron energy analysers. Photoemission spectra measurements of free core-shell nanoparticles [12] and X-ray photoelectron spectroscopy of isolated NP beams [13], which already exist to investigate X-ray photoelectron emission from non-metallic NPs of different sizes, could possibly be adapted to vaporized MNPs. On the other hand, the present distributions could be improved by more elaborate calculations of (1) the slowing down of electrons within the MNP, particularly in the low-energy regime; (2) the number of electrons emitted per initial interaction, (3) the number of electrons that recombine with the MNP and (4) the interaction with the MNP of electrons backscattered around and into the nanospheres. In such computations in biological media, it would be advantageous to consider the electrostatic potential created between the MNP and the escaping electron, including the charge-induced polarization of the medium and the multiple positive charges created in the MNP, which in turn influence the number and energy of emitted electrons.

Targeting of specific components of cancer cells (e.g., the nucleus) is possible with vector molecules that bind to MNPs [14,15]. However, for maximum efficiency, the MNP should be either coated with the shortest possible ligand, or be only partially coated, to reduce as much as possible, attenuation of

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3 *the short-range highly-destructive LEEs.* Finally, it should be noted that efficient X-ray therapy need  
4 not be limited to superficial tumors, if the MNP is accompanied by a therapeutic radioisotope. In other  
5 words, MNPs could improve localization of radiation energy in targeted radionuclide therapy (TRT)  
6 [15,17,19], where the emitted short-range particles are often accompanied by the emission of longer-  
7 range photons [16] that could be partially absorbed by the metal and produce LEEs. GNPs have already  
8 been complexed with chelators that bind to Auger-electron and beta-particle emitters used in TRT  
9 [15,17]. Encapsulation of such radionuclides in gold nanocages was also suggested to convert into LEEs  
10 particle radiation and the accompanying photon flux, which reaches healthy tissues during TRT [18].  
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### 20 **Concluding remarks and acknowledgments**

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22 Considering future quantification of the species produced around irradiated MNPs and ongoing  
23 refinement in the methods of targeting them to cancer cells, via intravenous administration of carriers  
24 [15, 19, 20] or direct intratumoral injection [21, 22], such particles should become highly efficient for  
25 treating tumors and their metastasis. These methods are also promising for extending the field of  
26 radiobiology to the nanoscopic level.  
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### 34 **Acknowledgements**

35 Financial support was provided by the Canadian Institute of Health Research (PJT-162325). The  
36 author is indebted to Dr. Fred Currell, Yi Zheng, Balder Villagomez and Andrew Bass for informative  
37 discussions.  
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# Modeling of MNP Effects with Monte Carlo

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## Status

The Monte Carlo (MC) method is a class of computational methods that simulates a process by randomly sampling its variables. For the modeling of Metallic Nanoparticle (MNP) effects, the radiation

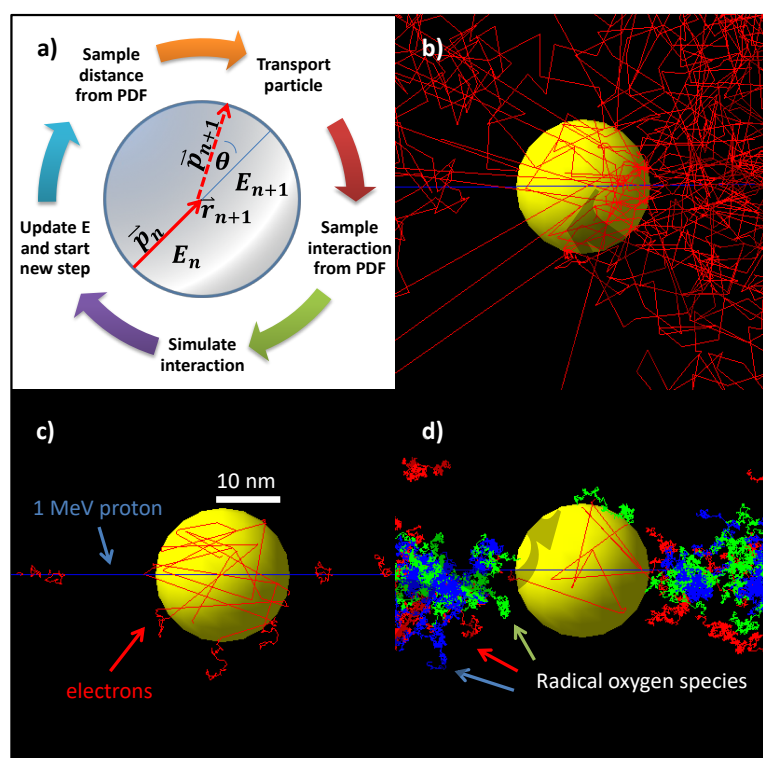


Fig. 1 a) MC codes simulate radiation transport by numerically calculating the change in position  $\mathbf{r}$ , momentum  $\mathbf{p}$  and energy  $\mathbf{E}$ . The transport of a 1 MeV proton through a 20 nm gold nanoparticle surrounded by water is shown for MC simulation using b) only CH physics processes in 10 nm steps (Geant4 Livermore EM physics), c) using TS in water (Geant4-DNA) and d) with water radiolysis included.

transport is simulated by sampling the probability density functions (PDFs) of various electromagnetic interactions. MC codes use two types of transport calculations: The condensed history (CH) approach groups multiple scattering processes together to reduce computation time for larger volumes, whereas the discrete model simulates the track structure (TS) event-by-event to calculate radiation interactions stepwise on a micro- and nanometer-scale. Examples of TS codes are NOREC, PARTRAC, KURBUC, TRAX, NASIC, general-purpose MC codes using CH are EGS and FLUKA, and PENELOPE,

MCNP and Geant4, which have also been extended with TS simulation capabilities for to low-energy processes in cellular media (Incerti, 2016a and references therein). The Geant4-DNA TS extension, for example, simulates electron interactions down to a few electronvolt (eV) in liquid water and has

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3 become popular in the medical physics research community as its features are also readily available  
4 through user-friendly interfaces such as TOPAS and GATE. MC simulations have been used to obtain  
5 electron emission spectra and radial dose distributions for single or clustered MNPs ( $^{26}\text{Fe}$ ,  $^{47}\text{Ag}$ ,  $^{64}\text{Gd}$ ,  
6  $^{78}\text{Pt}$  and  $^{79}\text{Au}$ ) for various geometrical parameters and radiation modalities (Zygmanski and Sajo,  
7 2016, McMahon *et al* 2015).

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10 While the physical dose enhancement by MNPs might be negligible for megavolt photons and particle  
11 beams, their impact in the chemical stage might be significant (Rudek *et al* 2019). Several MC codes  
12 offer simulation of radiolysis in a pure water environment (PARTRAC, Geant4-DNA, TRAXchem,  
13 KURBUCchem, RITRACKS and TOPAS-nBio). The increase in yield of radical oxygen species (ROS) scales  
14 with the physical dose enhancement when radial dose distributions are folded with radiolysis  
15 simulations in the surrounding water (Tran *et al* 2016). Experimental work demonstrated adverse  
16 effects such as catalytical production of hydroxyl at GNPs and binding of scavengers but also beneficial  
17 effects such as a reduction of ROS by superoxide dismutase or peroxide catalase. The actual chemical  
18 reactions of ROS and scavengers at the MNP surface are so far ignored in all MC codes. As MC codes  
19 already struggle with computational costs of pure water radiolysis, an implementation of such surface  
20 reactions that depend on dose, temperature and the chemical environment will dramatically increase  
21 the parameter phase space and is unlikely to happen anytime soon.

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24 Recently, the microscopic dose distribution shifted into focus to investigate biological effects of GNP  
25 distributions on cellular structures. The sensitivity enhancement predicted with an adaptation of the  
26 local effect model produced higher sensitization with kilovolt photons if GNPs were internalized into  
27 the cell (McMahon *et al* 2011). However, sensitization for megavolt photons and protons was only  
28 significant when GNPs were internalized into the nucleus (Lin *et al* 2015a). Organelles other than the  
29 nucleus such as the mitochondrial DNA may also be considered a target because some GNP  
30 configurations accumulate on the surface of mitochondria leading to locally increased energy  
31 deposition within the organelle (McNamara *et al* 2016, Kirkby and Ghasroddashti 2015). Imaging the  
32 experimental GNP distributions as input for MC simulations showed how important the geometry of  
33 radiosensitive structures and GNP clustering is for the prediction of cell survival (Sung *et al* 2018).  
34 Similarly, high dose spikes at the vascular wall, especially for hypo-fractionated treatment, have been  
35 found for accumulation of GNPs in the vesicles as typically observed in in-vivo experiments (Lin *et al*  
36 2015b).

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39 Simulations of macroscopic GNP distributions have also been carried out to show pathways of  
40 clinical translation. In virtual phantom scenarios, photon beam energies were optimized for arc therapy,  
41 and found to be dependent on GNP uptake, beam arrangements, and patient geometries (Koger and  
42 Kirkby 2018, Sung and Schuemann 2018).

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### Current and future challenges

Currently, TS MC codes are limited to simulations in water. Physics models need to consider the full deexcitation of electronically-excited heavy elements such as via long Auger cascades, simulate discrete ionization events and electron transport with low cut-off energies and benchmark these processes with updated cross section databases and scattering experiments on metallic nanostructures.

In addition, more systematic experiments are necessary to describe the chemical processes with MNPs. MC codes need to be extended to the chemical stage as recent experiments point out a significant impact of nanoparticles on radiolysis and scavenging of ROS. The implementation of selected test cases orientated on experimental benchmarks promises a major step forward in understanding the indirect effect by MNPs.

One significant challenge is that the calculation speed is slow not only for chemistry but already when only taking into account discrete physical processes compared to the condensed history approach.

Ultimately, the MC codes will be compared to experiments in living material. Cellular structural information is still limited due to current imaging capabilities. For example, transmission electron microscopy (TEM) only provides 2D structures for dead cells. Fluorescence probes may alter the original physiological conditions of the cell. Moreover, those studies are restricted only for in-vitro cell experiments.

### Advances in science and technology to meet challenges

Recently, the full deexcitation cascade in GNPs can be activated in Geant4, however, the initial ionization is limited to the K, L and M shells and the increase in total emitted energy was below 1% for all tested modalities (Incerti, 2016b). Discrete electron transport in gold has been implemented in Geant4-DNA and will be available in a future release (Sakata *et al* 2018). Recent versions of MCNP6 and PENELOPE include extended cross-sections down to 10 eV and 50 eV and calculate slightly higher doses than Geant4-DNA within the first few nm from the surface of the GNP (Jung *et al* 2018). Discrete electron transport in copper is available in PARTRAC (Dingfelder and Travia 2015) and an extension to gold is expected soon. However, molecular dynamics approaches such as the MBN Explorer will possibly allow to analyze the structure, composition and dynamics of atomic clusters and coated nanoparticles (Solov'yov *et al* 2012).

For ionization by particle beams, a discretization is not yet implemented in Geant4 and the cut-off at rather high mean ionization energies in the Bethe-Bloch theory for heavy elements compared to light elements is a possible source for the underestimation of dose enhancement observed for MNPs. A

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3 discretized ionization model based on cross sections calculated in the Binary Encounter Approximation  
4 as used in TRAX showed an increase of low-energy electron emission (<1 keV) of up to two orders of  
5 magnitude (Hespeels, 2019) which will push the overall dose enhancement closer to enhancements  
6 observed in cell experiments with particle beams. An inter-comparison such as the recent exercise by  
7 the European RAdiation DOSimetry (EURADOS) group is a helpful approach to ensure quality control  
8 of new implementations and various available codes. (Li *et al* n.d.)  
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11 Additionally, full track structure simulations with a mechanistic approach will offer a different method  
12 to the phenomenological models in the simulation setup. This approach may provide more  
13 mechanistic biological endpoints such as DNA breakages (J Schuemann *et al* 2018).  
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16 To improve the computational speed, new codes written for Graphics Processing Unit (GPU) cards have  
17 been developed and cloud computing, wherein computing resources are allocated from a third party,  
18 can also resolve the computational costs in track structure Monte Carlo simulations (Wang *et al* n.d.).  
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21 For a more realistic simulation setup, advanced molecular imaging is necessary to acquire more  
22 accurate 4D cellular structures. Clinical trials of nanoparticle-mediated radiotherapy will offer data on  
23 tumor control probability and normal tissue toxicity. Using imaged biodistributions in Monte Carlo  
24 simulations will help to interpret these new data.  
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### 30 31 32 **Concluding remarks**

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34 Monte Carlo simulations provide a comprehensive description of physical dose enhancements by  
35 MNPs. Advances in the physics modeling will further improve the accuracy especially in the low-energy  
36 range. The future challenge, however, lies in the implementation of indirect pathways via ROS and the  
37 modeling of biological damage and repair. Both approaches require substantial improvement of the  
38 underlying models, the available cross sections and the efficiency of the calculation. Future Monte  
39 Carlo studies should then be carefully designed to complement in-vivo and in-vitro observations.  
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### 45 46 **Acknowledgments**

47 BR acknowledges support from the German Research Foundation (DFG), project no. RU 2197/1-1.  
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# Chemical Effects in NPs Induced Radio-Enhancement

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## Abstract:

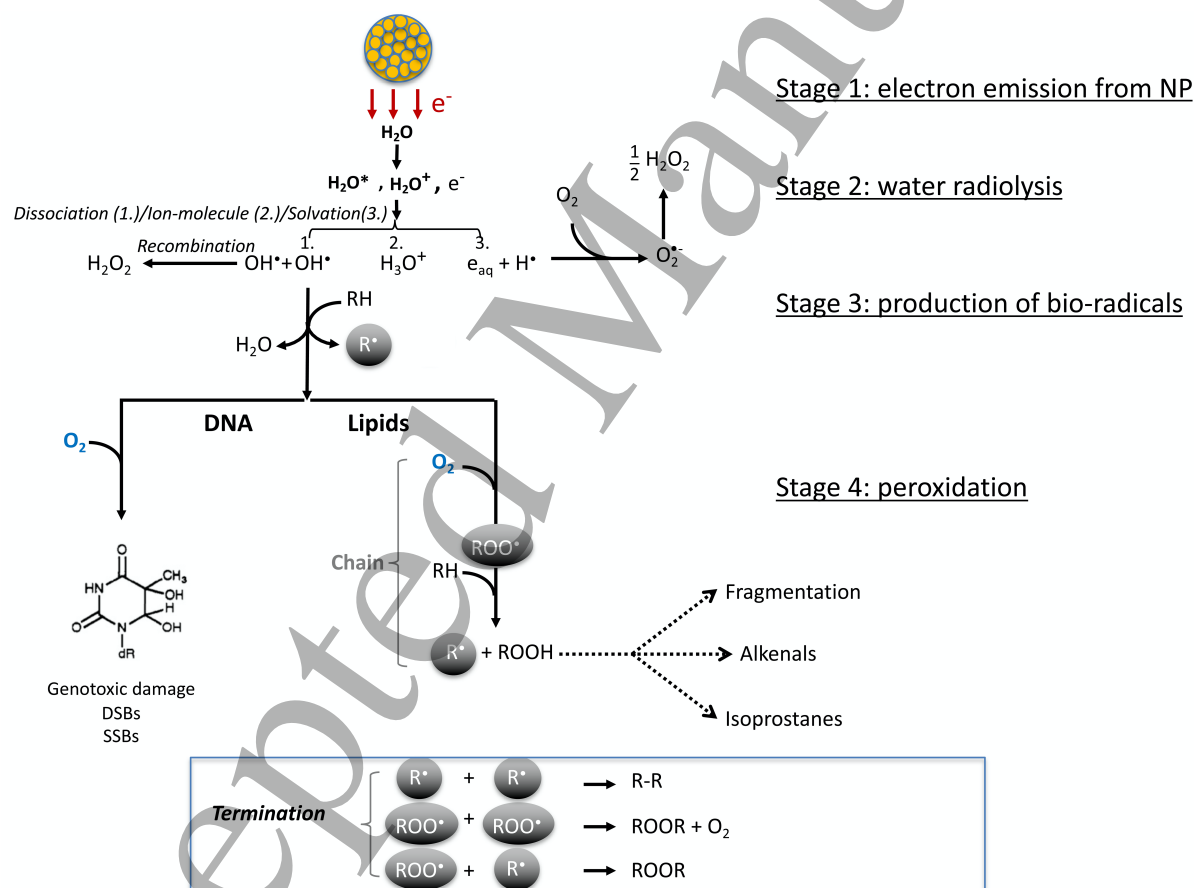
Nanoparticle enhanced radiation therapy is a breakthrough in the development of cancer treatment, which mainly aims at enhancing radiation effects (called radio-enhancement) in tumors selectively and can at the same time be used for tumor diagnosis or drug delivery using the same agent. The underlying principle of NP radio-enhancement is the amplification of radiation induced electron emission into a nanoscale volume using efficient electron emitters such as high-Z compounds, thereby amplifying the induction of lethal (complex) biological damage and the biological efficiency of radiation treatments. Various experimental and theoretical studies consolidated this description. However, the role of water radicals issued from the interaction of electron with surrounding water molecules, and, more surprisingly, the influence of molecular oxygen on the chemical reactions consecutive to the NP activation, are not yet much described in the existing models. This overview summarizes the chemistry that counts for the radio-enhancing effects of NPs.

## Status:

Tumor targeting of radiotherapy treatments and improvement of the therapeutic index for radioresistant cases remain great challenges. Adding nanoparticles able to improve radiation effects (radio-enhancers) into tumors is proposed as a promising strategy. The principle of this method is to use high electron emitters to amplify the local electron emission when activated by ionizing radiation. So far, complexes or nanoagents composed of high-Z atoms such as gold, platinum, gadolinium (AGuIX in particular, see Lux et al. (Lux *et al* 2018)) or hafnium have proven to be efficient radio-enhancers (Lux *et al* 2018, Porcel *et al* 2010, Bonvalot *et al* 2017). The advantage of small nanoparticles compared to metallic complexes stems from their capacity to concentrate in tumors via the Enhanced and Permeability Retention Effect (EPR). They also offer a high flexibility of surface functionalization, which allows implementing various modalities within the same agent (MRI by adding paramagnetic atoms,

PET using isotopes, cell imaging or other fluorescent based techniques by grafting various fluorescent markers) (Truillet *et al* 2016). The fast development of a radio-enhancement strategy – already used in clinic (see F. Lux *et al.* (Lux *et al* 2018)) – requires better understanding and simulation of the phenomenon with the goal to improve the design of new nanoagents and an implementation of this strategy in treatment planning systems. The difficulty of this work stems from the multiscale character of the phenomenon ranging from the early stage activation of the processes ( $<10^{-16}$  sec, nanoscale) to the impact in vivo (minutes to hours, macroscale). In between these scales sits the chemistry stage, which is often absent from the models although it is expected to play a crucial role in the observed outcome.

Below, the stages of radio-enhancement in the presence of NPs are described. The third and fourth steps are present in all radiation schemes of biological systems but were not yet investigated in the presence of NPs. Figure 1 illustrates the physical and chemical stages expected in the NPs induced radio-enhancement.



**Figure 1: Representation of chemical reactions induced after activation of NPs and consecutive amplification of electron emission: from water radiolysis to the production of water radicals and the induction of oxidized byproducts.**

### Step 1: Physical stage

The physical stage of the radio-enhancement effect has been extensively described and reviewed (Kuncic and Lacombe 2018).

Briefly, the radio-enhancement is attributed to a local amplification of electron emission due to the high ionization cross section of high-Z atoms compared to water. The activation of NPs by incident photons is initiated via the photoelectric effect for photons of energies below approx. 100 keV, and Compton effects for photons of higher energies. NPs may also be activated by incident charged particles, namely fast ions (protons or carbon ions as used in proton-and hadron-therapy), or electrons produced in the medium. In this case, Coulombic interactions are responsible for the activation of NPs (Kuncic and Lacombe 2018, Lacombe *et al* 2017). This model explains the improvement of radiation effects observed when NPs are added in the treatment of animals by x-rays or protons (Hainfeld *et al* 2004, Kim *et al* 2010). This effect was found to be correlated with the amplification of tumor cell killing when cells are treated with NPs prior to irradiation (Butterworth *et al* 2010). Using biomolecular probes in solution (without any metabolic effect), it was shown that the induction of nanosize lesions is enhanced in the presence of NPs (Butterworth *et al* 2008, Porcel *et al* 2012). This demonstrated the physical character of the NPs induced amplification of radiation lethality.

### Step 2: Chemical stage – water radiolysis

The production of water radicals by photon, ion or electron irradiations and the impact on living organisms is a very well-known phenomenon. In contrast, very few studies focused on the relative role of water radicals versus the role of the direct interaction with incident radiation in the radio-enhancement effect. The amplification of the radical production by NPs has been evidenced in experiments performed in water (Sicard-Roselli *et al* 2014) and in solution containing biomolecules (Porcel *et al* 2010). The few studies performed with cells using radical scavengers reported that, on average, hydroxyl radicals (OH), the most powerful oxidants of water derivatives ( $E^{\circ}=(^{\bullet}\text{OH}/\text{OH}^{\bullet}) = 1.90$  V at pH=7), account for 60-90% of the amplification of cell killing induced by high-Z compounds activated by high energy photons or ions (Usami *et al* 2008, Jeynes *et al* 2014). In parallel, it was demonstrated that the increase of nanosize lesions (>2 nm) is also due to OH (Porcel *et al* 2010, Butterworth *et al* 2008, Porcel *et al* 2012). So, the production of hydroxyl radicals is a key component of the radio-enhancement phenomenon. So far, very few simulation studies exist that consider this stage in simulation models (e.g. Geant 4 DNA (Schuermann *et al* 2019) and references therein, and TRAX for incident ion effects (Boscolo *et al* 2018)).

### Step 3 and 4: Chemical stage – production of organic radicals and peroxidation

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3 The chemistry that follows the production of hydroxyl radicals, strongly depends on the presence of  
4 molecular oxygen in biological systems. This phenomenon is well documented (Chapman *et al* 1973,  
5 Edwards *et al* 1984). However, the influence of NPs on these chemical reactions has never been studied  
6 nor even considered as important factor. In the view of future prospective, we remind here the basic  
7 principles of the chemistry that could be impacted by the presence of NPs.  
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13 Hydroxyl radicals ( $\cdot\text{OH}$ ) produced during the radiolysis phase (step 2) rapidly and efficiently undergo  
14 reactions with the main organic constituents (RH) of biological systems, namely, nucleic acids, proteins  
15 and lipids. These reactions lead to the production of carbon-centred radicals ( $\text{R}\cdot$ ) through hydrogen  
16 atom abstraction of a range of aliphatic or conjugated substrates RH. These species ( $\text{R}\cdot$ ) are highly  
17 reactive and short-lived ( $t_{1/2} < 5$  ms in mammalian cells in complete absence of oxygen).  $\text{R}\cdot$  have a high  
18 affinity for  $\text{O}_2$  yielding peroxyradicals ( $\text{ROO}\cdot$ ). This reaction is in competition with the annihilation of  $\text{R}\cdot$   
19 by electron donors (radical scavengers) that afford a high degree of radioprotection.  
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25 The interaction of hydroxyl radicals ( $\cdot\text{OH}$ ) with DNA (Chapman *et al* 1973, Cadet *et al* 2005) and with  
26 lipids (Sevanian and Hochstein 1985) has been extensively reported. Peroxyradicals formed from DNA  
27 ( $\text{ROO}\cdot$  with R corresponding to a nucleic acid chain) decay through bimolecular, tetroxide-based  
28 pathways in pyrimidines or 2'-deoxyribose ending in alcohols, aldehydes, cetones or bond breakage  
29 with frequent release of superoxide ( $\text{O}_2^{\cdot-}$ ) and oxygen in the singlet excited state ( $^1\text{O}_2$ ). Owing to lateral  
30 diffusion in phospholipid membranes, peroxyradicals formed from unsaturated lipids, namely lipid  
31 peroxy radicals ( $\text{ROO}\cdot$  with R corresponding to a lipidic chain) are able to abstract a hydrogen atom  
32 from other lipid molecules initiating a chain reaction. Chain termination is achieved by interaction of  
33  $\text{ROO}\cdot$  with scavengers (i.e.  $\text{H}\cdot$ ) yielding alkyl hydroperoxides ( $\text{ROOH}$ ) or by radical-radical recombination  
34 yielding organic peroxides ( $\text{ROOR}$ ) (Sonntag 1987). Disproportionation may also take place. Little is  
35 known of the fate of peroxyradicals formed from proteins. The well-known radiosensitizing effect of  
36 oxygen stems from these chemical cascades. Consistently, the partial pressure of oxygen in irradiated  
37 tissues is a crucial determinant of radiosensitivity.  $p\text{O}_2$  ranges between 1 and 10% in normal tissues,  
38 which defines normoxia, whilst the presence of hypoxic regions in solid tumors ( $p\text{O}_2 < 0.5\%$ ) explains  
39 the low therapeutic index of radiotherapy.  
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45 One study already reported that NPs have low effect when cells are irradiated in anoxic conditions ( $p\text{O}_2$   
46 close to 0) (Jain *et al* 2014). This result questions the efficiency of NPs to improve the treatment of  
47 hypoxic cancer cases. Thus, further experiments are needed to better characterize and quantify the  
48 effects of NPs depending on the cell type (if any) and their chemical environment. The localization of  
49 NPs in cell compartments (nucleus, lysosomes, mitochondria) may influence this effect.  
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3 In summary, the chemistry involved in NP induced radio-enhancement is crucial to characterize with  
4 the goal of designing more competitive agents and monitoring their impact in various tissues. This  
5 requires new investigations and theoretical models, which enclose reactions driven by water radicals  
6 and by molecular oxygen.  
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## 10 11 **Acknowledgements**

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15 This work was supported by the European Commission with the project ARGENT (FP7-  
16 PEOPLE-2013-ITN- 608163), by the Université Paris Saclay with the “Initiative de  
17 Recherche Stratégique” (IRS) NanoTheRad.  
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# Modelling the Biological Effects of Metal Nanoparticles

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## Status

The use of metal nanoparticles to enhance radiation therapy was originally proposed on very simple physical grounds – due to their high density and atomic number, heavy metals absorbed significantly more radiation than soft tissue, increasing the dose deposited and improving clinical outcomes. This effect can be readily quantified using Monte Carlo modelling on the macro- (Cho 2005, McMahon *et al* 2008) and nano-scales (Jones *et al* 2010, McMahon *et al* 2011).

However, while significant radiosensitisation was observed experimentally, it rapidly became clear that the observed effects differed significantly from those predicted on this simple physical basis. Two major observations were made: i) many experiments reported significantly greater enhancements than those predicted by physical dose modification alone; and ii) significant enhancement was observed with megavoltage photon irradiations, with only negligible differences in dose deposition (Chithrani *et al* 2010, Jain *et al* 2010, Butterworth *et al* 2012). These observations have been confirmed in numerous systems, clearly demonstrating that biological processes underlie experimental radiosensitisation, schematically illustrated in Figure 1. However, the exact nature of these processes is not well known. A range of different possibilities have been implicated by different studies, with some of the main candidates illustrated in Figure 1.

Additional sensitisation due to nanoscale dose enhancement has been by far the most extensively modelled process. As described elsewhere, models of MNP-radiation interactions make it clear that they have a highly heterogeneous impact on dose distributions on the nanoscale (Jones *et al* 2010, McMahon *et al* 2011). Analogies were drawn between these dose distributions and charged particle therapy, where similar sub-cellular localisation of dose leads to greater biological effects (Christian *et al* 2018).



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3 These effects have been extensively studied in charged particle therapy, with a number of  
4 models proposed to understand these effects, such as the Local Effect Model (LEM) (Elsässer  
5 *et al* 2008) and the Microdosimetric Kinetic Model (MKM) (Hawkins 2003). These models  
6  
7 have been applied to GNP-enhanced therapy, and demonstrated MNP dose heterogeneity  
8  
9 leads to greater biological effects and increased sensitisation for a range of radiation types  
10  
11 (McMahon *et al* 2011, Lechtman *et al* 2013, Lin *et al* 2014).  
12  
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14 However, such approaches involve assumptions about the uptake and distribution of MNPs  
15 throughout the cell which are not readily verified experimentally. While some progress has  
16  
17 been made through the use of detailed cell-level imaging enabling the prediction of cell-  
18  
19 specific dose distributions and associated radio-sensitisation effects, it is increasingly  
20  
21 apparent that nanoscale dosimetry cannot explain the full variation of experimental data.  
22  
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24 A major limitation of these models is that they typically consider MNPs as otherwise inert  
25  
26 modifiers of radiation dose, in contrast with experimental data showing that MNPs can  
27  
28 modify a range of chemical and biological processes. These effects may further modulate the  
29  
30 radiosensitivity of cells. For example, many nanoparticle preparations have significant  
31  
32 physico-chemical activity, acting as both sources and scavengers of free radicals (Ionita *et al*  
33  
34 2008), which is known to significantly impact on the yield and type of DNA damage,  
35  
36 potentially driving sensitisation at all energies.

37 Even in the absence of radiation, MNPs are not biologically inert, and have been shown to  
38  
39 impact on cells in a range of lethal and sub-lethal fashions. These include the induction of  
40  
41 DNA damage as single agents (Kang *et al* 2010), upregulation of cell death pathways  
42  
43 (Djurišić *et al* 2015), and disruption of oxidative stress regulation (Dayem *et al* 2017). These  
44  
45 effects have been shown to sensitise cells to other DNA damaging agents, underscoring that  
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47 MNPs can affect response even in the absence of physical dose modification (Zheng and  
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49 Sanche 2009, Jain *et al* 2010).  
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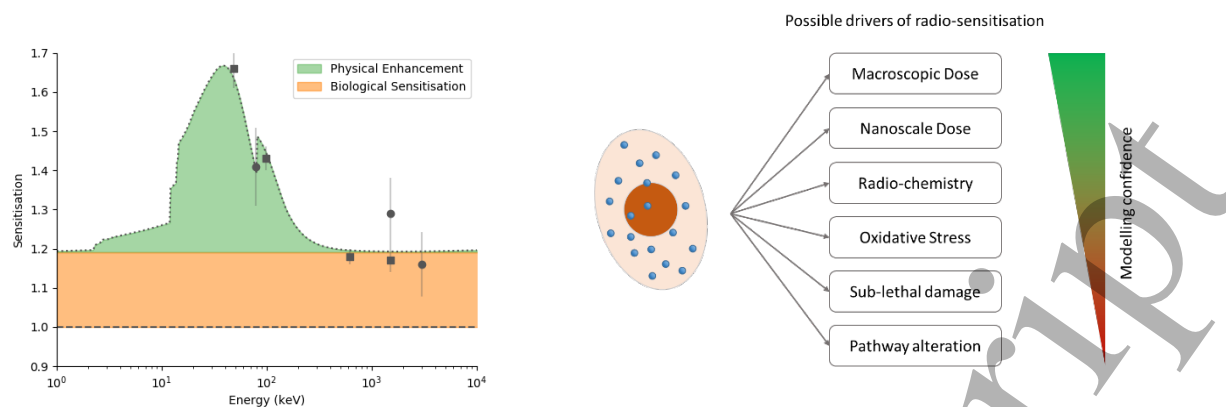


Figure 1 Left: Interplay between physical and biological sensitisation. Although MeV physical enhancement is expected to be only a few percents of keV enhancement, megavoltage sensitisation has been reported (squares: Chithrani 2010, circles: Jain 2010), indicating a large component of biological sensitisation. The absolute and relative magnitudes of the physical and biological effects are strongly MNP dependent, and are not easy to extrapolate between systems. Right: Illustration of possible drivers of radiationsensitisation in MNP-treated cells. These range from well-understood physical processes (top) to biological effects with almost no modelling (bottom).

Despite the importance of these effects— particularly at clinically-relevant energies – they are much less understood than physical dosimetry, and have seen little or no quantitative modelling. This represents a significant limitation in our understanding of MNP-based therapies.

### Current and Future Challenges

A major challenge in modelling MNP sensitisation is the extreme heterogeneity of particle types and model systems. While a small number of commercial MNPs have been used in multiple studies, most studies use locally-produced nanoparticles, frequently with specifically developed coatings and targeting molecules. While the dosimetric impact of these particles is not significantly affected by this functionalisation, it can profoundly change their biological activity. While uptake and localisation can be incorporated in physical dosimetry, purely biological effects are not meaningfully represented. In addition, the majority of published studies focuses on limited endpoints (e.g. cell killing). Together, this means there is insufficient evidence to fully characterise cellular toxicity and radiation sensitisation, and identify underlying mechanisms for each nanoparticle preparation. This challenge is compounded by the difficulty of translating *in vitro* observations into *in vivo* systems. Here, differences in the pharmacokinetics of different preparations and their impact through the course of a full treatment schedule must also be taken into account.

As a result, modelling in this area remains under-conditioned, with ad-hoc analysis of limited datasets producing models which are in reasonably good agreement with fitting data, but

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2  
3 which do not translate effectively to other systems. This approach is unlikely to generate  
4 models with wider applicability to support the development and optimisation of novel MNP  
5 treatments.  
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### 10 11 **Advances in science and technology to meet challenges**

12 The most efficient way to characterise a single nanoparticle preparation, remains an ad-hoc  
13 approach – that is, treating the nanoparticles as simple dose-modifying agents, using standard  
14 techniques to measure the additive and synergistic effects of the MNPs with radiation, and  
15 fitting a suitable empirical model. This has the advantage of being simple, and less  
16 experimentally demanding. Studies of the dependence of MNP radiosensitisation on  
17 irradiation type and energy may also enable a separation of “physical” and “biological”  
18 components of sensitisation. However, such ad-hoc approaches are not suitable for more  
19 general predictive tools. Instead, more general predictions require the development of  
20 relevant, systematic, mechanistic models to enable the integration of many heterogeneous  
21 datasets to produce useful predictions for a range of MNP.  
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31 A number of groups are beginning to develop tools which may address some of these  
32 limitations. For example, realistic Monte Carlo models of cellular DNA may enable the  
33 accurate prediction of radiation-induced DNA damage, integrated with models of free radical  
34 chemistry to incorporate radiation indirect effects (Meylan *et al* 2017, Lampe *et al* 2018). As  
35 these models mature, they may offer the capacity to incorporate MNPs as mediators of both  
36 direct and indirect damage providing a natural opportunity to understand these effects.  
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42 By contrast, models of purely biological mechanisms of MNP radiosensitisation remain  
43 limited. Although a number of individual models have considered some mechanistic aspects  
44 of DNA repair (Taleei and Nikjoo 2013, McMahon *et al* 2016, Henthorn *et al* 2017), in many  
45 cases these cannot easily incorporate the biological perturbations introduced by MNP. As a  
46 result, alternative approaches may be required to fully understand MNP radiosensitisation.  
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51 Systems biology and machine learning techniques present one alternative to more  
52 mechanistic model development. While these approaches have not seen wide application in  
53 MNP radiosensitisation, they have been applied to understand nanoparticle uptake and  
54 toxicity (Winkler *et al* 2014, Costa and Fadeel 2016). These may provide a framework to  
55 enable the complexity of cellular biology to be incorporated alongside mechanistic physical  
56 predictions in MNP radiosensitisation.  
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## Concluding Remarks

While it is clear that a large portion of MNP radiosensitisation is driven by biological effects which are largely independent of the physical dose modification, modelling in this area remains a significant weakness. Designing an optimised nanoparticle therapy necessitates the development of new approaches. This may involve the development of more mechanistic descriptions of MNP-cell-radiation interactions, or machine learning and systems biology approaches to leverage heterogeneous datasets for novel insights.

Regardless of the approach, there remains a pressing need for more high-quality, systematic experimental investigation of the impact of MNP across all stages of radiation response. Moreover, efforts should be made to present data in as accessible a format as possible, to enable incorporation of these disparate datasets in unified models.

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Accepted Manuscript

# Molecular Mechanisms of Metal Nanoparticle (MNP)-Mediated Radiation Enhancement

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## 1. Status

The unique physicochemical properties of high atomic number (Z) metal nanoparticles (MNPs) have made them attractive as potential radiosensitizers in cancer radiotherapy based on their strong photoelectric absorption coefficients in comparison to soft tissue. Since the seminal study by Hainfeld and colleagues [1], which demonstrated a significant increase in the survival of mice irradiated following injection of 1.9 nm gold nanoparticles (GNPs), a large body of preclinical evidence gained across multiple different MNPs, tumour models and radiation sources, has demonstrated the clear potential of MNPs as radiosensitizers [2,3]. However, these data are confounded as the majority of experimental observations cannot be accurately predicted from physical parameters including mass energy absorption and MNP concentration. This raises important questions concerning the underlying mechanisms of MNP-mediated radiosensitization which is not primarily driven by increased total dose delivered to target cells [2].

Over the past decade, the field has made concerted efforts aiming to resolve the disparity between predictions of MNP radiosensitization and observed biological effects. This has involved detailed studies of the underlying molecular mechanisms of MNP radiosensitization for gold, silver, platinum, hafnium and gadolinium particles. Our group has largely focussed on understanding the radiobiological effects of gold nanoparticles (GNPs) in studies using model systems which have evolved from simple plasmid DNA through to cellular



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3 systems and *in vivo* tumour models (Butterworth *et al* 2008, Jain *et al* 2011, Butterworth *et al*  
4 2016). For the first time, we demonstrated GNP-mediated radiosensitization at MV energies,  
5  
6 an unexpected finding due to the dominance of Compton effects in the MV energy range which  
7  
8 are unrelated to Z, and strongly implicated biologically driven radiosensitisation [5].  
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12 Subsequent reports from our laboratory and others have aimed to delineate the  
13  
14 molecular mechanisms of MNP-mediated radiosensitization (Reviewed in Butterworth *et al*  
15 2012, Rosa *et al* 2017). The majority of experimental reports have highlighted oxidative stress,  
16  
17 DNA damage response and cell cycle effects as critical response mechanisms (summarised in  
18  
19 figure 1). Furthermore, we reported that significantly elevated levels of DNA damage may be  
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21 a direct result of impaired mitochondrial functional manifested by increased oxidation and loss  
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23 of membrane potential [8].  
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29 Considering the broad range of experimental parameters and end-points investigated, it  
30  
31 is difficult to draw overall conclusions on a critical mechanism of action for MNP- mediated  
32  
33 radiosensitization. Clearly, the underlying mechanisms are the products of multiple and  
34  
35 complex physical, chemical and biological interactions, that ultimately result in enhanced cell  
36  
37 killing and have implications for the radiosensitivity, repair, reassortment, repopulation and  
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39 reoxygenation of tumour cells [9]. Importantly, these effects need to be better resolved to  
40  
41 improve the translation of MNPs to clinical evaluation and in realising their promise in cancer  
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43 radiotherapy.  
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### 49 ***Current and future challenges***

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51 Despite a significant body of evidence demonstrating the potential of MNPs to enhance  
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53 radiation response, only a very small number of agents have progressed to clinical trials. These  
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55 include nanoparticle formulations of gold (CYT-6091, Cytimmune, NCT00356980;  
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57 NCT00436410), gadolinium (AGuIX, NH TherAguix, NCT02820454; NCT03818386;  
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3 NCT03308604) and hafnium oxide (NBTXR3, Nanobiotix, NCT01433068; NCT02721056;  
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5 NCT02805894). Preclinical studies have provided considerable insight into the molecular  
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7 mechanisms of MNP radiosensitization, yet there remains a number of considerable challenges  
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9 to be addressed if the successful translation of investigative new MNPs to early phase trials is  
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11 to improve.  
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14  
15 One of the greatest challenges preventing MNPs from entering the clinic is the need for  
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17 a more comprehensive delineation of the impact of their physicochemical characteristics on  
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19 radiobiological response. The wide-ranging properties of MNPs that have been investigated in  
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21 radiation studies prevent a general description of the underlying mechanisms of  
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23 radiosensitization, which is a significant limitation in optimising MNP-based therapy. We have  
24  
25 begun to understand the influences of element, size, shape, surface modification and source  
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27 energy. As an example, our group demonstrated the thiol coating on a commonly used  
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29 commercial 1.9 nm GNP to significantly modulate cellular pathways relating to oxidative  
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31 stress, and inhibition of this effect almost entirely abrogated the radiosensitizing effect of the  
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33 GNP [10]. A precise understanding of how these variables control key biological interactions  
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35 and cellular localisation is required to inform the optimum physical parameters of MNPs, but  
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37 as of yet these have not been systemically investigated in standardised cell models.  
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43 Considering nuclear DNA as the critical target of radiation induced damage, it would  
44  
45 be expected that the subcellular localisation of MNPs would have a major impact on  
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47 radiobiological response. In particular, it may be expected that proximity to the nucleus is  
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49 critical due to the short range of Auger electrons emitted which cause a series of clustered  
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51 ionising events around the nanoparticle [11]. Using a soft X-ray microbeam targeting nuclear  
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53 or cytoplasmic cellular compartments, our group demonstrated that even in the absence of  
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55 nuclear localisation, cytoplasmic irradiation can drive significant DNA damage, again  
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57 identifying the mitochondria as a central mediator in GNPs radiosensitization [12]. These data  
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3 raise intriguing questions concerning subcellular localisation and critical organelle targets for  
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5 MNP effects, which will require more sophisticated imaging methods if they are to be better  
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7 understood.  
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10 Finally, most MNPs rely on the enhance permeability retention (EPR) effect for tumour  
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12 specificity due to abnormal vasculature. The development of improved methods for tumour  
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14 targeting has the potential to make a significant impact and lead to the application of MNPs for  
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16 precision medicine. For example, HER-2 targeted MNPs for use in breast cancer have been  
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18 postulated to have potential [13], but to date none of the many potential approaches in this area  
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20 have translated to the clinic.  
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### 26 ***Recent advances in science and technology to meet challenges***

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28 The future success of MNPs in the clinic is predicated on a more detailed mechanistic  
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30 understanding of the impact of key physical variables on cellular interactions and how these  
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32 determine cellular radiobiological response. As frequently occurs, technologic evolutions lead  
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34 to transformative impacts on scientific research, which will continue to propagate discovery in  
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36 the MNP research field.  
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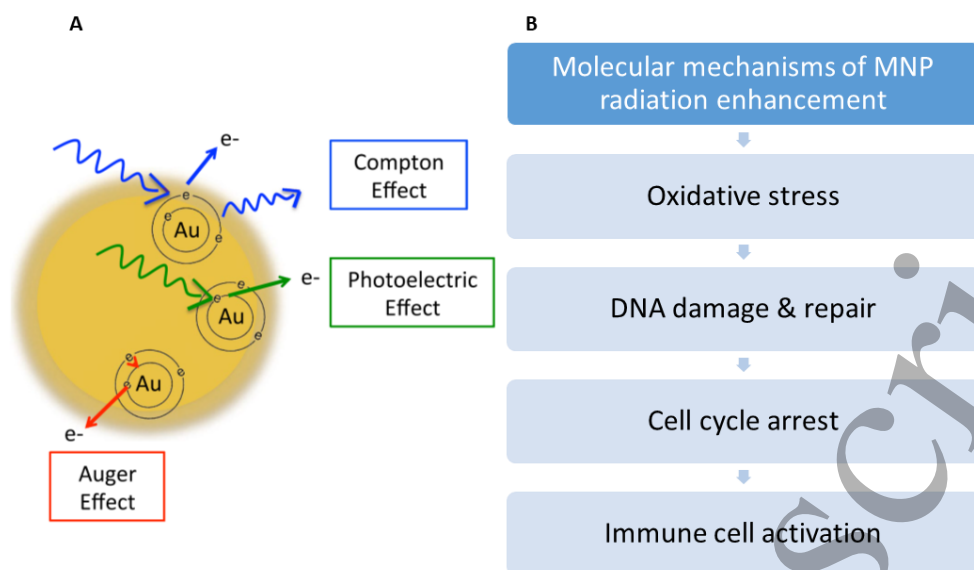
40 Recent advances in gene expression technologies such as single cell sequencing allow for  
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42 studies of the genomic and transcriptomic landscape of tumours at the single cell level [14].  
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44 This approach could provide critical insight in the multicellular complexity of MNP  
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46 radiosensitization along with spatial information about tissue context or volumetric localization  
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48 of gene expression changes. Advanced gene and protein-based methods may be used to  
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50 characterise alterations in immune cell types and improve understanding of interactions  
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52 between MNPs, the immune system and tumours. In the immunotherapy era, this approach  
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54 may be central to identifying novel opportunities for combining MNPs with immune-oncology  
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3 agents. Finally, these approaches could also identify biomarkers for the selection of patients  
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5 most likely to respond to MNP enhanced radiotherapy.  
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8 There is also a need to apply improved preclinical models to demonstrate the  
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10 radiobiological effects of MNPs in tumours and normal tissues. Approaches could include the  
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12 use of standardised cell models, syngeneic transplants or patient derived tumour models  
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14 established in humanised host animals. Tumour response data should be supported by  
15  
16 evaluation of MNP effects in disease relevant organs at risk, all of which should be evaluated  
17  
18 using precision small animal image guided radiotherapy [15]. These developments are likely  
19  
20 to synergise with new tools for improved theoretical modelling of MNPs utilising relevant,  
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22 systematic, mechanistic models and enabling the integration of many heterogeneous datasets  
23  
24 to produce useful predictions for a range of different MNP. The integration of these approaches  
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26 will undoubtedly improve our understanding of the fundamental processes underlying MNP  
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28 radiosensitization, leading to improved, biologically optimised treatments.  
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#### 35 **4. Concluding remarks**

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37 Technological advances and improved understanding of mechanisms of cellular  
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39 radiobiological response will continue to play critical roles in improving the efficacy of  
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41 radiotherapy. MNP agents have high potential to increase the therapeutic index and impact  
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43 standard of care in clinical oncology, yet there remain critical challenges towards clinical  
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45 translation. Addressing these challenges requires a multidisciplinary strategy focused on  
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47 applying state-of-the-art technologies to better understand the physical, chemical and  
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49 biological basis of MNP radiosensitization, and ultimately deliver optimised therapies to the  
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51 clinic.  
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**Figure 1.** Schematic illustration of the physics processes (panel A) and molecular mechanisms (panel B) of MNP-mediated radiation enhancement.

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# Optimization of GNP-based Cancer Therapeutics

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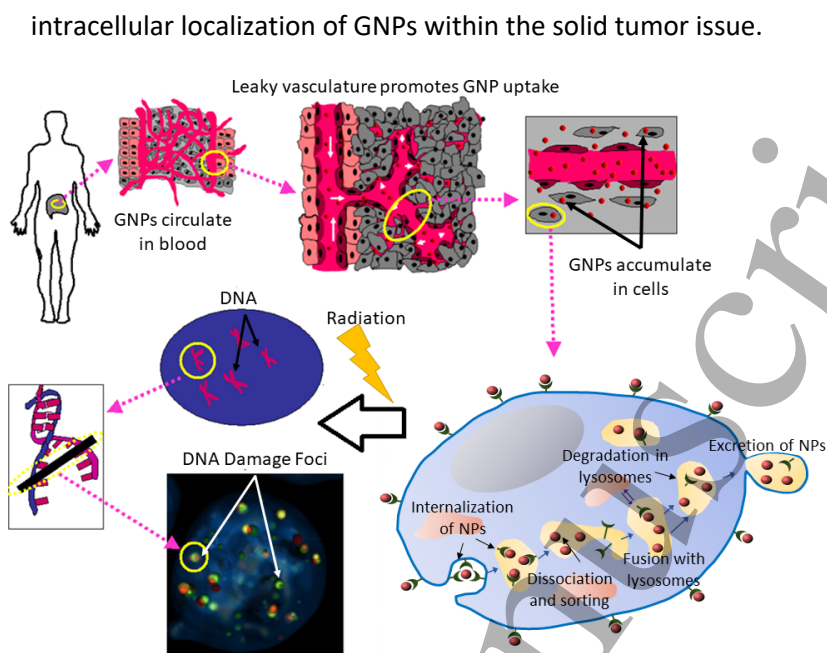
## Status

Over 20 years ago, it was demonstrated that iodine is a radiosensitizer of cells in culture[1]. It was also found that direct injection of iodine into tumors followed by radiotherapy (RT) suppressed the growth of 80% of tumors[2]. More recently, gold has been pursued as a radiosensitizer due to its higher atomic number compared to iodine ( $Z_{Au}=79$  vs.  $Z_I=53$ ) and favorable biocompatibility[3]. The high atomic number of gold nanoparticles (GNPs) means that they can enhance the effective radiation dose at the site of disease. During radiation therapy, the presence of GNPs enhances the cross section of low-energy electrons and other cell damaging species leading to increased cell death[4, 5]. This strategy is already being used by many groups to develop novel cancer treatments and the initial results are extremely promising[6]. The ultimate goal of these GNP-based platforms will be the targeted delivery of tumors using radiotherapy while causing minimal side effects to normal tissue.

## Current and future challenges

Irradiation with kilovolt X-rays following intravenous administration of 1.9 nm GNPs to mice bearing subcutaneous tumors was the first study to show an increased radiosensitization effect in tumors[3]. A comparison of the mass concentrations of iodine and gold across different studies suggests that in the absence of intracellular uptake, *in vivo* intra-tumoral concentrations of  $\sim 5 \frac{\text{mg}}{\text{g}}$  were required for radiosensitization[7]. In contrast, whenever GNPs were internalized *in vitro*, radiosensitization was achievable at concentrations as low as  $1 \frac{\text{ng}}{\text{g}}$  [8]. Based on these recent observations, the current challenge is to achieve radiosensitization effects at dramatically lower concentrations through



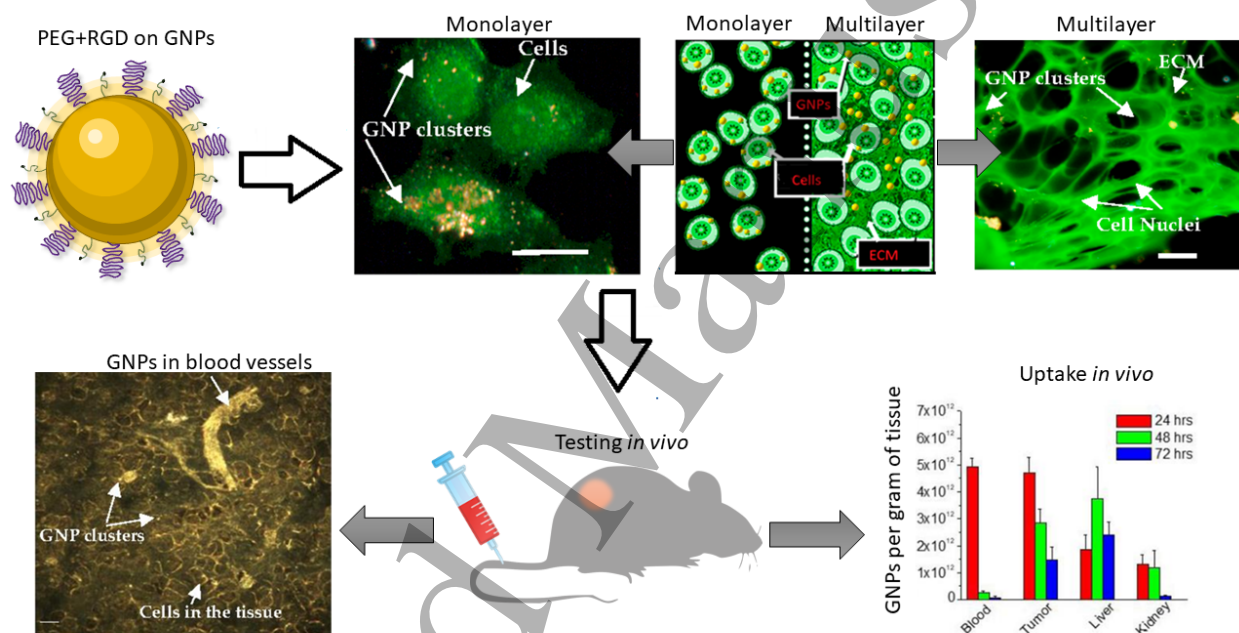


**Figure 1.** Roadmap to optimizing GNP-mediated radiation response. Accumulation of a higher percent of injected NP dose within the tumor cells involves enhanced blood circulation of NPs, penetration within the tumor tissue once they leave the leaky tumor blood vessels, and finally internalization of GNPs within every tumor cell. The ultimate goal is to enhance the DNA damage using GNPs as a radiosensitization agent.

### Advances in science and technology to meet the challenges

Advances in building novel lipid-based platforms for efficient intracellular delivery of GNPs has shown remarkable results[9]. This can also be achieved through optimizing physicochemical properties of GNPs for individual particle delivery[10]. Successful delivery of GNPs into the intracellular space of individual cells also depends also on the efficiency of crossing a few boundaries, including tumor blood vessels to tumor tissue followed by their penetration successfully through the tumor tissue as illustrated in Figure 1. A tumor with a well-developed collagen network or extracellular matrix (ECM) can be considered to be physically resistant to NP-based therapies[11, 12]. For tumors with a strong ECM, treatments that reverse or inhibit collagen production and assembly could be performed prior to NP-based therapies [11]. Hence, downregulation of ECM may become a promising adjuvant therapeutic strategy for ECM-rich tumors [12]. Another approach could be to use ECM-degrading enzymes to modify the collagen structure to further improve the distribution of NPs in solid tumors.

Features of solid cancers not accounted for by three-dimensional (3D) *in vitro* models are variable interstitial fluid pressure (IFP), the influence of convection, and stromal cells[13]. Although direct *in vivo* assessment has the advantage of duplicating the clinical environment more closely, these 3D *in vitro* models offer the advantage of being able to examine and evaluate isolated results with less complicated parameters[14]. As illustrated in Figure 2, a recent study has shown that the size and surface functionality of the NPs could be tailored using these 3D *in vitro* models to produce a higher accumulation within the tumor in an *in vivo* tumor model[15].



**Figure 2.** Optimization of NP-based delivery systems at monolayer and tissue-like multilayer models leads to their successful accumulation in *in vivo* models.

Radiation therapy, in particular, damages cancer cells with a less pronounced effect on the vasculature[16]. It has been shown that nano-sized molecules enter radiation-treated tumors at a rate 2.2-fold higher than non-irradiated tumors[17]. Radiation killed well-oxygenated cancer cells near tumor blood vessels leading to higher vascular permeability[16]. However, excessive radiation damage to blood vessels can shut down blood flow, which affected the nano-therapeutic agent delivery negatively. Moreover, the effects of radiation on the ECM must be considered since radiation could damage the long polymer chains of hyaluronan and elastin resulting in premature stiffening of tissue[18]. Hence, the effect of radiation on the ECM and tumor blood vessels should be taken into account when designing NP-based

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3 treatment protocols. In addition, rapid tumour growth results in leaky vessels allowing NPs to leak into  
4 the tumour and NPs are retained due to the lack of a functional lymphatic system. However, the lack of  
5 lymphatic drainage within the tumour also increases IFP which can also limit delivery of therapeutic  
6 agents[13]. Hence, it could be important to consider administration of agents that renormalize  
7 vasculature temporarily to improve the blood flow [13, 19].  
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### 11 Concluding remarks

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14 The incorporation of cancer nanomedicine into current therapeutic protocols requires the careful  
15 consideration of multiple factors to overcome the barriers for intracellular NP delivery within the tumor.  
16 Three-dimensional tumor models could be further improved to test NP-based therapeutics in parallel to  
17 animal models to improve our understanding and overcome barriers to NP transport while understanding  
18 the mechanisms of tumor cell death in a less complex tumor microenvironment. Introduction of GNPs to  
19 current chemoradiation protocols could potentially be another addition to the current toolbox that could  
20 benefit some patients[20]. Most of the *in vitro* and *in vivo* data have shown promising results with clinically  
21 relevant MV photon beams and lower concentration of GNPs. Hence, clinical translation of GNP-mediated  
22 radiosensitization could be achieved with a collective effort from research groups around the world.  
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# Targeting Tumor Vasculature with Nanoparticles for Radiation Therapy

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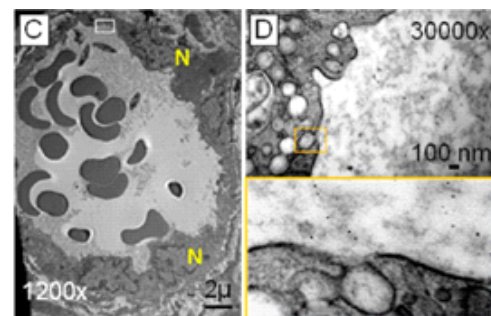
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## Status

Limitations of some nanoparticle-based cancer therapies include inadequate diffusion into the tumor interior and poor tissue penetration of stimulating agents (optical, IR, UV, kV x-rays, etc.). While relying solely on the inherently leaky tumor vasculature limits nanoparticle delivery to tumor cells, this challenge can be turned into an advantage by targeting tumor vasculature. Nanoparticles of a certain size tend to be trapped in tumor blood vessels and nanoparticle design can further enhance vascular accumulation. Situations in which passive tumor targeting confronts various physiological barriers (*e.g.* vascular walls, tissue, collagen, ECM matrix, etc.), nanoparticles actively targeted to tumor blood vessels have direct access to the therapeutic target. Several peptides have been introduced to actively target tumor endothelial cell receptor expression, increasing accumulation in these sites. For example, Arginine-Glycine-Aspartic Acid (RGD) has been shown to increase the nanoparticle concentration in and near tumor endothelial cells (1) (**Figure 1**).

Tumor endothelial cells provide reliable receptor expression compared to other tumor cellular targets. In cancer cells, receptor expressions and density can vary depending on the tumor type, origin, stage, and mutational characteristics. All these factors make tumor endothelial targeting a more consistent approach across different disease sites. In addition, the range of the local radiation dose enhancement – and subsequent biological damage - via the production of low energy electrons (2) is relatively low (several microns), meaning that nanoparticles need to be close to the target cells. Therefore, vascular targeted nanoparticles will confer major damage to tumor blood vessels after irradiation.



**Figure 1. TEM imaging of tumor endothelial cell uptake in a mouse tumor model.** Vascular targeted gold nanoparticles are shown in tumor endothelium 1 h after IV administration. Magnified images show uptake via clathrin/caveolae vesicles. Reprinted with permission from (Kunjachan Nano Letters 2015). Copyright 2015 American Chemical Society.

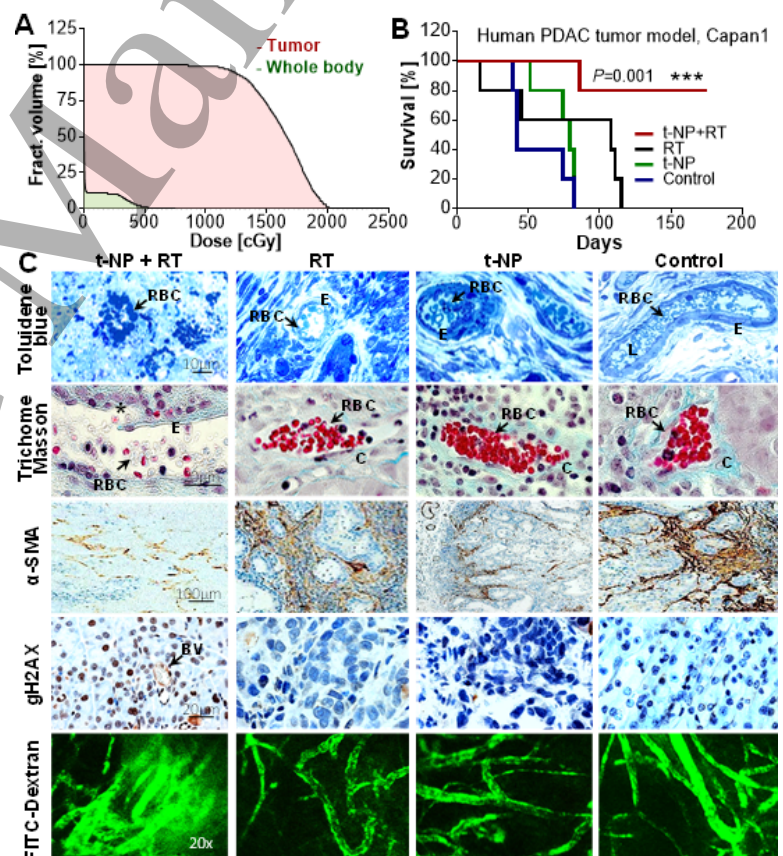
There is strong evidence that tumor vasculature plays an important role in radiation therapy (3-5). Garcia-Barros *et al.* proposed that damage to tumor vasculature during radiation therapy may be more therapeutically beneficial than clonogenic cell death (3). A review by Park *et al.* presented experimental evidence linking radiation-induced tumor vascular damage to the success of stereotactic body radiation therapy (SBRT) procedures (5). Similarly, vascular disruption of hepatocellular carcinoma with injected Y-90 microspheres is a proven clinical technique (6).

The concept of targeting tumor vasculature with nanoparticles to amplify radiation dose for vascular disruption was first proposed by Berbeco *et al.* (7). It has since been demonstrated that radiation therapy combined with vascular-targeted gold nanoparticles destroys tumor endothelial cells while largely sparing normal tissues (8, 20). In that work, gold nanoparticles were targeted to tumor blood vessels via RGD prior to radiation therapy. Imaging and histology assays demonstrated the endothelial cell damage and subsequent vascular disruption (Figure 2). This group also demonstrated increased model nanodrug delivery after vessel disruption, suggesting a benefit for combined therapies (20).

Vascular therapies improve the effects of radiation therapy (9). Preclinical experimental results indicate a synergistic relationship between vascular disruption and radiation therapy (10). However, no clinical trials have yet been reported with chemical vascular disrupting agents (VDAs) and radiation therapy. Negative results of a Phase 3 trial of chemical VDAs with chemotherapy (11) have been attributed to clinical factors (12). Using targeted GNPs rather than chemical VDAs should reduce off-target toxicities because GNPs are inactive outside the high radiation volume.

### Current and Future Challenges

Changes in tumor physiology after vascular disruption may present both benefits and challenges for radiation therapy. For example, tumor vascular disruption may increase hypoxia. Hypoxic tumor regions represent



**Figure 2. Vascular disruption after delivery of targeted gold nanoparticles and radiation therapy.** Endothelial destruction is observed via histopathology and confocal microscopy. (from Kunjachan *Scientific Reports* 2019)

1 resistant cell populations requiring additional therapeutic attention and failure to address hypoxia has  
2 implications for tumor therapy response as well as metastatic potential. The hypoxic conditions that are  
3 associated with tumors create a cellular response in which alternative metabolic pathways are enabled by  
4 factors such as HIF-1 $\alpha$  which has been shown to be an exclusive regulator of carbonic anhydrase 9 (CA IX)  
5 activation. CA IX is largely absent from normal tissues and its over-expression is a clinical biomarker for poor  
6 prognosis (13).  
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11 The tissue penetration of high energy (MV) photons is an advantage of external beam radiation therapy (EBRT)  
12 as a nanoparticle “activating” agent. These photons have significant skin sparing properties and deliver >60% of  
13 their maximum dose at 10 cm depth, for example. However, the penetration benefits of the high energy beam  
14 are somewhat mitigated by the physical interaction properties. The probability of a photoelectric interaction  
15 between an atom in the nanoparticle and an incident photon is proportional to  $1/E^3$ , where  $E$  is the energy of  
16 the incident photon. EBRT beams are composed of a spectrum of photon energies peaking well outside the  
17 range of photoelectric interactions (*e.g.* 6 MV). For this reason, combining nanoparticles with EBRT has been  
18 dismissed as impractical(14). However, roughly 95% of clinical radiation therapy procedures utilize this energy  
19 range, therefore a solution is necessary for nanoparticles to be clinically translated.  
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### 31 **Advances in Science and Technology to Meet Challenges**

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33 The advancement of non-invasive hypoxia imaging will provide an opportunity to study temporary and  
34 permanent physiological changes within the tumor microenvironment to better understand the effects of  
35 nanoparticle targeted vascular disruption therapies. Current modalities for hypoxia identification include  
36 oxygen electrodes for direct measurement of pO<sub>2</sub> levels, exogenous markers such as HIF-1 $\alpha$ , and imaging  
37 modalities such as positron emission tomography (PET) and magnetic resonance imaging (MRI). Although  
38 oxygen electrodes provide a direct pO<sub>2</sub> measurement, readings are limited to invasive, point measurements.  
39 HIF-1 $\alpha$  and pimonidazole are used currently in clinical practice, however the results are limited to the area of  
40 the biopsy. PET imaging with hypoxia specific radiotracers such as [<sup>18</sup>F] labeled misonidazole (FMISO) is non-  
41 invasive but is limited due to the cost as well as permeation limitations of small molecules. In the preclinical  
42 setting, there are additional optical imaging methods to image CA IX expression *in vivo*(15). An emerging  
43 noninvasive modality is functional MRI based diffusion-weighted (DWI) and blood/tissue oxygen level  
44 dependent (BOLD/TOLD) imaging. Both use gradient echo pulses to image the relative paramagnetic properties  
45 of oxygenated and deoxygenated hemoglobin within the whole tumor (16). Advantages of MRI-based  
46 approaches include non-invasive, serial imaging capabilities and translational potential to human scales.  
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1 Once the time-course of induced hypoxia has been identified, new techniques can be used to mitigate the  
2 therapeutic impact. Identification of these regions has begun transitioning cancer therapy towards improving  
3 tissue oxygenation. Current clinical trials are underway studying the use of hypoxia-activated prodrugs such as  
4 tirapazamine, hyperbaric chambers, and radiation dose painting based on hypoxia maps. Other strategies  
5 include increasing hypoxic cell radiosensitivity via misonidazole, decreasing oxygen consumption via metformin,  
6 and specifically targeting hypoxia surface markers such as HIF-1 $\alpha$  (17).  
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11 There is ample evidence that disrupting tumor blood vessels can increase drug delivery, improving outcomes  
12 (18). Ultrasound stimulated microbubbles (USMB) is a well-researched preclinical approach and has resulted in  
13 positive clinical results of a Phase 1 trial (19). USMB vascular modulation is limited, however, as it is not  
14 appropriate in all parts of the body. Published work with vascular-targeted gold nanoparticles demonstrated  
15 increased perfusion of an injected dye after disruption (20) (**Figure 2**) and further quantification by optical and  
16 MR imaging showed increased permeability and accumulation of model nanodrugs (20). Recent advances in  
17 drug nanoformulations will improve tumor delivery, particularly after vascular disruption.  
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25 While EBRT beams peak at high energies, they also contain substantial contributions of low energy photons. Key  
26 radiation treatment planning parameters will also affect the proportion of low energy photons at the site of  
27 disease (21). At the patient surface, the fraction of low energy (<150 kV) photons is small (0.5%). Due to  
28 scattering processes, this increases to 8% at 10 cm depth and 11% for a flattening filter free (FFF) beam.  
29 Additional modifications of the linear accelerator can be made to further increase the low energy photon  
30 content without compromising the overall dose conformity (22).  
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### 36 **Concluding Remarks**

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38 Recent studies have demonstrated the therapeutic benefit of targeting tumor blood vessels with nanoparticles  
39 prior to radiation therapy. Induced hypoxia after vascular disruption needs to be considered and mitigating  
40 actions should be included if needed. While preliminary results indicate that the subsequent vascular disruption  
41 will increase model drug delivery, careful studies in advanced translational models should be undertaken to fully  
42 understand the combined impact on tumor growth. The tools for these steps are currently available and  
43 therefore it is anticipated that clinical translation of this therapeutic approach will follow.  
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# Clinical Translation of Gold Nanoparticles for In-Vivo Use

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## Status

Currently there are 12 active or completed clinical trials involving gold nanoparticles listed on [clinicaltrials.gov](http://clinicaltrials.gov), as shown in **Table 1**. Despite a slow start, clinical translation of gold nanoparticles is accelerating toward the clinic, led by Clene Nanomedicine who is actively recruiting for Phase 2 clinical trials. Unlike many of the other technologies which utilize IV delivery, CNM-Au8 is orally administered and does not suffer from many of the off-target accumulation issues associated with systemic delivery. However, this is still a very significant milestone because it marks the first gold nanoparticle technology to move into Phase 2 effectiveness studies.

**Table 1:** List of active or completed clinical trials of gold nanoparticles from [clinicaltrials.gov](http://clinicaltrials.gov).

Clinical Trial Identifier	Year Listed	Phase	Sponsor/Company	Material	Conditions
NCT00356980	2006	Phase 1	NIH Clinical Cancer / Cytimmune	Aurimmune: TNF-target conjugated and PEGylated gold nanosphere	Unspecific Adult Solid Tumor

NCT00436410	2007	Early Phase 1	NIH Clinical Cancer / Cytimmune	Aurimune: TNF-target conjugated and PEGylated gold nanosphere	Adrenocortical Carcinoma, Breast Cancer, Colorectal Cancer, Gastrointestinal Cancer, Kidney Cancer, Liver Cancer, Melanoma (Skin), Ovarian Cancer, Pancreatic Cancer, and Sarcoma
NCT00848042	2009	Not Applicable	Nanospectra Biosciences	AuroLase: PEG coated gold nanoshell	Head and Neck Cancer
NCT01270139	2011	Not Applicable	Ural Medical University	Silica-gold nanoparticle	Cardiovascular
NCT02680535	2016	Not Applicable	Nanospectra Biosciences	AuroLase: PEG coated gold nanoshell	Neoplasms of the Prostate
NCT02837094	2016	Phase 1	Cardiff University / Midatech Pharma	MidaCore MTX102: ~1.5nm diameter Gold nanoparticle coated with	Type 1 Diabetes

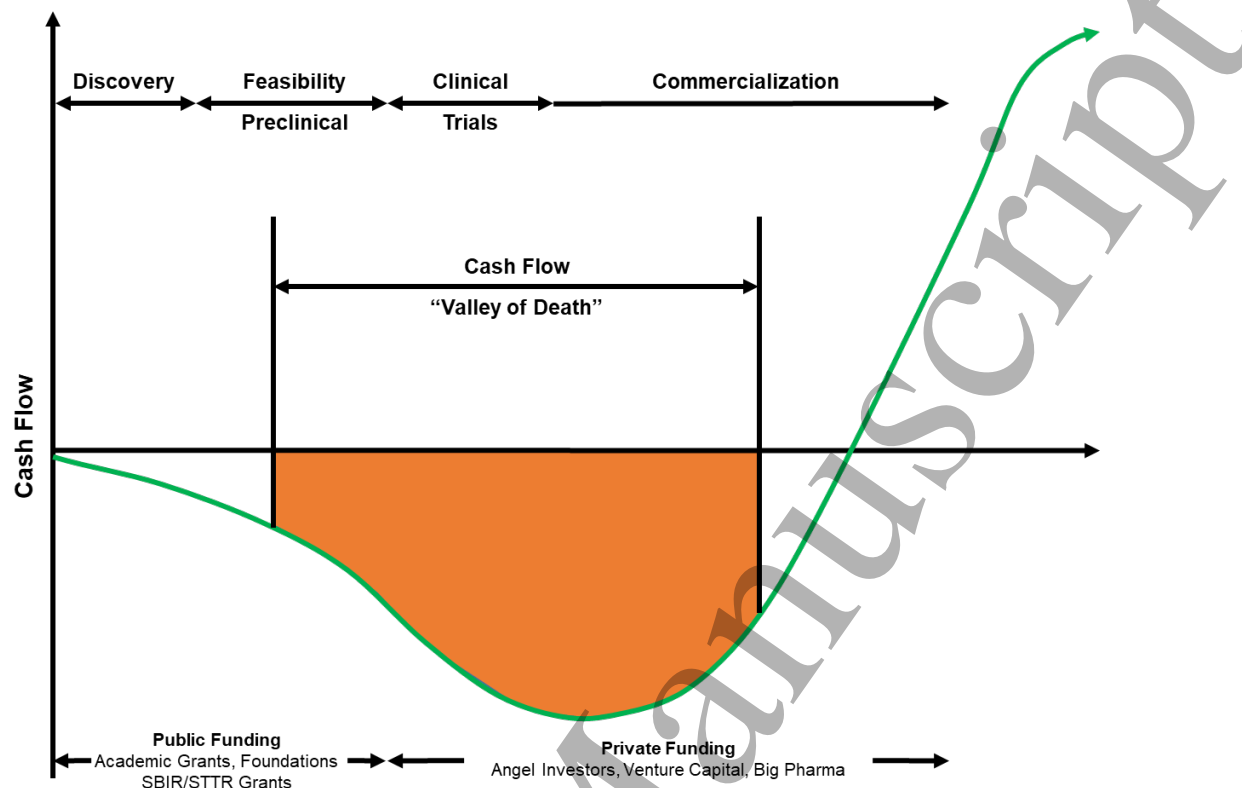
				C19-A3 peptides, L- glutathione, and glucose	
NCT02755870	2016	Phase 1	Clene Nanomedicine	CNM-Au8: ~10nm diameter Gold Nanocrystal	Healthy Volunteers - Male and Female
NCT03020017	2017	Early Phase 1	Northwestern University	Gold Nanoparticles coated with NU-0129 Spherical Nucleic Acid	Gliosarcoma Recurrent Glioblastoma
NCT03669224	2018	Not Applicable	Cairo University	Nano Care Gold: Gold and silver nanoparticles suspended in 70% isopropyl alcohol	Dentistry
NCT03536559	2018	Phase 2	Clene Nanomedicine	CNM-Au8: ~10nm diameter Gold Nanocrystal	Relapsing Remitting Multiple Sclerosis, Optic Neuropathy, and

					Optic Neuritis With Demyelination
NCT03843710	2019	Phase 2	Clene Nanomedicine	CNM-Au8: ~10nm diameter Gold Nanocrystal	Amyotrophic Lateral Sclerosis
NCT03815916	2019	Phase 2	Clene Nanomedicine	CNM-Au8: ~10nm diameter Gold Nanocrystal	Parkinson's Disease

### Current and future challenges

Despite more than 50,000 articles listed on Google Scholar with the keywords gold nanoparticles and *in vivo*, no gold nanoparticle-based intervention has received FDA approval for clinical use. Many of these technologies could have significant clinical merit, but that alone is not enough to garner clinical translation. Most of the work with gold nanoparticles has been performed in academic laboratories, and in order to achieve some sort of publication the investigators must demonstrate some sort of feasibility that their gold nanoparticle-based technology can work for a particular application. Although many of these publications are significant contributions, most of the work is devoted to scientific discovery and not clinical translation. Unfortunately, this leaves a gap between academic development and clinical product development. Once feasibility in a technology has been established, financial funding is the main determinant of clinical translation. As shown in **Figure 1**, public grants can only take a technology so far, and eventually will need to rely on private funding.





**Figure 1.** Cash flow diagram adapted from [1]. The cash flow as a function of development stage (time) with typical funding sources at various stages. SBIR, Small Business Innovative Research; STTR, Small Business Technology Transfer.

The finances involved with any clinical product development are very substantial and generally investors weigh the finances with how long to receive FDA approval and return on investment. Gold nanoparticle technologies developed based on scientific discovery generally have a lot of innovative aspects to them, which equals additional regulatory oversight and a longer path to clinic. Both public (e.g., business grants) and private investors are generally less enthusiastic about these type of technologies as they can often be considered too high risk investments.

Assuming that the technology has the correct mix of innovation while being simple enough to have a relatively quick path to the clinic, there are a variety of different funding sources, as shown in **Figure 1**. Because many technologies start in academic laboratories, the initial funding source is government grants. A technology can be backed by public funding for a

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3 significant portion of the clinical product development. There is precedent that public funding  
4 has taken technologies as far as early clinical trials, however most technologies must make the  
5 jump from public to private funding sometime before Good Manufacturing Practices (GMP)  
6 production is required.  
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10 Gold nanoparticles are not trivial to manufacture, requiring high purity ingredients and  
11 reactors. Further complicating the manufacture is that gold nanoparticle formation is a  
12 stochastic process and requires extremely expensive characterization equipment (e.g.  
13 transmission electron microscopy). If a biomolecular targeting group is involved (e.g. antibody,  
14 peptide, nucleic acid, affibody, or anything that could cause an immunologic response) then the  
15 required quality controls (QCs) and quality assurances (QAs) are even more complex. Once the  
16 quality systems are established and the gold nanoparticle manufacture is fully integrated into  
17 current GMP, then the next step will be extensive toxicity studies. To be cleared by the FDA to  
18 perform a clinical trial, these toxicity studies must be performed under Good Laboratory  
19 Practices (GLP). Maintaining a GLP facility is extremely expensive and therefore most  
20 technologies outsource these studies to a GLP-certified contract research organization (CRO).  
21 The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for  
22 Human Use (ICH) has outlined most of the guidelines for toxicity studies, and depending on the  
23 method of action of the gold nanoparticle, will determine the number of animal species and  
24 testing burden required. For simple gold nanoparticle interventions, ones with no biologic and  
25 only a single low dose, the GLP toxicity study costs can be less than \$100,000. However,  
26 complex interventions that include a biologic, or a known toxic agent, and multiple dosing could  
27 escalate costs to over \$10,000,000.  
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43 Investments of this level are not readily available for a technology class that has not  
44 been approved by the FDA. Therefore, many of the technologies entering clinical trials are  
45 generally considered as 'high-risk high-reward'. Due to the nature of these technologies, clinical  
46 testing will likely proceed for quite some time until approval can be obtained. Once one gold  
47 nanoparticle-based technology is approved, then some of the required GLP toxicity studies and  
48 clinical trials may be bypassed. This will greatly reduce commercialization times and costs for  
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3 subsequent products seeking FDA clearance, encouraging more private investments and driving  
4 more gold nanoparticle-based technologies to the clinic.  
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### 8 **Advances in science and technology to meet challenges**

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10 The major concerns with the FDA is safety and effectiveness, and until recently none of  
11 the gold nanoparticle technologies have moved past Phase 1 in-human safety studies. Although  
12 this may not necessarily be the case of these technologies, some gold nanoparticles are known  
13 to suffer from poor clearance from the body.[2] It has been shown that gold largely  
14 accumulates in the mononuclear phagocyte system (MPS) (also known as the  
15 reticuloendothelial system) because the body cannot break-down gold and most technologies  
16 require gold nanoparticles too large to be cleared by the kidneys. Therefore, if the technology  
17 uses gold as a drug delivery vehicle, there are questions as to why gold should be chosen as  
18 opposed to other existing technologies. If the technology uses gold as an energy converter for  
19 therapy (e.g., photothermal therapy or radiation therapy) or imaging contrast (e.g.,  
20 photoacoustic/optoacoustic imaging), then questions about dosage and biodistribution are at  
21 the forefront. Therefore, current systemically delivery applications of gold nanoparticles are  
22 limited to high-morbidity indications.  
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34 Advances in delivery and clearance will probably have the most profound influence on  
35 the future of clinical translation of gold nanoparticles. Effective delivery, either by improving  
36 bioavailability for lower effective doses or molecular targeting strategies, could have a  
37 profound effect on the potential safety profile of gold nanoparticles. If the clearance problem is  
38 solved, then long-term accumulation concerns will be reduced and could open gold  
39 nanoparticle applications for low-morbidity interventions.  
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### 47 **Concluding remarks**

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49 The clinical presence of gold nanoparticle is on the rise, with at least eight new clinical  
50 trials listed in the past three years. Despite the financial burden to get a technology through  
51 feasibility and preclinical testing just to get a shot of testing into humans, several  
52 companies/groups have demonstrated that it can happen and is happening at an increasing  
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3 rate. The presence of the Phase 2 trials is really encouraging for the gold nanoparticle field,  
4 since it signifies the first time that gold nanoparticles have moved past Phase 1 safety studies.  
5 Hopefully these pioneering clinical trials pave the way for many more gold nanoparticle-based  
6 technologies, ushering in entirely new classes of diagnostics and therapeutics.  
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# Next-Generation Metallic Nanoparticles for Cancer Radiation Therapy

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## **Status**

High Z elements (heavy metals) behave as potent radiosensitizers because they can produce a cascade of Auger electrons when ionized by X-ray irradiation [1]. In fact, the increment of resonant energies in heavy metals after irradiation is adequately high to ensure significant penetration in body tissue [2]. Laboratory experiments, using heavy metals injected into tumors and then irradiated with high-energy X-rays, have exhibited considerable reduction in tumor sizes [3]. Most importantly, the in situ deposition of radiation energy, followed by secondary photon and electron emission, will be confined at the irradiated tumor site. Due to these benefits, various formulations made of heavy metals have been conceived and developed to improve the radiation dose enhancement for cancer treatments.

## **Current and future challenges**

Among high Z elements, gold ( $Z=79$ ) has been the prime choice for radiation therapy due to its biocompatibility and low toxicity. Although the earliest studies with bulk or larger particles of gold to enhance radiation dose were successful in vitro, the larger

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3 gold particles failed to show their effectiveness in vivo. Alternatively, gold nanoparticles  
4 (AuNPs) with diameter (1-5 nm) were produced and intravenously injected to enhance  
5 the accumulation of these ultrasmall particles in tumor tissues. As a consequence, when  
6 the tumor was irradiated, the 1-year survival was 86% compared to 20% with radiation  
7 alone and 0% with gold alone [3]. Following the successful result, innumerable studies  
8 have focused on the optimization of composition and size of AuNPs, uptake of  
9 nanoparticles into cells, and the dose of applied radiation.  
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19 A major problem of AuNPs is their short circulation in the blood stream after injection,  
20 requiring immediate irradiation. But, a high dose of AuNPs, required for satisfactory  
21 treatment of tumors, is impractical for human use, partly due to toxicity concerns. To  
22 use the AuNPs for clinical applications, the size, surface chemistry and targeting ability  
23 of AuNPs could be tuned for longer circulation time and enhanced accumulation in  
24 specific tumors. A vast array of chemical agents (cysteamine, glucose, choline, citrate,  
25 folic acid), biomolecules (nucleotides, peptides, antibodies) and polymers (synthetic,  
26 natural) are available to conjugate to AuNPs to facilitate the higher uptake of particles in  
27 tumors. Although targeting agents may ameliorate the delivery of AuNPs to tumor sites,  
28 it is not fully understood whether these modified particles really assist radiation for  
29 tumor therapy. Again, optimization of AuNPs with optimum density of targeting agents  
30 and delivery routes (intravenous, intratumoral) is necessary prior to clinical translation.  
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To date, only a few clinical trials (<10) of AuNPs are currently underway for cancer treatment [4] [5] [6].

**Advances in science and technology to meet challenges**

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3 In pursuit of alternatives to AuNPs, other high Z elements such as bismuth (Z=83),  
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5 platinum (Z=78) and gadolinium (Z=64) are currently being investigated to prepare the  
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7 formulations for radiation therapy. Under similar conditions of size and concentration of  
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9 particles, bismuth provides higher dose enhancements than gold while platinum shows  
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11 equivalent efficacy to gold [7]. Particularly, bismuth oxide nanoparticles (BiNPs) are  
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13 verified as efficient dose enhancing agents demonstrating their potential application in  
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15 clinical radiotherapy [8]. Bismuth selenide nanoplates have shown significant radiation  
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17 dose enhancement both in vitro and in vivo [9]. Of note, these nanoplates were  
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19 selectively accumulated in tumor tissue after intraperitoneal injection into mice. The  
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21 higher concentration of nanoplates not only enhanced the contrast of X-ray  
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23 computerized tomography (CT) images but also led to damage on the tumor cells when  
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25 exposed to radiation.  
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31 A targeted radiotherapeutic agent was developed from bismuth nanosheets coated  
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33 with chitosan and RGD peptide [10]. The nanosheets exhibit targeting ability to  $\alpha\beta3$   
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35 integrin-overexpressing HeLa cells with higher radiosensitization efficiency. When the  
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37 mice bearing HeLa tumors were irradiated after treatment with the nanosheets by  
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39 intravenous or intratumoral injection, the tumor volume decreased significantly.  
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41 Importantly, the nanosheets can be used for photoacoustic imaging and magnetic  
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43 resonance (MR) imaging to study their targeting ability and therapeutic effects  
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45 together. Thus, this study reveals an effective theranostic agent for next-generation  
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47 cancer radiotherapy. In another study, functionalization of BiNPs with hyaluronic acid  
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49 enhanced water solubility, biocompatibility and targeting ability to cancer cells  
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3 overexpressing CD44 receptors [11]. These particles offer great promise for target-  
4 specific CT imaging and radiosensitization of tumors.  
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7 Although nanoparticles of high Z elements are used as radiosensitizers to enhance  
8 radiation therapy, the non-degradable nature of the nanoparticles, that can cause long-  
9 term toxicity when accumulated inside body, is a major bottleneck in translating  
10 nanoparticle radiosensitizers into clinical application for cancer treatment. To address  
11 the issue, a new formulation of folate and red blood cell membrane (F-RBC) combined  
12 BiNPs was developed, where folate targets tumor and cell membrane coating provides  
13 longer blood circulation time [12]. When F-RBC BiNPs were used to sensitize radiation  
14 for breast cancer treatment, there was significant tumor inhibition in mice resulting in  
15 improved survival ratios. Histological and in vivo bio-distribution analyzes revealed F-  
16 RBC BiNPs were excreted after 15 days from the animal body with no evident damage  
17 or inflammatory response in major organs.  
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20 Gadolinium (Z=64) is another alternative to gold which has been routinely used as a  
21 contrast agent in MR imaging. For instance, gadolinium oxide nanoparticles behave as  
22 efficient positive contrast agents for MR imaging and show radiosensitizing effects in  
23 gliosarcoma rat models [13]. In a new report, bovine serum albumin coated gadolinium  
24 nanoclusters were developed as multifunctional theranostics that could be used for  
25 CT or MR imaging and photothermal or radiation therapy of tumor treatment [14].  
26 Moreover, the low toxicity and efficient renal clearance of these theranostics suggest  
27 their practical applications for cancer diagnoses and therapies. A new gadolinium  
28 nanoparticle agent, named AGuIX (Activation and Guidance of Irradiation by X-Ray),  
29 has recently been established as a potent radiosensitizer in cancer treatment. AGuIX  
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3 has high capacity of absorption of photons delivered by a radiotherapy beam which  
4 generates an enhanced local dose deposit. Accordingly, combining AGuIX with  
5 radiation therapy greatly enhanced tumor cell death, and improved the survival ratio of  
6 animals with multiple brain melanoma metastases [15]. Currently, the first phase I trial is  
7 going on to study the side effects and optimum dose of AGuIX when given together with  
8 whole brain radiation therapy in treating patients with multiple brain metastases [16].  
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10 Magnetic particles are another option to use in cancer radiation therapy in combination  
11 with hyperthermia. Hyperthermia is a treatment of cancer by exposing tumor tissues to  
12 high temperatures which can damage and kill cancer cells, usually with minimal harm to  
13 normal tissues. Hyperthermia may induce some cancer cells to be more sensitive to  
14 radiation or damage the cancer cells that radiation cannot kill. Iron oxide nanoparticles  
15 (FeNPs), due to their low toxicity and ability to emit heat energy when excited by  
16 magnetic fields, are generally used in hyperthermia. Additionally, FeNPs are used as  
17 contrast agents for MR imaging. Due to these benefits, several varieties of FeNPs have  
18 been produced for cancer therapy. Typically, dextran-coated FeNPs have shown to  
19 decrease tumor growth in a breast cancer model using hyperthermia and irradiation  
20 [17]. Most importantly, both intracranial and intratumoral hyperthermia using FeNPs  
21 combined with radiotherapy could be safely applied on patients with glioblastoma  
22 multiforme [18] [19]. A few studies have also focused on the radiosensitization  
23 properties of FeNPs. Irradiation on human prostate carcinoma cell line (DU145) with  
24 FeNPs (1 mg/ml) produced a dose enhancement factor of approximately 1.2 [20]. In  
25 another study, several tumor cells were irradiated in the presence of citrate- or malate-  
26 coated FeNPs, where a drastically increased concentration of reactive oxygen species  
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3 in the tumor cells verified these nanoparticles as potent radiosensitizers for radiation  
4 cancer therapy [21].

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7 Recently, Iodine nanoparticles (INPs) are developed for radiotherapy to overcome the  
8 drawbacks of AuNPs in clinical translation [22]. The major drawbacks of AuNPs are due  
9 to cost, skin discoloration and poor body clearance. In contrast, INPs have unique  
10 characteristics; they are almost colorless, non-toxic, and cost-effective in manufacturing.  
11 Above all, INPs show higher body clearance from the liver (50% in 6 months) compared  
12 to AuNPs (9% in 6 months) [23]. Experimentally, INPs when combined with  
13 radiotherapy or chemotherapy extended the survival period of the treated groups [22]. In  
14 addition, INPs have compatible size (20 nm) for better tumor penetration compared to  
15 liposomes (>100 nm) [24], and they, being coated with PEG, have longer blood half-life  
16 (40 hours) for better tumor uptake. When injected through intravenous route, INPs  
17 strongly accumulates in tumors at high levels providing extended-time for high contrast  
18 vascular and tumor imaging [26].  
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### 38 **Concluding remarks**

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40 Although significant progress has been made in radiation therapy for cancer treatment  
41 using metallic nanoparticles, the inherent radioresistance and inaccuracy of localization  
42 of nanoparticles in tumors weaken the clinical implementation. Hence, the development  
43 of nanosized particles with versatile properties (biocompatibility, low toxicity and  
44 targeting efficacy) to use in imaging-guided radiation therapy has always been a top  
45 priority in biomedical research. Next generation metallic nanoparticles decorated with  
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3 biocompatible coating and functionalized with ligands specific to cancer cells will solve  
4 the current problems of imaging and therapy.  
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# Theranostics Metallic Nanoparticles

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## Status

In recent years, the field of nanomedicine has experienced significant growth in research interest. The use of metallic nanoparticles (MNPs) has been especially notable due to both the number of scientific publications advancing the field and the scope of applications. Advancements in synthesis, conjugation, transport and distribution patterns of nanoparticles have allowed for their use in numerous biomedical applications. Coupling the diagnostic and therapeutic capabilities of these MNPs to form theranostic agents is an active area of research. The therapeutics research area seeks to increase nanoparticle targeting, treatment dose distribution, and multidrug delivery. Concurrently, the diagnostics area provides improved image quality and enhanced contrasts. Nanoparticle theranostics harnesses the multi-functionality of MNPs to allow for continuous diagnosis or assessment of patients before, during and after therapy to better understand and increase treatment efficacy. This would allow for the development of more tailored therapies, and improvements in patient prognosis<sup>1,2</sup>.

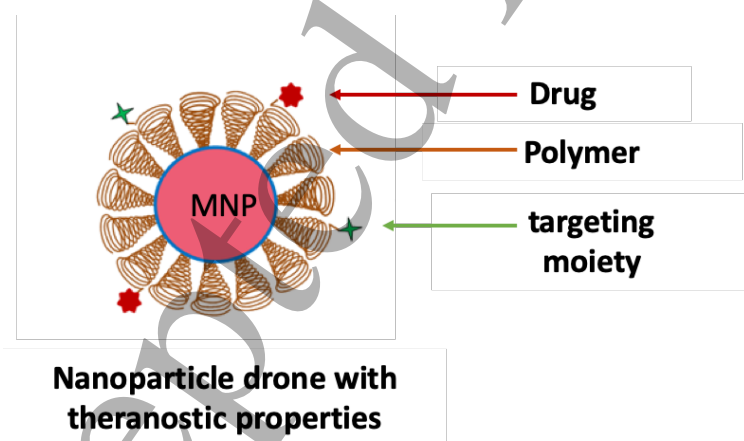


Figure 1: Metallic Nanoparticle designed for theranostic applications.

The synthesis of nanoparticle theranostic agents can occur in several ways. Therapeutic nanoparticles can be conjugated with contrast agents such as radioisotopes or additional imaging contrast agents to induce additional diagnostic imaging functionalities. Conversely, imaging

nanoparticles can be conjugated with therapeutic agents such as drugs, radio and photosensitizers, and therapeutic moieties to induce therapeutic functionalities<sup>3</sup>. Certain metallic nanoparticles have intrinsic theranostic (therapeutic and imaging contrast) capability (e.g. gadolinium or gold nanoparticles). Other nanoparticles may be hybrid nanoparticles—composed of more than one metal. Such combination promises to be more effective as it can make use of the inherent positive characteristics of each metal making up its composition<sup>4</sup>. Much focus has been placed on refining nanoparticle synthesis as it is an important determinant of its functionality.

Due to the inherently complex nature of biological systems, nanoparticles must be optimized to have specific and measurable mechanics. They must have large enough blood circulation half-lives to allow them to reach their targeting site. In their therapeutic applications, they must be able to efficiently deliver a specific dose of drugs to a localized region so as to decrease toxicities. Once delivered, they must be able to be rapidly and specifically accumulate within biological targets of interest in order to reduce collateral damage and to increase treatment specificity. In their diagnostic applications, they must be able to analyze and report the biological, biochemical and morphological characteristics of the tissue or disease of interest in an effective way. After performing their theranostic tasks, they must be effectively degraded into noncytotoxic byproducts or be cleared from the body quickly and effectively without inducing any toxicities<sup>3</sup>.

### **Current and Future Challenges**

Most nanoparticles when used in solid tumors for imaging and drug delivery rely on the Enhanced Permeability and Retention (EPR) effect to localize within the tumor microenvironment. This EPR effect is induced by aberrant tumor vasculatures that are characteristic of most solid tumors<sup>5-7</sup>. Unfortunately, it also leads to extensive difficulties when attempting to determine how to best optimize nanoparticles for specific targeting—as it becomes significantly difficult to determine environmental conditions and create predictable diffusion or transport mechanisms<sup>5,8</sup>. Engineering nanoparticles that have dynamic active targeting capabilities is an important field of interest.

A significant hurdle in the use of nanoparticle theranostics in clinical settings is due to an inability to generate theranostic agents capable of meeting all the above-mentioned nanoparticle

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3 optimization criteria. In the current state of nanoparticle synthesis, strides in one direction often  
4 require that compromise be made in other directions. For example, increasing blood circulation  
5 half-life often leads to increased toxicity. Conversely, conjugating nanoparticles with tumor-  
6 specific ligands for increased binding often leads to decreased blood circulation half-life.  
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10 Discerning methods to simultaneously achieve these criteria without sacrificing any is an active  
11 area of research<sup>1,3</sup>  
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14 Several parameters that must be optimized for the use of these nanoparticles in everyday  
15 clinical practice includes: optimizing nanoparticle materials based on their intended goals,  
16 increasing the efficiency of ligand conjugations, decreasing the cost of nanoparticle synthesis  
17 and conjugation, and development of methods that allow for consistent reproducible nanoparticle  
18 synthesis such that their size, density, and material composition remains homogenous.  
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20 Furthermore, nanoparticles must be designed to be able to avoid innate immune recognition,  
21 though they could also be tailored to enhance immunotherapy.  
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### 27 **Advances in science and technology to meet challenges**

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29 Metallic nanoparticles have intrinsic physicochemical and biocompatible properties that  
30 make them strong candidates for theranostic applications. Gold nanoparticles have been shown  
31 to be relatively noncytotoxic, to increase the therapeutic ratio of radiotherapy, and to improve  
32 local imaging<sup>9-13</sup>. Recent advancements in this space have led to the development of metallic  
33 nanoparticle conjugates capable of selectively binding tumor-specific ligands. One such example  
34 utilizes the angiogenic markers characteristic of many tumors. A second mechanism is the  
35 generation of folate-conjugated porphyrins based nanoparticles which utilize the fact that the  
36 folate receptor, a glycosylphosphatidylinositol-linked membrane protein, is highly expressed in  
37 many tumors and has a high affinity for folic acid<sup>14-17</sup>.  
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45 Because nanoparticle theranostics is a combination of the diagnostic and therapeutic  
46 fields, independent progress in any of these two fields, theoretically can advance theranostics;  
47 one type can simply be conjugated with the characteristics of the other to form theranostic  
48 capabilities<sup>6</sup>. The Food and Drug Administration (FDA) has successfully accepted more than 35  
49 nanoparticles for therapeutic and image diagnostic use in clinical trials<sup>2,3</sup>. These nanoparticles  
50 can be employed as platforms to incorporate theranostic agents and thus be able to reach clinical  
51 trials which will allow for expedited approval for clinical trials.  
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5 Substantial effort has gone toward formulating mechanisms that increase the circulatory  
6 half-life of theranostic nanoparticles. By coating nanoparticle theranostics with cellular  
7 membrane of leukocytes and red blood cells, their susceptibility to immune recognition is  
8 considerably minimized<sup>18</sup>. These nanoparticles can also be synthesized to show active targeting  
9 of select tumors by conjugating multiple targeting ligands to the nanoparticle. Furthermore, one  
10 active area of research looks to map out the diffusion and accumulation of varying nanoparticle  
11 sizes on breaking through the vasculature epithelium to accumulate in tumor  
12 microenvironments<sup>8</sup>.  
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## 22 Conclusion

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24 Nanoparticle Theranostics using MNPs has become a very active field of research and  
25 promises to both enhance therapeutic delivery and retention of chemotherapeutics,  
26 photosensitizing and radiosensitizing agents, as well as improving imaging quality. Although  
27 only few nanoparticle platforms have reached clinical trials, the significant amount of interest  
28 and research activity in this space promises to quickly change that. The use of nanoparticles as  
29 theranostic agents will help enhance personalized disease therapy by enabling continuous and  
30 concomitant diagnosis and treatment. Developing agents capable of long blood circulation,  
31 specific therapeutic dose delivery, decreased toxicity and an ability to specifically accumulate  
32 within a target region are all avenues of ongoing studies.  
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# Metallic Nanoparticles for Enhancing Radiotherapy and Immunotherapy

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## Status

The treatment of cancer has been transformed by immunotherapy, which now represents a fourth pillar of modern cancer therapy with surgery, radiotherapy, and chemotherapy. For many cancers, immunotherapy is combined with standard therapies to improve locoregional tumor control and overall survival rates. However, many patients receiving immunotherapy fail to achieve long-term, durable responses. A recent clinical trial for head and neck cancer showed that 20% of patients achieved a radiographic treatment response to immunotherapy, while responses in melanoma, renal cell carcinoma, and lung cancer range from 20–25%. [1,2] Innovative strategies are needed to bolster immunotherapy responses.

In certain clinical contexts, radiotherapy can act synergistically with immunotherapy. Radiotherapy triggers immunological cell death (ICD), release of tumor-associated antigens (TAAs), and radiation-induced neoantigens. Modulation of the tumor microenvironment (TME) following radiotherapy elicits immunostimulatory effects such as cytotoxic T-cell infiltration. While immunosuppressive factors in the TME offset some of the salutary effects of radiation, coupling radiotherapy with immunotherapy can tip the scale towards more immunogenic effects. [3] Recent data from patients treated with radiotherapy combined with immunotherapy suggest that this approach may have clinical utility. [4–6]

An area of interest for radiotherapy and immunotherapy synergy is the abscopal effect. Proponents posit that the local effects of radiotherapy can activate systemic anti-tumor immunity and cause regression of distant, non-irradiated tumors. While the abscopal

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3 effect has been reported in preclinical models and case reports, approaches to  
4 reproducibly elicit abscopal responses in patients remain undefined.  
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7 Given the role of metallic nanoparticles (MNPs) as radiotherapy enhancers, there is now  
8 interest in determining whether MNPs may likewise enhance immunotherapy. The  
9 delivery of immunostimulatory agents into the TME may also be facilitated by  
10 nanoparticle carriers. Through their dual roles as radiotherapy enhancers and carriers of  
11 immunostimulatory agents, nanomedicines could have an increasing role in the next  
12 generation of immunotherapy-based treatments.  
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### 21 **Current and Future Challenges**

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23 Initial case reports of the abscopal effect generated significant interest and have led to  
24 hundreds of preclinical studies and clinical trials combining radiotherapy and  
25 immunotherapy.[7] However, it remains uncertain how to reproducibly elicit abscopal  
26 effects in patients. For nanomedicine to impact immuno-oncology, at least three  
27 questions must be addressed: (1) Can nanomaterials amplify radiotherapy-induced ICD  
28 and release of TAAs? (2) Can nanomaterials modulate the immunosuppressive TME?  
29 (3) Can nanomaterials-based treatment strategies limit immune-related systemic  
30 toxicities?  
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37 Preclinical data suggest that the radiation dose per fraction is correlated with activation  
38 of anti-tumor immunity. Doses greater than 8 Gy per fraction increased anti-tumor  
39 immunity via the cGAS/STING pathway as measured by interferon-beta (IFN- $\beta$ )  
40 expression, activated dendritic cells (DCs), and infiltrating cytotoxic T-cells. Doses  
41 above 20 Gy per fraction diminished anti-tumor immunity via activation of the DNase  
42 Trex1, suggesting an optimal window in which radiotherapy bolsters anti-tumor  
43 immunity.[8] A recent study of ablative radiotherapy combined with CTLA-4 immune  
44 checkpoint inhibitors reported increased T-cell diversity and cytotoxic T-cell expansion  
45 and decreased immunosuppressive regulatory T-cells.[9] MNPs including gold and  
46 hafnium oxide nanoparticles are well-established as radiosensitizers via the increased  
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3 production of secondary electrons.[10] Emerging data suggest that MNPs may lead to  
4 increased anti-tumor immunity by amplifying the biological effects of radiotherapy.  
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7 The interactions between nanomaterials and the immune system are multifaceted.  
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9 Nanomaterials bolster immune activity by several mechanisms, including delivering  
10 immunoadjuvants to stimulate DC maturation, increasing TAA release, and modifying  
11 the TME to promote T-cell infiltration.[11] However, the innate and adaptive immune  
12 systems have evolved mechanisms to recognize and remove 'non-self' entities including  
13 nanomaterials. Circulating and tumor-resident macrophages can efficiently phagocytose  
14 nanomaterials to limit therapeutic benefit. The complex give-and-take between  
15 nanomaterials and the immune system presents multiple opportunities for improvement.  
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17 For example, coating nanoparticles with CD47, a protein which evades phagocytosis,  
18 leads to reduced nanoparticle clearance rates.[12]  
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25 Delivery of therapies and circulating cells into tumors is limited by transport barriers  
26 including blood flow, extravasation, and interstitial diffusion. Radiotherapy may promote  
27 anti-tumor immunity by modifying these physiologic transport barriers.[13,14] A  
28 comprehensive understanding is required at the molecular, cellular, and tissue levels to  
29 delineate how MNPs dynamically modify the TME and the resulting biological response,  
30 including infiltration and efflux of immune cells. The extension from these efforts would  
31 focus on developing nanomaterials that modify the TME to optimize activation of anti-  
32 tumor immune pathways.  
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39 An important consideration when combining radiotherapy and immunotherapy is the  
40 potential for immune-related toxicity affecting multiple organ systems. While studies  
41 have shown combined radiotherapy and immunotherapy to be well-tolerated, others  
42 have reported increased toxicity rates. [15] Many nanomaterial-based strategies have  
43 focused on improving therapeutic efficacy, but there may be beneficial opportunities for  
44 nanomaterials to reduce immune-related systemic toxicity.  
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### 53 **Advances in science and technology to meet challenges**

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55 *Increasing immunological cell death with nanomaterials*  
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3 MNP  
4 generate secondary electrons to amplify the radiation dose within tumors. Gold  
5 nanoparticles are established as radiotherapy enhancers via increased secondary  
6 electron production and reactive oxygen species (ROS) formation.[10] Investigators  
7 have established that gold nanoparticles can serve as radiosensitizers for megavoltage  
8 (MV) energies commonly used in the clinic.[16] Future work will clarify whether gold  
9 nanoparticles and radiotherapy can modulate anti-tumor immunity. Hafnium oxide  
10 nanoparticles are currently in clinical trials for head and neck cancer, soft tissue  
11 sarcoma, prostate cancer, and liver cancer. Initial data suggest that hafnium oxide  
12 nanoparticles combined with radiotherapy may increase ICD, T-cell infiltration,  
13 activation of adaptive immunity, and cytokine signaling compared to radiotherapy  
14 alone.[17] While intriguing, more data is needed through prospective clinical trials to  
15 determine the immunomodulatory properties of hafnium oxide nanoparticles. Additional  
16 nanomaterials including gadolinium, platinum, and cerium oxide have been explored as  
17 radiotherapy enhancers.[18] Because of their shared mechanisms for enhancing  
18 radiotherapy, approaches combining MNPs with chemical oxygen mimetics such as  
19 nimorazole or misonidazole may be beneficial for local control of treatment-refractory  
20 hypoxic tumors.  
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### 32 33 *Immune-Nanomaterial interactions in the tumor microenvironment*

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35 Nanomaterials designed to modulate the TME are engineered to have pharmacokinetic  
36 (PK) and biodistribution properties that permit accumulation in the TME. Numerous  
37 studies have found that for particles of similar charge and hydrophilicity, the principal  
38 systemic clearance mechanisms are renal and hepatobiliary excretion for smaller and  
39 larger particles, respectively.[19] An additional factor is the interaction of nanomaterials  
40 with circulating and tissue-resident macrophages. CD47-coated nanoparticles showed  
41 reduced rates of phagocytosis by activated M1 macrophages, demonstrating that  
42 surface modifications of nanomaterials can alter the interaction between nanomaterials  
43 and immune cells.[12] Incorporating immunoadjuvants into nanomaterials represents a  
44 promising approach to modify the TME. While radiotherapy can be transiently  
45 immunostimulatory, opposing immunosuppressive factors in the TME may negate this  
46 effect.[3] Nanomaterials localized to the TME that deliver a sustained release of  
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3 immunostimulatory molecules may be beneficial for activating anti-tumor immunity.  
4 Research aimed at deciphering the multiscale interactions between nanomaterials, the  
5 TME, and immune system will be informative toward these efforts. Advanced imaging  
6 techniques such as intravital microscopy, genetically-engineered mouse models  
7 (GEMMs), and fluorescently-labeled cells and nanomaterials may be combined in  
8 innovative ways to reveal dynamic interactions in tumors.  
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#### 14 *Nanomaterials to reduce toxicity of radiotherapy and immunotherapy*

16 Using nanomaterials to deliver immunotherapy to the TME and surrounding lymphatics  
17 may offer an approach for minimizing toxicities of systemic immunotherapy. A recent  
18 study investigated this approach in a preclinical model in which tumors received  
19 radiotherapy with intratumorally-administered anti-CD40 antibody in an emulsified  
20 formulation that permitted sustained local release.[20] Maximizing the intratumoral  
21 concentration of nanomaterials to reduce systemic toxicity can be achieved by at least  
22 several mechanisms. Direct intratumoral injection of nanomaterials is being explored in  
23 preclinical and clinical studies. Delivery of molecules using nanoparticle carriers can  
24 also increase local concentrations of immunotherapies while reducing systemic  
25 toxicities. Efforts to target nanomaterials would include cataloging secreted proteins in  
26 the TME and those upregulated following radiotherapy to identify potential targeting  
27 ligands. Inter- and intra-tumor variability are relevant factors, as tumor sequencing has  
28 revealed considerable genetic and molecular heterogeneity between tumors.  
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#### 42 **Concluding Remarks**

44 While immunotherapy has transformed modern oncology, many patients fail to achieve  
45 long-term, durable responses. Efforts are underway to bolster responses by combining  
46 radiotherapy with immunotherapy. Given the role of MNPs as radiotherapy enhancers, it  
47 follows that combinations of MNPs, radiotherapy, and immunotherapy may improve  
48 local and distant tumor control. We have highlighted several challenges in this emerging  
49 field. Can MNPs increase ICD following radiotherapy? Can MNPs evade macrophages  
50 and other clearance mechanisms *in vivo*? Can MNPs reduce the toxicity of systemic  
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3 immunotherapy? The multiscale complexity of this work requires collaborations between  
4 biologists, chemists, engineers, and clinicians. Based on early results, there is cause for  
5 cautious optimism that nanomaterials-based solutions will maximize immunotherapy  
6 and radiotherapy responses in cancer patients.  
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## Concluding Remarks:

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This special collection of mini-articles on metal nanoparticles in radiation therapy provides a series of commentaries on the state-of-the-science and where the field is headed in the future, both in the laboratory and in the clinic. Drawing on the expertise of a non-complete selection of thought leaders in the field, this collection charts out a roadmap for future research activities. Each commentary outlines lessons learned on the path to where the field is today, highlights perceived challenges as the field advances towards the future, and finally discusses scientific and technological advances that might help overcome hurdles in the future. Although this is a compilation of several commentaries, the terseness of each commentary (mandated by the journal and us) makes for easy reading of multiple viewpoints from multiple perspectives and disciplines, all coalesced under one umbrella. We believe such a compilation of opinion pieces is especially interesting for an uninitiated reader who is looking for a high-level overview in broad brushstrokes. More in-depth information is readily available in articles referenced by the authors, many of which are co-authored by the same experts who wrote the commentary. For more advanced readers, these articles offer a summary of current challenges and an outlook of where we envision the field is headed.

A theme that emerges across all commentaries is that the science of fabricating, characterizing, decorating, imaging, and modeling of metal nanoparticles is mature and highly sophisticated. Nanoparticles are now used for a large variety of applications, most notably for functional imaging that has already advanced into the clinic. In the theranostic or radiation dose enhancing realm, the understanding of the underlying physical, chemical and biological consequences of interactions between these nanoparticles and ionizing radiation is evolving rapidly. Preclinical data across research teams are exciting and consistent in their ability to enhance radiation therapy effectiveness via physical and biological radiation sensitization. Against this background of palpable excitement are broad efforts to translate these technological advances to clinical reality after addressing safety, biocompatibility and scale-up issues. These clinical adoption strategies may span a variety of metallic nanoparticle constructs, a spectrum of targets within the tumor microenvironment, and combinations with other therapies including chemotherapy or immunotherapy. The enormous progress made in our understanding of fundamental nanoparticle-radiation interactions is especially meaningful because it has required the concerted efforts of scientists in disparate disciplines working individually or, more often, in unison to achieve these goals. While challenging questions remain regarding the best way to synergize nanoparticle use with radiation therapy without collateral toxicity, the overall therapeutic approach remains highly promising. As can be seen from the articles, all of the authors share the vision of advancing the outcome of radiation therapy using various constructs of nanoparticles by providing better imaging or therapeutic effects or a combination of both. Widespread clinical translation is long overdue. We hope this collection of research reflections and anticipated future directions will play a small role in making our vision of routine clinical use of metallic nanoparticles to enhance therapy a reality.