Nanoparticle radio-enhancement: principles, progress and application to cancer treatment

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Nanoparticle radio-enhancement: principles, progress and application to cancer treatment

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Abstract
Enhancement of radiation effects by high-atomic number nanoparticles (NPs) has been increasingly studied for its potential to improve radiotherapeutic efficacy. The underlying principle of NP radio-enhancement is the potential to release copious electrons into a nanoscale volume, thereby amplifying radiation-induced biological damage. While the vast majority of studies to date have focused on gold nanoparticles with photon radiation, an increasing number of experimental, theoretical and simulation studies have explored opportunities offered by other NPs (e.g. gadolinium, platinum, iron oxide, hafnium) and other therapeutic radiation sources such as ion beams. It is thus of interest to the research community to consolidate findings from the different studies and summarise progress to date, as well as to identify strategies that offer promising opportunities for clinical translation. This is the purpose of this Topical Review.

List of abbreviations and acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AuNP</td>
<td>Gold nanoparticle</td>
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<tr>
<td>BP</td>
<td>Bragg peak</td>
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<tr>
<td>GdNP</td>
<td>Gadolinium based nanoparticle</td>
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<tr>
<td>HfNP</td>
<td>Hafnium based nanoparticle</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>LET</td>
<td>Linear energy transfer</td>
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<td>NP</td>
<td>Nanoparticle</td>
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<td>NRE</td>
<td>Nano-radio-enhancer</td>
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<tr>
<td>PtNP</td>
<td>Platinum nanoparticle</td>
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<tr>
<td>SOBP</td>
<td>Spread-out Bragg peak</td>
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<tr>
<td>SPION</td>
<td>Super-paramagnetic iron-oxide nanoparticle</td>
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1. Introduction

Nanoparticle strategies for enhancing the therapeutic efficacy of cancer treatment modalities continue to be actively investigated (Davis et al 2008, Mura and Couvreur 2012). In the context of radiotherapy treatment, high atomic number (Z) nanoparticles (NPs) have attracted increasing interest for their capacity to enhance radiation damage. Indeed, the introduction of high-Z NPs into a tumour offers the possibility of amplifying radiation damage effects selectively in the tumour as compared to healthy tissues, thus enhancing therapeutic efficacy. The property specific to high-Z nanoparticles is the enhancement of electron release in the medium compared to water due to atomic excitation by incident radiation. Note that high-Z NPs have been referred to as radiosensitisers, similar to drugs that sensitisce cells to radiation (e.g. DNA repair drugs). In this review, we distinguish these two categories of agents and refer to NPs used to amplify radiation effects as ‘nano-radio-enhancers’ (NREs).

Most studies to date have focused on gold NPs of different sizes, shapes and surface coatings, which offer the advantage of being easily synthesised and stable, as well as exhibiting good biocompatibility and
pharmacokinetics (Jiang et al. 2008, Toy et al. 2014), as well as the capacity to internalise within cells and enhance radiation effects therein (e.g. Chithrani et al. (2010) and Bhattarai et al. (2017)). Other NPs have been developed in parallel including theranostic agents, which are now attracting growing interest for their potential to enhance not only targeted radiation effects but also contrast in magnetic resonance imaging and other imaging modalities (e.g. Luchette et al. (2014)).

1.1. Motivation and rationale for this review
This Topical Review is motivated by the rapidly increasing number of studies investigating the potential of NREs to enhance external beam radiotherapy (photons and ions) in a clinical setting. While a number of reviews of this topic have been published in the literature, these have focused almost exclusively on gold NPs and photon irradiation (e.g. Hainfeld et al. (2008), Butterworth et al. (2012, 2013), Jain et al. (2012) and Schuemann et al. (2016)). With recent developments using other NREs, particularly gadolinium, which is a theranostic agent (reviewed by Sancey et al. (2014) and Lux et al. (2015)), and using other radiation treatment modalities such as proton therapy and heavy ion therapy, there is a need to:

i. consolidate our understanding of and rationalise the various approaches to nano-enhanced photon and particle based radiation therapies;
ii. identify strategies to support the next phase of development of nano-enhanced radiation therapy approaches with high potential for clinical translation;
iii. support the development of relatively under-explored capabilities for nanoparticle radio-enhancement combined with particle therapy;
iv. expose the added value of emerging opportunities such as internal radionuclide targeted therapy techniques combined with nano-agents and new nano-carriers as highly promising tumour targeting theranostic and patient adaptable platforms.

1.2. Scope of review
This review aims to provide:

– an up-to-date overview of the current understanding of the principles of high-Z nanoparticle activation by low-energy (keV) and high-energy (MeV) photons, as well as by electrons and incident ions, and the subsequent impact on the surrounding biological medium, with a focus on the initial physical processes involved;
– a summary of progress to date from experimental, simulation and modeling studies investigating radio-enhancement effects by gold NPs activated by photons or ions;
– a review of the development of other emerging and promising NREs, such as gadolinium based nano-agents, platinum nanoparticles, as well as hafnium oxide, iron oxide, cerium oxide and nanodiamonds;
– an overview of recent simulation, theoretical and modeling studies on radio-enhancement by NREs in general.

The structure of this review is as follows: section 2 presents the physical principles underlying high-Z nanoparticle radio-enhancement; sections 3–5 review the progress made to date from multiscale experimental studies—from in vivo to molecular and cellular scales—that have contributed to the increasing interest in NREs to improve photon and also particle beam radiation therapy; section 6 summarises corresponding modeling studies for incident photons and ions; and section 7 briefly summarises emerging strategies that present particularly promising opportunities for clinical translation. Finally, in section 8, we draw the main conclusions from our review and present our own perspective on the outlook for nanoparticle-enhanced radiation therapies.

2. Physical processes
The physical processes involved in the interactions of photons and ions with condensed matter have been extensively presented in the literature (see e.g. Hatano et al. (2010)).

Briefly, the physical stage consists of the interaction of the primary beam (typically photons or fast ions) as well as the interaction of secondary particles (photons and electrons) produced along the primary radiation trajectory with the high-Z constituent atoms of nanoparticles embedded in biological tissue. This early excitation/ionisation stage is promptly followed by the de-excitation of the system via redistribution/rearrangement of electronic states, resulting in fluorescent photon emission (predominantly K-shell transitions) and Auger electron emission (predominant for inner shells other than the K orbital) (Evans 1955). Provided they are emitted in a dense medium (nanoparticle or biosystem), these secondary photons and electrons of energies ranging from...
few eV (Auger electrons) to MeV (delta electrons) may successively ionise surrounding biomolecules as well as neighbouring nanoparticles. This cascade is expected to result in the enhancement of the radiation-induced perturbation highly localised to the nanoparticle-loaded biosystem. The basics of these processes are represented schematically in figure 1 and summarised in this section.

2.1. Interaction of photons with high-$Z$ nanoparticles

Photoelectric absorption is the dominant interaction process of incident keV energy photons (typically up to $\approx 500$ keV) with high-$Z$ atoms ($Z \approx 60–80$ amu). The most studied high-$Z$ nanoparticles considered as NREs are typically comprised of gold ($Z = 79$), platinum ($Z = 78$), hafnium ($Z = 72$), or gadolinium ($Z = 64$). The photoelectric cross-section strongly depends on $Z$, especially at photon energies $E$ just above the absorption edges of the atom, where $\sigma \propto (Z/E)^n$, with $n \approx 3–4$. Thus, high-$Z$ nanoparticles are able to transfer energy from radiation to the medium, through photoabsorption and subsequent electronic emission, much more effectively than water.

Ejection of a photoelectron, in principle from any shell, results in an electronic perturbation around the nanoparticle. Multiple photoelectrons emitted from many atoms in a high-$Z$ NP (and indeed from multiple NPs) can thus give rise to an enhancement in dose, relative to that which would be measured without the presence of NPs. However, an additional enhancement effect arises from the ensuing atomic de-excitation processes (discussed below in section 2.3) that amplify the electronic perturbations in the immediate vicinity of NPs. We refer to this as ‘nanoscale enhancement’ of the radiation effects. The range of the ejected photoelectron is determined by the difference between $E$ and the shell binding energy, $E_B$. For example, a $100$ keV photon incident on a gold atom ($Z = 79$) is most likely to eject an electron from the $K$-shell ($E_K \approx 80.75$ keV). As a result, a photoelectron, which is ejected with a kinetic energy of $19.25$ keV, may travel to a range in water of approximately $9 \mu$m, which is comparable to the size of a single cell. On the other hand, a $200$ keV photon incident on gold may induce a $119.25$ keV $K$-shell photoelectron, with a range $\approx 200 \mu$m, sufficient to traverse several cells. An incident photon with $E < E_K$ can instead eject an electron from a shell higher than $K$-shell (i.e. $L$, $M$ shells), although the photon mass attenuation and absorption coefficients rapidly become comparable to that of soft tissue at low energies, so that only the inner shells ($K$, $L$, $M$) are potentially capable of producing dose enhancement through photoabsorption (e.g. the mass attenuation coefficient of soft tissue is comparable to gold at energies just below its $M$-edge, $\approx 2–3$ keV).

Figure 2 presents plots of the mass attenuation and absorption contrast against water for $1\%$ Au ($Z = 79$) or $1\%$ Gd ($Z = 64$), elements commonly used as NREs. Absorption differs from attenuation because above the $K$-edge, energy transferred from the incident photon to the atom can be converted into fluorescence emission, whereas below the $K$-edge, characteristic radiation is emitted predominantly via low-energy Auger electrons, which are locally absorbed (see table 1). Byrne et al (2017) showed with Monte Carlo (MC) simulations that the enhanced photoelectric absorption contrast against water in a target with $1\%$ atomic gold can not only result in dose enhancement in the target, but can also reduce out-of-target scatter dose. This effect is offset by fluorescence emission and so is most effective for incident x-ray energies below the $K$-edge.

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![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Schematic illustration of inelastic interactions with a high-$Z$ nanoparticle for: (a) incident keV photons (orange clouds represent photoelectric events); (b) incident MeV photons (blue and yellow clouds represent Compton scatter and pair production events, respectively); (c) incident electrons (blue and red clouds represent large and small impact parameters leading to ionisation and bremsstrahlung, respectively); and (d) incident ions (orange clouds indicate impact ionisation events). Image provided by Gholami, University of Sydney. Reproduced with permission from Y Gholami.
At photon energies $>500\text{ keV}$, Compton scattering becomes the dominant interaction process with high-$Z$ atoms and the atomic cross section varies as $\sigma \propto Z$. At MeV photon energies, pair production can also occur. The atomic cross-section is $\sigma \propto Z^2$, which potentially offers some contrast against water, although pair production is less important than Compton scatter at clinically relevant energies (below 10 MeV).

### 2.2. Interaction of charged particles with high-$Z$ nanoparticles

Particle therapy is a modality that is developing rapidly worldwide (Durante et al 2017). The application of NREs to improve particle therapy performance is also progressing rapidly (see the review by Lacombe et al (2017)). Charged particles interact with a nanoparticle comprised of high-$Z$ atoms via Coulomb interactions with a cross section that depends primarily on the kinetic energy and charge of the incident particle and on the impact parameter (radial distance from the particle’s track to the target). For high-$Z$ targets, nuclear stopping is negligible and fast ions or electrons, typically with energies $>1\text{ MeV u}^{-1}$, lose energy mainly via inelastic Coulomb collisions with the atomic electrons.

The electronic stopping power is mostly due to interactions with outer valence shell electrons until the incident ion or electron energy has sufficiently slowed to energies comparable to the mean orbital energy of inner shell (e.g. $L$, $K$) electrons (Hatano et al 2010). An important difference between ions and electrons is their track structure: electrons are readily deflected by collisions, whereas ions, being relatively much heavier, undergo mostly small angle deflections, which gives rise to the characteristic highly localised Bragg peak at the end of their trajectories, with heavier ions (e.g. carbon) exhibiting a more pronounced Bragg peak than protons.

The mass collision stopping power, $S$, has only a weak logarithmic dependence on $Z$, with $S \propto -\ln I$ given by the Bethe–Bloch formula, where $I$ is the mean ionisation/excitation potential that generally increases with $Z$. 

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**Table 1.** Total probability of Auger electron emission for $K$, $L$, and $M$ shell transitions in atoms commonly used as NREs (data from the ENDF flexible database explorer: [www-nds.iaea.org/exfor/e4explorer.htm](http://www-nds.iaea.org/exfor/e4explorer.htm)).

<table>
<thead>
<tr>
<th></th>
<th>Gd ($Z = 64$) (%)</th>
<th>Hf ($Z = 72$) (%)</th>
<th>Pt ($Z = 78$) (%)</th>
<th>Au ($Z = 79$) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$ shell</td>
<td>6.5</td>
<td>4.8</td>
<td>4.2</td>
<td>4.1</td>
</tr>
<tr>
<td>$L$ shell</td>
<td>86.2</td>
<td>80.5</td>
<td>75.5</td>
<td>74.6</td>
</tr>
<tr>
<td>$M$ shell</td>
<td>99.5</td>
<td>99.1</td>
<td>98.6</td>
<td>98.5</td>
</tr>
</tbody>
</table>
Thus, the mass collision stopping power decreases with $Z$ of the target for a given incident charged particle species and energy. For example, gold has $I \approx 790$ eV, which is an order of magnitude larger than that for water $I \approx 75$ eV. This means that radio-enhancement by high-$Z$ nanoparticles exposed to ions or electrons is primarily due to the density contrast of the nanoparticles against tissue. This is different from the case for keV photons, where the contrast against tissue is provided by the strong $Z$-dependence of photoabsorption (see figure 2). Ions and electrons are nevertheless capable of activating a nonlinear avalanche of electron emission from high-$Z$ nanoparticles through impact ionisation and ensuing Auger cascades. Importantly, the secondary electrons generated by these events can continue to excite and ionise surrounding biomolecules and neighbouring nanoparticles.

Solov’yov and co-workers also proposed that, for ion beam radiation, additional physics may take place that is based on the induction of a propagating shock wave in the medium surrounding a high charge ion track (Verkhovtsev et al 2016b). They developed a multiscale model to predict the induction of biodamage due to the combination of multiple mechanisms including shock-induced excitation/ionisation of the medium, transport of secondary particles, chemical interactions, and thermo-mechanical damaging pathways. Although their multiscale model does not explicitly consider nanoparticle radio-enhancement, it does nevertheless consider processes initiated at the nanoscale and thus potentially offers new insights into NRE in the context of ion-induced radiation damage effects.

### 2.3. De-excitation processes

Following ionisation of an inner shell by either photons or charged particles (electrons or ions), an excited atom can de-excite via fluorescent photon emission or Auger electron emission (Evans 1955). The relative importance of each process depends on the target atomic number $Z$. For high-$Z$ atomic targets, $K$-shell de-excitation occurs predominantly via fluorescence emission, e.g. for $Z > 55$, the probability of atomic de-excitation by $K$-alpha fluorescence is higher than 95%. De-excitation of high-$Z$ atoms via Auger electron emission occurs predominantly from inner shells higher than the $K$-shell, e.g. for $Z > 60$, the total probability of atomic de-excitation by Auger $L$-shell transitions is 60–80% (see table 1). Note that the Auger process takes place after $K$ shell ionisation and subsequent $L$ shell electron decay into the $K$ shell.

Fluorescent photons generally travel further than Auger electrons and thus delocalise the local perturbation away from a nanoparticle. For gold, for example, the $K$ alpha transition energy is $\approx 69$ keV, corresponding to a photon attenuation length of $\approx 5$ cm in tissue, whereas typical Auger electrons (energies $< 100$ eV) correspond to a range $< 10$ nm in tissue. Although the mass collision stopping power of low-energy electrons traversing a high-$Z$ medium is not well known, they may be readily stopped within a high-$Z$ nanoparticle by its high density. Thus, the size of a high-$Z$ nanoparticle determines how many Auger electrons can effectively escape self-absorption (McMahon et al 2011a). Similarly, nanoparticle coating can also attenuate Auger electrons that would otherwise contribute to radio-enhancement (Xiao et al 2011). For smaller nanoparticles, the chain of escaping multiple low-energy Auger electrons may result in a highly reactive nanoparticle with highly charged atomic states. The nanoparticle atoms, excited by intra-atomic Auger decay, can de-excite via inter-atomic/molecular electronic decay involving neighbouring water and other biomolecules (Stumpf et al 2016). Both inter-atomic Coulomb decay (Jahneke et al 2010, Mucke et al 2010, Goldberg et al 2014), driven by energy transfer, and electron-transfer mediated decay, driven by charge transfer (Stumpf et al 2013), are ultra-fast relaxation processes that may ionise surrounding molecules and produce slow electrons, thereby amplifying radiation damage beyond that predicted by Auger decay alone.

At very low-energies (typically tens of eV), an excited atom can also de-excite via collective transport processes that redistribute energy across electronic states (Fano 1992, Hatano et al 2010). Some noble metal atoms such as gold and platinum can de-excite via decay of surface plasmons, resulting from the delocalisation of electronic excitations into coherent motions of valence electrons (Kittel 1963, Verkhovtsev et al 2015a, 2015b). Solov’yov and co-workers demonstrated the yield of low-energy ($<50$ eV) electrons can be significantly enhanced, compared to water, by damping of surface plasmons as well as giant atomic resonances in high-$Z$ nanoparticles excited by ion irradiation (Verkhovtsev et al 2015a, 2015b). Following an excitation event such as a low-energy Coulomb collision, thermal equilibrium is eventually restored through electron–phonon interactions (Kittel 1963). Equilibrium is also recovered through recombination of thermalised electrons, although sub-excitation electrons can also undergo dissipative electronic electron attachment to surrounding water and other biomolecules, causing further radiation-induced damage (Boudaiffa et al 2000, Pan et al 2003).

Although a detailed exposition of the physico-chemical stage of radiation-induced effects is beyond the scope of this review, it is important to highlight the critical role that free radicals play in NRE effects. NPs have the capacity to release copious low-energy electrons in nano-scale volumes, which enhances the production of free radicals in the vicinity of the NPs. This high density of radicals can not only create a positive feedback on NP activation, but can also trigger biological signalling pathways that result in enhanced cell death.
3. **In vivo studies—proof of concept**

The amplification of radiation effects with high-\(Z\) compounds was first demonstrated *in vivo* using iodine complexes combined with keV synchrotron x-rays (Adam *et al* 2003). The first proof of concept using nanoparticles as radio-enhancers was demonstrated with gold nanoparticles (AuNPs) activated by keV x-rays (Hainfeld *et al* 2004).

### 3.1. Gold nanoparticles

Gold nanoparticles (AuNPs) are the most studied NREs owing to their high electronic density, which is favorable to amplification of radiation effects. In addition, small (<20 nm) AuNPs have a relatively straightforward synthesis route, high stability in biocompatible solvents, low toxicity, and also good biodistribution and pharmacokinetics (Perrault *et al* 2009, Laurent *et al* 2016). The effect of AuNPs activated by photons has been expertly reviewed by Butterworth *et al* (2012, 2013) and Jain *et al* (2012) (see also the earlier review by Hainfeld *et al* (2008)) and more recently by Haume *et al* (2016). Here, we summarise major findings in a broader context as well as reviewing more recent studies that have since been published in the literature.

Hainfeld *et al* (2004) were the first to demonstrate the ability of AuNPs to enhance the effect of x-ray radiation. They showed that intravenous injection of 1.9 nm AuNPs (up to 2.7 g Au/kg) improves the survival of mice bearing subcutaneous EMT-6 mammary carcinomas treated with 26 Gy of 250 kVp x-rays. A major breakthrough of this study was the preferential tumour targeting that these small AuNPs exhibited, an important attribute that was not observed in earlier studies (e.g. Herold *et al* (2000)). This was partly attributed to the small AuNP size, which possibly favours enhanced permeability and retention (EPR) through the impaired vasculature structure around tumours (although whether the EPR effect is relevant in humans has been questioned—see e.g. Danhier (2016)). Since this pioneering *in vivo* study, the field has seen a proliferation of other studies that have confirmed the radio-enhancement property of AuNPs.

Continuing on from their pioneering *in vivo* studies, Hainfeld *et al* (2013) studied the image contrast and radiotherapy enhancement effects of 11 nm AuNPs intravenously injected into mice bearing a highly malignant brain tumor. After delivering 35 Gy of 100 kVp x-rays, they found significant improvement in long-term (>1 year) survival of mice receiving AuNPs (4 g Au/kg) and radiation (≈56%) compared to radiation alone (≈19%) and no treatment (0%) (see figure 3). The radiation dose enhancement factor was estimated to be ≈300%. Although they demonstrated a localization to the brain after injection and observed a 19:1 tumor to normal brain uptake, the majority of AuNPs were blocked by the normal blood-brain barrier. Hainfeld and co-workers took advantage of this accumulation of AuNPs and performed contrast-enhanced, high-resolution imaging of the brain tumor using micro-CT.

CT-guided radiotherapy using AuNPs to enhance both CT image contrast and radiation effects in a tumour was studied by Al Zaki *et al* (2014). They found a 1.7-fold longer median survival time of mice bearing a HT-1080 human fibrosarcoma tumour treated with AuNP-assisted radiotherapy compared with radiotherapy alone (6 Gy at 150 kVp) using 650 mg Au/kg. The NPs consisted of 1.9 nm AuNPs encapsulated within polymeric micelles (hydrodynamic diameter ≈75 nm), which provide longer circulation time, better tumour uptake and more efficient excretion than core AuNP of the same size, as well as rapid clearance and reduced accumulation from the reticuloendothelial system. The high tumour uptake of these NPs resulted in better CT image contrast, thus demonstrating the potential for improving delineation of tumour margins.

In a similar theranostic study, Zhang *et al* (2015) used clusters of 2 nm AuNPs protected by a glutathione shell and also found high uptake in U14 cervical carcinoma bearing mice, as demonstrated by enhanced CT
image contrast relative to mice without tumour. They also found a \(\approx 66\%\) decrease in tumour volume following 5 Gy treatment with \(^{137}\text{Cs}\) gamma rays with the peptide-protected AuNP clusters, with a concentration of 5.9 mg Au/kg (injected intraperitoneally), compared to radiation only.

A different approach altogether was taken by Kunjachan et al. (2015), who investigated NP-enhanced radiation damage to the tumour neo-endothelium as a treatment strategy for tumours that otherwise respond poorly to standard therapies. Using PEGylated AuNPs (2–3 nm core, 8–10 nm hydrodynamic diameter), surface functionalised with a neo-vascular targeting ligand, they treated pancreatic tumour xenograft mice with 10 Gy of a 220 kVp x-ray beam. After excising the tumours 24 h after treatment, they found a 3-fold increase in DNA damage compared to control groups.

As pointed out by McQuade et al. (2015), an important limitation of using AuNPs as a theranostic platform is the significant disparity between the high gold concentration required to enhance CT image contrast (typically of the order of grams Au/kg body weight) compared to the tumour concentration required for radio-enhancement (up to three orders of magnitude lower). McQuade and co-workers instead developed a theranostic approach that uses magnetic resonance (MR) imaging, with contrast enhanced by 14 nm superparamagnetic iron oxide nanoparticles (SPIONs) loaded into pegylated micelles together with 2.2 nm AuNPs (total hydrodynamic diameter \(\approx 100\) nm). Similar to the study by Al Zaki et al. (2014) and McQuade et al. (2015) demonstrated a radio-enhancement effect of these NPs in HT-1080 tumour-bearing mice treated with 6 Gy of 150 kVp x-rays and 400 mg Au/kg, with a \(\approx 1.5\)-fold improvement in mean survival of mice treated with AuNP-SPION-loaded micelles compared to radiotherapy only. Additionally, they demonstrated an enhanced contrast of the MR tumour image, confirming tumour uptake of the AuNP-SPION-loaded micelles, and observed a correlation between MRI contrast-enhancement and tumour volume decrease, suggesting contrast-MRI can predict tumour response to treatment.

In a similar rat study, Miladi et al. (2014) coated \(\approx 2\) nm AuNPs with gadolinium chelates to show that MR contrast imaging can be used to optimise tumour radio-enhancement by monitoring the AuNP biodistribution and delaying irradiation until tumour uptake is observed. They found a significant peak in tumour uptake compared to healthy tissue internalisation approximately 5 min after intravenous injection (see figure 4(a)). Using the European Synchrotron to deliver Microbeam Radiation Therapy (MRT—with an x-ray spectrum spanning 50–350 keV), they found a 78% increase in median survival, compared to MRT only, of rats bearing the highly radioresistant 9L gliosarcoma brain tumour when treated with MRT 5 mins after injecting the Gd-chelated AuNPs (0.7 ml with [Au] \(= 50\) mM, corresponding to \(-23\) mg Au/kg for a 300 g rat) (see figure 4(b)).

More recently, the same team has observed that relatively small changes in the chemical formulation of the macrocyclic chelators used to graft gadolinium complexes on AuNPs can significantly affect the size, biodistribution and pharmacokinetics of AuNPs as well their radio-enhancement effect. They found that \(\approx 2.3\) nm AuNPs coated with TADOTAGA–Gd perform the best in improving the life span of 9L gliosarcoma bearing rats treated by kV x-ray MRT owing to their ability to penetrate deeper into the tumour volume (Laurent et al. 2016).

The studies summarised above used kV x-rays as radiation modality to take advantage of the high photoelectric absorption cross-section and consecutive release of secondary electrons from NREs to amplify radiation effects (see section 2). This physical basis notwithstanding, other recent in vivo studies have reported nanoparticle radio-enhancement using higher energy x-rays, including clinical MV beams.

For example, the \(\approx 66\%\) decrease in tumour volume reported by Zhang et al. (2015) using clusters of 2 nm AuNPs (5.9 mg Au/kg) was achieved using a \(^{137}\text{Cs}\) gamma source (which has a spectrum extending from kilovoltage x-rays to gamma-rays, peaking at 662 keV).
Similarly, Su et al (2015) delivered 5 Gy with a $^{60}$Co source (mean gamma energy 1.25 MeV) to treat tumour-bearing mice injected with AuNPs ($\approx$50 mg Au/kg body weight) conjugated with a tumour-targeting peptide and found more apoptosis and tumour volume reduction compared to radiotherapy using AuNPs without targeting peptides.

Using a clinical 6 MV beam, Wolfe et al (2015) investigated the effects of gold nanorods (AuNRs; 31 nm x 9 nm) conjugated with a tumour-targeting agent (goserelin) in a mouse prostate cancer model. The relatively high tumour cell accumulation of the AuNRs due to the targeting agent (and also to the EPR effect in rodents), allowed a relatively low concentration of gold to be used effectively (10 mg Au/kg body weight). A biodistribution study showed that the intratumoural accumulation of unconjugated (but pegylated) AuNRs was approximately 3-fold less. Radiotherapy (5 Gy) was delivered 24 h post-injection, and the team found enhancement of MV radiation effects by the goserelin-conjugated AuNRs, as evidenced by reduced tumour growth compared to MV treatment using unconjugated AuNRs and radiotherapy only. They attributed the MV radio-enhancement to clustering of the targeting AuNRs inside the tumour cells and the consequent increase in ionization density within the cytoplasm.

A similar conclusion was drawn by Dou et al (2016) who injected tumour-bearing mice with 13 nm pegylated AuNPs (delivering 60 nmol Au/kg, corresponding to just 12 $\mu$g Au/kg) and found dramatic inhibition of tumour growth following radiotherapy with clinical 6 MV beam treatment when compared to radiotherapy alone. Moreover, they observed complete eradication of tumour cells when treatment was delivered 4 h post intravenous injection, thus demonstrating again the importance of circulation time. They postulated that smaller AuNPs are more efficient than larger ones because they can form higher density clusters and release more electrons than larger AuNPs.

Popovtzer et al (2016) evaluated the efficacy of combined treatment of nanoparticle with radiation to the treatment of head and neck cancer cases. They showed that the treatment of mice with a clinical 6 MV flattening-filter-free (FFF) beam combined with intravenous injection of 6 mg Au/kg 30 nm AuNPs coated with cetuximab (an inhibitor of the epidermal growth factor receptor which is overexpressed in head and neck and other cancers) resulted in suppressed tumour growth compared to radiation only and radiation with cetuximab. Furthermore, using immunohistological markers, they established that the overall impact of AuNPs is due to a combination of physical radio-enhancement and biological radio-sensitization (in particular enhanced tumour apoptosis) effects.

These in vivo studies demonstrated the capacity of AuNPs to enhance the effects of MV beam radiation. This is somewhat unexpected because at these energies, the dominant photon interaction process is Compton scattering. Although the Klein–Nishina cross-section (Nishina 1928) differs from the energy dependence of the Klein–Nishina cross-section, the effective probability of a Compton interaction is given by the Klein–Nishina cross-section, which increases with energy for a given atomic number (Z).

These data indicate that AuNPs are particularly effective in enhancing the effects of MV beam radiation. However, more research is needed to fully understand the mechanisms behind these effects, and to determine the optimal conditions for their use in clinical applications.

Less evidence is available for AuNP radio-enhancement combined with incident proton or heavy ion beams compared to MeV x-rays. Kim et al (2010) were the first to demonstrate that small (1.9 and 14 nm diameter) AuNPs (and also 13–15 nm SPIONs) enhance the effect of proton irradiation. In their pioneering study, they observed $\approx$90% and $\approx$75% volume reduction of CT26 mouse tumours following intravenous injection of AuNPs and SPIONs (100 or 300 mg NP/kg), respectively, and irradiation with a 41.7 MeV spread out Bragg peak (SOBP) beam (see figure 6(a)). Note, however, that relatively high concentrations were injected to achieve these therapeutic effects: 15–55 g Au/kg body weight (and 5 or 15 g Fe/kg body weight). Nevertheless, from consecutive in vitro CT26 cell experiments, they established the link between in vivo impact and increased cell killing in the presence of NPs. They initially proposed that the observed radio-enhancement is due to particle induced x-ray emission (PIXE), although this is now suggested to play a negligible role in early stage mechanisms (see e.g. Cho et al 2016).

Indeed, in a similar follow-up study, Kim et al (2012) observed enhanced production of reactive oxygen species (ROS) in cells which they attributed primarily to secondary electron emission from NPs following activation either directly by proton bombardment or indirectly via ROS produced by proton water radiolysis.
Interestingly, they also found that proton and NP treatment of the same tumour in mice was less effective when using the Bragg peak (BP) than when using the traversing beam; complete tumour regression and 100% long-term (1 year) survival was found for the group receiving 31 Gy and 300 mg NP/kg (see figure 6(c)). Note, however, that high Au (and Fe) injected doses were again used, with tumour uptake reported to be 59 mg Au/kg tissue and 41 mg Fe/kg tissue. Despite these high concentrations, no toxicity effects were reported in mice receiving only 300 mg NP/kg body weight treatment, without radiation (see figure 6(c)). To our knowledge, these are the only in vivo studies to date that provide evidence of the potential for metal core NREs to improve proton beam treatment. Additional in vivo studies are needed to reproduce these results and elucidate the underlying mechanisms responsible for the observed difference in treatment outcomes for the traversing and BP proton beam components.

### 3.2. Gadolinium-based nanoparticles

Gadolinium (Gd) is a lanthanide commonly used as a magnetic resonance image (MRI) positive contrast agent because of its 7 unpaired electrons in its 4f subshell, which are not involved in bonding when Gd is in its most...
common oxidation state, Gd$^{3+}$. With Gd’s relatively high atomic number ($Z = 64$) and mass absorption contrast against tissue (see figure 2), the potential radio-enhancing properties of Gd-based nanoparticles (GdNPs) are also being explored (see Saney et al (2014) and Lux et al (2015) for reviews). Unlike AuNPs, GdNPs are not metal core nanoparticles but rather compounds usually comprised of Gd chelates with a relatively small number of Gd atoms. Tillement and co-workers carried out a number of studies investigating a new class of ultrasmall (<5 nm hydrodynamic diameter) GdNP, called AGuIX® (manufactured by NH TherAguix, Lyon, France), for MRI-guided radiotherapy. AGuIX® consists of $\approx 3.0 \pm 1.0 \text{ nm gadolinium chelates surrounding a polysiloxane core}$ (Bridot et al 2007, Mignot et al 2013). Tillement and co-workers found that AGuIX® was able to cross the blood-brain-barrier in glioma-bearing rats, but not in healthy mice, with a rapid increase in MR signal intensity 1 min after intravenous injection (Le Duc et al 2011, Saney et al 2014). The signal remained saturated up to 24 h post-injection of AGuIX® with [Gd] = 40 mM. Enhancement of radiation effects was demonstrated both in vitro and in vivo. The median survival time of gliosarcoma-bearing rats treated with MRT was found to increase by a factor of $\approx 2$, from 46 d to 96 d, when AGuIX® was intravenously administered 24 h before treatment compared to treatment only, i.e. without AGuIX® (Le Duc et al 2011, Saney et al 2014) (see figure 7). Similar results were also achieved with kV radiotherapy and AGuIX® delivered to mice bearing multiple brain metastases (Kobt et al 2016). These treatment outcomes were achieved with milligrams of Gd/kg body mass, which is considerably lower than the typical doses of gold used in previous in vivo studies. Indeed, in a follow-up study, Tillement and co-workers obtained x-ray fluorescence maps of 9LGS bearing rat brains after intravenous injection of AGuIX® and demonstrated that as little as 15 ppb of Gd in a tumour is sufficient to enhance the efficacy of the treatment (Dufort et al 2016).

In a slightly different approach, Detappe et al (2016) investigated AGuIX® GdNPs and showed that in addition to enhancing MR image contrast, they also enhanced sensitivity to both pre-clinical kV and clinical MV x-ray beams. In their study, pancreatic tumour-bearing mice were injected with 0.25 mg g$^{-1}$ of the GdNPs and a treatment plan was delivered using a small-animal irradiator (220 kVp beam, 10 Gy) or a clinical linac (6 MV flattening filter free beam, 10 Gy). Significant tumour growth suppression ($\approx 3$-fold decrease in tumour volume) and improvement in overall survival (from 45 d to 85 d) was observed for the mice treated with the GdNPs compared to those treated without them using the pre-clinical kV beam. For the clinical MV beam, tumour growth was less suppressed, but tumour volume was still approximately halved compared to treatment without GdNPs and survival was extended by $\approx 40$ d, similar to the pre-clinical kV irradiation results. Similar results were found in a related subsequent study (Detappe et al 2017) using AGuIX® complexed with bismuth (Bi), primarily to enable CT contrast imaging.

The effect of GdNPs combined with particle therapy has not yet been demonstrated in vivo but promising results have been obtained in vitro (see section 5).

### 3.3. Hafnium and iron oxide based nanoparticles

Radio-enhancement by hafnium ($Z = 72$) oxide (HfO$_2$) nanoparticles (HfNPs) has been investigated with in vivo studies as well as with in vitro and simulation studies by Maggiorella et al (2012). They used a commercial product, NBTXR3 HfNPs (hydrodynamic diameter $\approx 50 \text{ nm}$), developed for intratumoural injection (IT). An A673 human sarcoma model was xenografted into mice which then received 15 Gy from a $^{60}$Co source, 24 h after IT injection of NBTXR3 (64 mg ml$^{-1}$; the amount injected was not provided). They found a significant increase in tumour growth inhibition (82%) for mice treated with radiotherapy and NBTXR3 compared to those...
receiving radiotherapy only (72%). Furthermore, they also investigated an epithelial tumour model (HCT116) in mice treated with $^{207}$Th brachytherapy with either two doses of 4 Gy or a single dose of 8 Gy, 24 h after IT injection NBTX3. For both cases, they observed complete tumour remission 20 d post treatment, whereas tumour growth continued in mice that received brachytherapy without NBTX3. Separate in vitro studies showed that the NBTX3 NPs accumulated inside endosomes within the tumour cells.

Superparamagnetic iron oxide nanoparticles (SPIONs) were investigated by Choi et al (2012) for their potential as NREs in photon activation therapy (PAT) with monochromatic synchrotron x-rays. Choi et al (2012) treated CT26 tumour-bearing mice with 13 nm SPIONs (100 mg kg$^{-1}$) 30 min prior to irradiation with 10 Gy synchrotron x-rays at 7.1 keV (just above the Fe K-edge). They observed an increase in tumour concentration of magnetite up to 40 μg Fe/g tissue (a contrast ratio of 17 relative to surrounding muscle tissue) and 80% complete tumour regression, with relapse-free survival for up to 6 months, whereas 80% fatality was observed in the control group receiving radiation only. The authors suggest that SPION-PAT offers a potentially effective treatment strategy for superficial tumours. They did not suggest combining this with MRI to develop a theranostic approach, but this seems an obvious direction for further development.

As mentioned in section 3.1, SPIONs were also investigated by Kim et al (2012) for their potential as NREs for proton therapy. They found similar results compared to AuNPs when treating CT26 cells and tumour-bearing mice with 300 mg SPIONs/kg body weight followed by 41.7 MeV proton beam radiation (31 Gy); complete tumour regression and 100% long-term (1 year) survival. It is worth re-iterating here that this impressive treatment outcome was achieved using the traversing component of the proton beam, whereas treatment using the BP component was less effective.

3.4. Summary of in vivo studies

Table 2 summarises in chronological order in vivo studies carried out to date and their key findings. It is evident from this table that the reporting of results is varied, making it difficult to compare and synthesise the findings. Given the unique value of in vivo studies in providing direct evidence for the effectiveness of radio-enhancement, we suggest that future studies report a standard set of results. In particular, we recommend experimental protocols that report on survival over tumour regression, as this provides a link to in vitro cell survival studies. Protocols should also include a sufficient sample number to allow statistically significant results to be reported, including both the median and mean survival, with variance, as well as fixed-term survival (e.g. 90 d, 6 months, 1 year). Table 2 shows that critical parameters that determine the in vivo efficacy of NREs are the administered concentration (e.g. mg Au/kg body weight) and the tumour-to-normal tissue uptake. Hence, these parameters should be explicitly reported in future studies.

4. Molecular scale studies—evidence of elementary processes

The first evidence of molecular scale radio-enhancement was evidenced by Guo and co-workers (Carter et al 2007), who conjugated small (≈3 nm) AuNPs to supercoiled plasmid DNA (≈10 AuNP per ≈5600-bp plasmid) (see figure 8) and irradiated with 100 kVp x-rays. They observed enhancement of single and double strand breaks in the presence of AuNPs. More importantly, using a radical scavenger (Tris base) to reduce the diffusion of hydroxyl radicals down to just a few nanometers, they observed a decrease of radio-enhancement effect. With this method they attributed the AuNP-mediated NRE effect to the production of hydroxyl radicals by secondary electrons released in the nano-size volume.

Following a similar experimental protocol, McMahon et al (2011c) quantified the effect of AuNPs on plasmid DNA damage induced by kV x-rays. They found that the induction of single strand breaks does not correlate with the variation of energy absorption coefficient, which questions the relation between radio-enhancement and the photo-electric process. Surprisingly, they observed that the damage did not depend on AuNP diameter or on radical scavenging environment (Tris-EDTA). The effect of AuNPs and x-rays on the induction of double strand breaks was not considered here.

Kobayashi et al (2002) have pioneered this field and were the first to propose platinum complexes to enhance effects of x-rays. Plasmids were used as nano-bioprobes and the role of water radicals in radio-enhancement (using DMSO as radical scavenger) was evidenced and quantified (see Kobayashi et al (2010) for a review).

Later Porcel et al (2010) highlighted the capacity of platinum to improve the performance of medical carbon ions (C$^{6+}$, 276 MeV amu$^{-1}$) (see figure 9(a)). They used an experimental procedure similar to Kobayashi et al (2002) and Carter et al (2007) to compare the capacity of platinum complexes and small (3 nm) Pt nanoparticles (PtNPs) to amplify molecular damage, in particular nano-size biodamage. More interestingly Porcel et al (2010) attributed the higher NRE observed with PtNPs than with Pt complexes to collective electronic processes that take place in activated nanoparticles only. They also confirmed the key role of water radicals. Porcel et al (2012) then found that PtNPs also enhance the effect of He$^{2+}$ (2.3 keV μm$^{-1}$) and gamma rays (0.2 keV μm$^{-1}$) to produce nano-size damages with the same efficacy as the carbon ions at the end of the track (100 keV μm$^{-1}$).
Table 2. Summary of in vivo studies, in chronological order, and key results.

<table>
<thead>
<tr>
<th>Reference and type of study</th>
<th>Type of NRE and administered dose</th>
<th>Type of radiation and delivered dose</th>
<th>Key results</th>
</tr>
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</table>
| Hainfeld et al (2004)       | 1.9 nm AuNPs 2.7 g Au/kg IV injection (7 mg Au/kg in tumours) | 250 kVp x-ray beam, 26 Gy | • High tumour-to-normal tissue uptake ratio (up to 8.6)  
• 1-year survival fraction improved by factor ≈4.3 over radiotherapy alone |
| Kim et al (2010)            | 1.9 nm AuNPs and 14 nm ligand-coated AuNPs; 13–15 nm alginate-coated SPIONs 15–55 g Au/kg; 5 and 15 g Fe/kg; IV injections (<1% uptake in tumours) | 41.7 MeV protons SOBP, 100 Gy | • Consistent tumour regression with up to 90% volume reduction following proton therapy with either NP; tumour regrowth for proton therapy alone |
| Le Duc et al (2011)         | <5 nm GdNPs (AGuIX®) 9LGS tumour in rats | European Synchrotron MRT, 50-350 keV x-rays (mean ≈ 90 keV) | • Median survival time increased by factor 2 for MRT treatment with AGuIX over MRT only  
• MRI used to optimise treatment outcome by delivering MRT at peak tumour uptake after AGuIX injection (24h) |
| Maggiorella et al (2012)    | ≈50 nm HfNPs (NBTXR3) | 60Co gammas (mean: 1.25 MeV), 15 Gy (A673 mice) | • 10% more tumour growth inhibition than radiotherapy alone (A673 mice)  
• Complete tumour remission after 20 d, regardless of fractionation; (HCT116 mice) |
| Choi et al (2012)           | 13 nm SPIONs | 7.1 keV monoenergetic x-rays (photon activation therapy—PAT), 10 or 40 Gy | • High tumour-to-normal tissue uptake (up to 17)  
• 80% complete tumour regression for 10 Gy PAT-SPIONs  
• 100% complete tumour regression for 40 Gy with both PAT-only and PAT-SPIONs, but achieved earlier with PAT-SPIONs |
| Kim et al (2012)            | 5 nm AuNPs and 12 nm ligand-coated AuNPs; 13–15 nm alginate-coated SPIONs 100 or 300 mg NP/kg IV injections (Au and Fe injection doses not provided; tumour uptake 59–203 mg Au/kg tissue and 41–132 mg Fe/kg tissue, which was <1% injected dose) | 45 MeV protons: BP, 41 Gy; and traversing beam, 10, 21, 31 Gy | • Significantly greater tumour regression after proton therapy with NPs compared to without NPs  
• Traversal beam treatment more effective than BP treatment, with complete tumour regression and 100% long-term survival observed for highest NP dose and proton dose (31 Gy) |

(Continued)
<table>
<thead>
<tr>
<th>Reference and type of study</th>
<th>Type of NRE and administered dose</th>
<th>Type of radiation and delivered dose</th>
<th>Key results</th>
</tr>
</thead>
</table>
| Hainfeld et al (2013)      | 11 nm AuNPs                      | 100 kVp x-ray beam, 35 Gy         | - High tumour-to-normal brain uptake ($\approx 19$)  
- 1 year survival fraction improved by factor $\approx 3$ over radiotherapy alone  
- Gd-contrast MRI used to monitor uptake of AuNPs to tumour, before delivering MRT  
- Median survival time increased by factor $\approx 1.8$ (Au@DTDTPA-Gd) and $\approx 3$ (Au@TADOTAGA-Gd) over MRT only |
| Miladi et al (2014) and Laurent et al (2016) 9L gliosarcoma in rats | 2 nm AuNPs, Gd-chelate-coated: Au@DTDTPA-Gd (Miladi et al) and Au@TADOTAGA-Gd (Laurent et al) 0.7 ml IV injection: [Au] = 50 mM ($\approx 23$ mg Au/kg) | European Synchrotron MRT, 50-350 keV x-rays (mean $\approx 90$ keV). Entrance peak dose $\approx 400$ Gy (valley dose $\approx 20$ Gy) | - Median survival time increased by factor $\approx 1.8$ (Au@DTDTPA-Gd) and $\approx 3$ (Au@TADOTAGA-Gd) over MRT only |
| Al Zaki et al (2014)       | 1.9 nm AuNPs encapsulated in polymeric micelles (hydrodynamic diameter $\approx 75$ nm). 650 mg Au/kg IV injection | 150 kVp x-ray beam, 6 Gy | - Mean survival time increased by factor $\approx 1.5$ over radiotherapy only  
- 90 d survival increased by factor $\approx 3$ over radiotherapy only  
- Cell survival decrease for $D > 4$ Gy and DER $\approx 1.2$ inferred from LQ model D_{37} |
| McQuade et al (2015)       | 2.2 nm AuNPs and 14 nm SPIONs loaded into pegylated micelles (hydrodynamic diameter $\approx 100$ nm). | 150 kVp x-ray beam, 6 Gy | - Mean survival time increased by factor $\approx 1.5$ over radiotherapy only  
- 90 d survival increased by factor $\approx 2.8$ over radiotherapy only  
- MRI contrast confirmed tumour uptake; correlated with tumour volume decrease |
| Kunjachan et al. (2015)    | 2–3 nm spherical AuNPs, PEGylated and functionalised with neo-vascular targeting ligand (hydrodynamic diameter $\approx 8$–10 nm) 1.2 g Au/kg IV injection | 220 kVp x-ray beam, 10 Gy | - H&E staining of excised tumour 24 h after treatment—massive cell death in tumours treated with AuNPs compared to controls  
- γH2AX staining of excised tumour 24 h after treatment—3-fold increase in DNA damage for AuNP treated tumour compared to controls |
| Zhang et al (2015)         | <2 nm Au nanoclusters enclosed by glutathione shell 5.9 mg Au/kg IP injection | $^{137}$Cs gammas (mean: 662 keV), 5 Gy | - 66% decrease in tumour volume compared to radiotherapy without NPs |
| Su et al (2015)            | 20 nm peptide-conjugated AuNPs 50 mg Au/kg IV injection | $^{60}$Co gammas (mean: 1.25 MeV), 5 Gy | - 33% lower tumour volume growth using NPs with tumour-targeting peptide compared to non-targeting NPs |

(Continued)

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**Table 2.** (Continued).
<table>
<thead>
<tr>
<th>Reference and type of study</th>
<th>Type of NRE and administered dose</th>
<th>Type of radiation and delivered dose</th>
<th>Key results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolfe et al (2015)</td>
<td>31 nm × 9 nm goserelin-conjugated pegylated Au nanorods 10 mg Au/kg IV injection</td>
<td>6 MV clinical beam, 5 Gy.</td>
<td>● 3-fold more accumulation of Au nanorods in tumour when goserelin used ● Tumour growth delayed by ≈17 d compared to RT with/without non-targeted Au nanorods</td>
</tr>
<tr>
<td>Dou et al (2016)</td>
<td>13 nm pegylated AuNPs 4.7 µg Au/kg IV injection</td>
<td>6 MV clinical beam, 6 Gy</td>
<td>● Tumour growth rate significantly reduced</td>
</tr>
<tr>
<td>Popovtzer et al (2016)</td>
<td>30 nm AuNPs coated with cetuximab 6 mg Au/kg IV injection</td>
<td>6 MV FFF clinical beam, 25 Gy</td>
<td>● Tumour growth suppressed ● Enhanced tumour apoptosis</td>
</tr>
<tr>
<td>Detappe et al (2016)</td>
<td>4.5 nm SiGdNPs (AGuIX®) 0.25 mg NPs/kg IV injection</td>
<td>220 kVp SARRP pre-clinical beam, 10 Gy; and 6 MV FFF clinical beam, 10 Gy</td>
<td>● Tumour delineated using MRI after GdNP injection ● For kVp treatment with GdNPs, ≈3-fold decrease in tumour volume and ≈90% increase in overall survival, compared to without GdNPs ● For MV treatment with GdNPs, tumour volume ≈50% smaller and ≈66% increase in overall survival, compared to without GdNPs</td>
</tr>
<tr>
<td>Detappe et al (2017)</td>
<td>4.5 nm SiBiGdNPs (AGuIX® complexed with Bi) 420 mg NPs/kg dry weight (no other information provided)</td>
<td>6 MV FFF clinical beam, 10 Gy</td>
<td>● MRI used to determine optimal time to deliver radiotherapy (30 mins post-injection, maximal tumour uptake 3.5%) ● Significant improvement in tumour growth delay and survival of mice receiving radiation following NP injection compared to radiotherapy alone</td>
</tr>
</tbody>
</table>
In a more recent study, Schlatholter et al (2016) compared the molecular effects induced by Pt and Gd nanoparticles activated by 150 MeV protons at the entrance and the end of their track (see figure 9(b)). They found that induction of nano-size damage is amplified by both types of nanoparticles. More importantly, the effect was found to be stronger at the end of the proton track (3.6 keV μm⁻¹). Here again the major role of hydroxyl radicals was evidenced (see figure 10). The enhancement of bio-damage at the end of the proton track is promising for the...
use of nanoparticles to concentrate NRE effects in a tumour. Additional in vitro and in vivo studies are needed to corroborate this result.

It is important to note that DNA plasmids are not representative of nuclear DNA. Rather, they are commonly used as nano-bioprobes to quantify the efficacy of NPs to enhance the induction of complex bio-damage, since double strand breaks are indicative of damages larger than 2 nm (distance between the two strands).

5. In vitro studies—cellular impact

NRE effects of NPs have been evidenced in vivo by measuring tumour volume, growth rate, and post-treatment survival (section 3). In parallel in vitro studies are needed to characterise cellular-scale impact of NRE including cell toxicity, uptake dynamics and radiation-induced cell survival, with the scope to optimise and tailor treatments as summarised below.

5.1. Gold nanoparticles

In vitro studies with AuNPs activated by incident photons have been intensively carried out. Key conclusions are summarised in this section.

Using lipid-encapsulated AuNPs (2.2 nm diameter core) to enhance membrane penetration, Yang et al. (2014) found greater intracellular spread in melanoma cells than in breast carcinoma cells. Indeed, following irradiation with 4 Gy $^{137}$Cs, they observed a 3-fold increase in melanoma cell death in the presence of NPs but no amplification in the breast cancer cell line. They attributed this difference to limited uptake of lipid-encapsulated AuNPs into the cytosol of breast carcinoma cells, as demonstrated by confocal microscopy.

Taggart et al. (2014) measured cell survival and 53BP1 foci for breast cancer cells (MDA-MB-231), prostate cancer cells (DU-145) and glioma cells (T98G) treated by 225 kVp x-rays. They observed significantly reduced survival of the breast cancer and glioma cells when 1.9 nm AuNPs were present, compared to irradiation without AuNPs. However, no significant AuNP NRE was observed for the prostate cell line. No microscopy was performed in this case, while other studies (e.g. Kong et al. (2008), Zhang et al. (2008) and Antosh et al. (2015)) demonstrated the relation between NRE effects and cellular uptake using AuNPs conjugated with membrane-targeting agents. In Taggart et al. (2014) the authors obtained DEFs of 1.23 up to 1.90 for the different cell lines incubated with AuNPs. Interestingly, elevated DNA damage, mitochondrial membrane alterations and mitochondrial oxidation were found in all three cell lines exposed to AuNPs, which demonstrated the impact of coating-free AuNPs with biomolecules. Upon irradiation, DNA damage (measured 24 h post-irradiation) and mitochondrial membrane polarisation (measured 1 h post-irradiation) was increased in the breast and prostate cancer cell lines incubated with AuNPs, but not in the glioma cells. Early mitochondrial response to gamma irradiation was also observed by Kam et al. (2013), who found significant nonlinear changes in the expression of mitochondrially-encoded genes. These results corroborate those of Butterworth et al. (2013), who surmise that oxidative stress is implicated as the key regulator of radiation damage responses. These studies highlight the important role of mitochondria and the complex nature of biological responses to radiation, a complete understanding of which requires considering the whole cell rather than nuclear DNA only (Kuncic et al. 2012, Byrne et al. 2013, McNamara et al. 2016). This is especially important when considering radio-enhancement by nanoparticles, which generally do not localise to the nucleus.

Currell and co-workers (McQuade et al. 2016) reached the same conclusion after studying the response of AuNP-loaded cells irradiated with monoenergetic synchrotron x-rays (in the range 8–60 keV) using both clonogenic and DNA damage assays. They found negligible difference in long-term cell killing due to high nanoscale doses when AuNPs were deposited in the nucleus compared to the rest of the cell.

The effect of metal core NPs activated by fast protons has also been studied. In addition to their in vitro experiments using a CL29 tumour model and a 41.7 MeV proton beam (see section 3), Kim et al. (2010) were the first to establish a relation between the localization of NPs (AuNPs and SPIONs) and their impact on cell killing, together with consecutive tumour growth regression. In a follow-up study (Kim et al. 2012), they also established the major role of reactive oxygen species (ROS) in amplifying cell kill, as illustrated schematically in figure 10. This interpretation of cascade events when metallic based NPs are activated by fast particles is consistent with molecular scale experiments that first demonstrated the proof of principle (see section 4).

The ability of AuNPs (44 nm diameter internalized in phages) to enhance the effects of protons (160 MeV at the SOBP location) was also observed by Polf et al. (2011) in in vitro experiments. They found that the radiobiological effectiveness (RBE) increases by 15% (at SF 10%) to 19% (at SF 50%) for human prostate tumour cells loaded with AuNPs (1 ng Au/cell).

Kirkby and co-workers (Jeynes et al. 2014) found no statistically significant in vitro evidence for NRE effects due to AuNPs (50 nm) activated by 3 MeV protons. Using results from simulation studies, they attributed this to negligible enhancement in secondary electron production from AuNPs for low-energy protons compared to high-energy protons (e.g. 250 MeV). On the contrary, Li and co-workers observed significant amplification of
cell killing with A431 epidermoid carcinoma cells loaded with PEG coated AuNPs (5 and 10 nm) and treated with 2 MeV protons. Interestingly they highlighted the influence of the beam linear energy transfer (LET). Indeed they observed radio-enhancement with protons of 25 keV · µm⁻¹ LET but not with 10 keV · µm⁻¹. Moreover the effect was found to be higher with 5 nm AuNPs. This was attributed to the location of AuNPs in the nucleus, a result that differs from most other AuNP studies (e.g. Kong et al (2008), Zhang et al (2008) and Antosh et al (2015)).

NRE by AuNPs has been demonstrated in vitro also using high-energy carbon ions as incident radiation, a modality that is used in particle therapy—also called hadrontherapy—with ions of energies above 200 MeV amu⁻¹. As a first attempt, Kaur et al (2013) showed in vitro that glucose capped AuNPs (6 nm) enhance HeLa cell killing using 62 MeV carbon ions (290 keV · µm⁻¹ LET). They observed a dose enhancement factor (DEF) close to 40% RBE. More recently, a similar result was obtained by Liu et al (2015, 2016) with HeLa cells loaded with 15 nm citrate-capped AuNPs irradiated with carbon ions, with cell killing enhancement of ≈25%.

The major role of hydroxyl radicals was clearly evidenced using coumarin as a radical marker. As found in other in vitro studies, the nanoparticles were located in the cytoplasm.

In summary, small AuNPs (<20 nm) are able to enhance radiation effects in vitro when activated by photons and also by charged particles (protons or carbon ions). Their activation depends on the beam characteristics (energy and LET) but the interactions between the beam and AuNPs has not yet been adequately described and simulations are needed to elucidate the effect of ion tracks interacting with NPs.

Apart from AuNPs, several other NREs have been tested in vitro as described below.

5.2. Gadolinium-based nanoparticles

Summarised here are studies investigating gadolinium as a NRE, in particular AGuIX®, the Gd-based nanoparticle (GdNP) developed by the group of O. Tillement (University Lyon, France). Irradiation of various cancer cell lines incubated with GdNPs and irradiated by kV and MV photons revealed survival decreased by up to 2.5 times compared to irradiation without NPs (Luchette et al 2014, Sancey et al 2014, Stefancikova et al 2014). Note that GdNPs were found localised in the lysosomes of human glioblastoma cells but not in the nucleus (Stefancikova et al 2014) (see figure 11).

Similar results were found using pancreatic cancer cells (Detappe et al 2015) and rat glioma cells (Taupin et al 2015). In this case, NRE with high-energy photon radiation (gamma-rays and MV beams) was not as strong as with kV x-rays. More importantly, Taupin et al (2015) compared the effect of GdNPs (AGuIX®) with a commonly used Gd contrast agent (Magnevist®). They found that both Gd compounds enhanced the cell kill of clonogenic glioma cells treated with 4 Gy x-rays at 65 keV, with maximum enhancement ratios of 2.44 for GdNPs and 1.5 for the Gd contrast agent at the same Gd concentration in the cell culture medium (2.1 mg ml⁻¹). However, only the GdNPs enhanced radiation effects when ⁶⁰Co gamma rays (mean energy 1.25 MeV) were used as the incident radiation. The authors attributed this difference to the much lower cellular uptake of Gd contrast agent relative to the GdNPs (by a factor ≈30). Interestingly they found that cell cycle and cell proliferation were impacted similarly for all incident photon energies. Last but not least, Stefancikova et al (2014) observed that cell killing amplification is not related to nuclear DNA damage, as shown with foci quantification, a result that corroborates previous studies and suggests cytoplasm-driven biological pathways.

The only study reported to date on NRE by GdNPs combined with incident ions is that of Porcel et al (2014). They found amplification of radiation effects using carbon ions (150 MeV amu⁻¹, 2.23 keV µm⁻¹) and helium.
ions (150 MeV amu⁻¹, 2.23 keV μm⁻¹) used as incident particle beams. The effect was found to be slightly more pronounced with carbon ions (≈19%) compared to helium ions (≈11%). With the use of GdNPs, this study opens up the possibility of nano-theranostics in particle therapy, where GdNPs provide both an NRE effect as well as MRI contrast to monitor the nanoparticle biodistribution and tumour uptake, thereby identifying the optimal time for delivery of the particle beam to maximise therapeutic efficacy.

5.3. Other nanoparticles: platinum, hafnium oxide, iron oxide, cerium oxide nanoparticles, and nanodiamonds

*In vitro* studies with NREs other than gold and gadolinium are fewer in number and generally less comprehensive. For instance, concentrations used and incubation times are generally not reported.

Pt complexes were one of the first NREs used to amplify the effect of photon or ion irradiation. In particular carboplatin, a chemotherapeutic agent, enhances the production of x-ray-induced strand breaks (Yang et al 1995). In contrast, Corde et al (2002) did not find any cell killing enhancement when cells were treated with cisplatin prior to x-ray radiation. On the other hand, Usami et al (2008) observed a strong cell killing enhancement of mammalian cells treated with terpyridine platine prior to carbon ion and helium ion radiation. This study highlighted for the first time the location of the radio-enhancing agent in the cell cytoplasm.

Molecular scale studies (see section 4) highlighted the radio-enhancement properties of platinum nanoparticles (PtNPs) (Porcel et al 2010, 2012), however no cellular scale studies have been carried out. Yet the innate anti-cancer activity of PtNPs alone has been demonstrated by increased DNA damage and apoptosis associated with genotoxic stress (Asharani et al 2010). It has been proposed that the damaging effects of PtNPs may be caused by the release of soluble Pt ion species that form a complex with DNA similar to that of cisplatin (Gehrke et al 2011).

Radio-enhancement by hafnium oxide NPs (NBTXR3—Nanobiotix, France) has been demonstrated by Maggiorella et al (2012). In addition to *in vitro* studies (see section 2.3), *in vivo* studies were performed with human fibrosarcoma HT1080 cells used as models to investigate the NRE effects of these NPs combined with ⁶⁰Co gammas and 6 MV x-rays. For both radiation sources, significant amplification of cell killing was found in the presence of NBTXR3. Using an *ex vivo* clonogenic assay with HT1080 cells from an excised xenograft tumour in mice treated with 4 Gy and 8 Gy of ⁶⁰Co, 24 h after injection of NBTXR3, they found dose enhancement factors exceeding 1.5, relative to tumour cells from mice that received only radiotherapy.

The properties of SPIONs were explored by Choi et al (2012) who performed *in vitro* studies in tandem with *in vivo* studies to probe radio-enhancement using monochromatic synchrotron x-rays tuned to just above the Fe K-edge (see section 3.3). An MTT assay showed CT26 tumour cell viability significantly reduced (by up to 70%) with increasing SPION concentration when cells were irradiated 24 h after incubation with the SPIONs. Klein et al (2014) also investigated enhancement of radiation effects with SPIONs using different cell lines (tumour and healthy). However, their study focused on the enhancement of ROS production resulting from the interactions of low-energy x-rays with the ligands used to coat the SPIONs, rather than the interaction of the radiation with the high-Z magnetic core, which was not addressed. Interestingly, they observed faster uptake dynamics of SPIONs in tumour cells than in healthy cells, which they attributed to the relatively higher metabolic activity of tumour cells.

Another oxide nanoparticle, comprised of CeO₂, is particularly interesting because it exhibits dual radio-enhancement and radioprotection properties. Previous studies revealed CeO₂ NPs to be differentially toxic to cancer cells and to provide protection to normal cells by capturing reactive oxygen species, thus making them ideal for optimising therapeutic efficacy (see review by Gao et al (2014)). Briggs et al (2013) showed that CeO₂ NPs are also effective NREs, due to the relatively high atomic number of Ce (Z = 58). They found significant effects in survival of radioresistant 9L cells upon treatment with CeO₂ NPs and either 10 MV or 150 kVp x-ray beams, with more lethal effects observed for the kVp x-rays. Subsequent simulation studies have also been carried out by this group to investigate the potential for CeO₂ NPs to enhance proton therapy (McKinnon et al 2016).

Lastly, an alternative strategy has been proposed by Grall et al (2015) who explored the capacity of hydrogenated nanodiamonds (NDs) to enhance radiation effects. Contrary to other NREs reviewed here, NDs do not possess high-Z elements. Nevertheless, hydrogenated NDs exhibit NRE properties due to their semi-conductor behaviour and negative electron affinity, which are conducive to photo-induced emission of low-energy electrons from their surface (electrons can escape once excited into the conduction band). *In vitro* studies by Grall et al revealed enhanced double strand breaks and cellular senescence in different radioresistant cancer cell lines treated with hydrogenated NDs and gamma rays (¹³⁷Cs, 4 Gy). Interestingly, enhanced production of reactive oxygen species was also found, similar to high-Z NREs.

6. Modelling studies

Different models developed for understanding the impact of radiation on biological systems have been adapted to describe the impact of nanoparticles. Most of these models are based on Monte Carlo simulations. Monte
Carlo (MC) particle simulations can be used to model the physical and physico-chemical processes of radiation interactions with biological targets, but cannot be used to study radio-enhancement effects because the biological outcomes cannot be directly simulated (Kuncic 2015). However, MC-based models have been developed which integrate MC simulations with mathematical radiobiological modeling (e.g. McMahon et al (2011a) and Lechtman et al (2013)). Other modeling approaches have been developed to address the multiscale aspects of radiation damage effects, to better integrate the effect of oxygen in tumour (oxic and anoxic situations), and to bring new understanding when photons or fast ions are used as ionising radiation. Verkhovtsev et al (2016a) present a comprehensive overview of theoretical and computational studies of physical phenomena arising from photon, electron and ion collisions with nanoparticles.

### 6.1. Photon radiation

Stand-alone MC models have proven useful for providing valuable insights into the relevant importance of primary photon and secondary electron processes and calculating nanoparticle-induced enhancement in dose and ionisations for a range of parameters (e.g. target geometry, nanoparticle size and distribution, radiation source energy), which would otherwise be difficult and in most cases impractical to measure experimentally. The first MC studies to investigate dose enhancement by high-\(Z\) atoms were carried out by Robar et al (2002) for gadolinium and iodine contrast agents and MV photon beams. For Gd, their results predicted significantly more enhancement for a FFF beam (\(\approx 11\%\) for 6 MV) compared to a flattened beam due to the additional low energy photons in the FFF beam spectrum.

The MC study by Cho (2005) was the first to investigate dose enhancement by AuNPs for typical MV treatment beams, finding less than 1% enhancement. Carter et al (2007), investigated specifically the low-energy electrons released by AuNPs upon kV irradiation, and the ensuing nanoscale energy deposition distribution. They carried out MC simulations to complement their DNA plasmid experiments (see section 4) and concluded that AuNPs need to be located in close proximity to DNA to maximize tumour cell kill by free radicals generated by the low-energy electrons (see figure 12). A number of subsequent MC studies then focused on different kV sources and sizes of AuNPs and their effect on the characteristics of low-energy electrons produced (Cho et al 2009, Lechtman et al 2011, Leung et al 2011, McMahon et al 2011a). Lechtman et al (2011) also investigated the effects for a clinical 6 MV source compared to a range of kV sources and found that significant cross-firing occurs due to energetic photoelectrons, implying that AuNPs could potentially enhance radiation damage effects even without localising into all tumour cells. They concluded, however, that AuNP radiosensitisation using a 6 MV source is not clinically feasible because of the unacceptably high concentration of AuNP required (approximately 1600 mg g\(^{-1}\) tumour is needed to double a prescribed dose of 2 Gy). On the other hand, the MC studies by Berbeco et al (2011) and McMahon et al (2011b) demonstrated that inhomogeneous accumulation (i.e. clustering) of AuNPs results in steep nanoscale dose gradients that can also arise for a clinical MV source due to the low-energy component of the spectrum (see figure 5).

Douglass et al (2013) developed a sophisticated 3D randomized cell model geometry which they incorporated into Geant4 to investigate dose enhancement resulting from AuNP clusters randomly positioned inside the compartmentalized cells irradiated with both 80 kVp and 6 MV x-ray beams. In particular, they were able to...
study the effect of Auger electrons by activating and de-activating atomic de-excitation processes in Geant4. They showed that the Auger electrons, by virtue of their very low energies, have a negligible effect on dose enhancement within a cell, and that dose enhancement depends on the location, as well as the size, of the AuNP cluster, with AuNP clusters attached to the cell nucleus resulting in a larger enhancement compared to clusters located in the cytosol or membrane.

Brivio et al (2015) adopted the approach of modelling a realistic clinical kV treatment scenario, radiosurgery with AuNPs for neovascular age-related macular degeneration. Here, a 4 mm diameter 100 kVp beam is aimed at the macula to deliver a single ablative dose. The MC model, developed using MCNP6, investigated dose enhancement by AuNPs to the diseased macular endothelial cells. For 20 nm diameter AuNPs of concentration 32 g kg\(^{-1}\), the model predicted a dose enhancement factor of nearly 2, implying significantly reduced risk to surrounding critical organs (i.e. retina/optic nerve) as a result of requiring less radiation dose to achieve the same outcome as treatment without AuNPs.

Given that experimental in vitro studies show that NPs tend to localise in the cytosol or organelles other than the nucleus (see section 5), McNamara et al (2016) carried out a Geant4 study with a realistic compartmentalised cell model based on measurements of the chemical composition of a lymphocytic JURKAT cell, including separate composition measurements of the nucleus and mitochondrion. They found that the presence of 1% gold in the cytosol, to represent AuNP uptake, increases energy deposition by \(\approx 1.8\%\) due to the additional photoelectrons. Moreover, the dose enhancement is greater in the mitochondrial volumes than in the nuclear volume due to the larger surface area to volume ratio of the mitochondrion compared to the nucleus. These results demonstrate that physical dose enhancement effects in cells are not restricted to the vicinity of NPs and also point to the mitochondria, and mitochondrial DNA, as being viable radiation targets (see also preliminary follow-up simulation studies by McMahon et al (2017)).

In another Geant4 study, Zygmanski et al (2013) addressed the several orders of magnitude difference in scale between clinically relevant macroscopic dose and the nanoscale transport processes that occur in the immediate vicinity of irradiated AuNPs. They analysed the transition in phase space from macro- to micro-beams incident on AuNPs and investigated the influence of micron-scale source-target geometry, including beam size and AuNP clustering, using both monoenergetic (11 keV–1 MeV) and spectral (50 kVp) x-ray sources. They found both the magnitude and spatial distribution of dose enhancement exhibits a strong nonlinear dependence on AuNP cluster size, thus demonstrating that MC simulation results for dose enhancement due to single AuNPs and specific source-target geometries cannot be simply extrapolated or scaled-up from nanoscales to micron-scales. However, the density and size of nanoparticles are also important because they determine the escape probability of secondary electrons and hence, the dose enhancement in surrounding biological tissue (see e.g. Lechtman et al (2011) and McMahon et al (2016)).

Owing to the disproportionately large number of studies to date, AuNPs now present a useful benchmark for other NREs. In a comprehensive modeling study, McMahon et al (2016) compared the macroscopic and nanoscale effects resulting from nanoparticle-radiation interactions for a range of high-Z elements, including gold, irradiated by kV x-rays just above the K-edge of each material and also a 6 MV linac beam. MC simulations using Geant4 showed that electron impact emission dominates in the case of MV beam irradiation while Auger electron emission dominates in the immediate vicinity of a high-Z nanoparticle irradiated with kV x-rays. A mathematical radiobiological model developed specifically for nanoscale effective dose deposition following kV exposure of high-Z nanoparticles (McMahon et al 2011a) was then applied and predicted an optimal radiobiological effectiveness for \(Z \approx 34\) and \(Z \approx 68\), corresponding to elements at or near germanium and gadolinium.

Edouard et al (2010) performed a MC study using MCNPX to compare dose enhancement by iodine, gadolinium and gold contrast media commonly used in synchrotron stereotactic radiotherapy. Using a head phantom model based on clinical CT scans, they considered different x-ray beam qualities, including monochromatic synchrotron x-rays, kVp x-ray beams and a 6 MV linac beam. Their simulations predicted dose enhancement factors in the range of 2–2.4 for all three contrast agents with concentration of 10 mg ml\(^{-1}\) using monochromatic synchrotron 40–90 keV x-rays. Slightly lower enhancement factors, but still near 2, were predicted for kV beams with energies 80–120 kVp. For a 6 MV linac beam, a negligible dose enhancement was predicted for all the contrast agents. Another contrast-enhanced radiotherapy simulation study was carried out by Garnica-Garza (2011) using PENelope for a pancreatic tumour model. The results predicted that 0.75 mg g\(^{-1}\) of Gd distributed uniformly in surrounding healthy tissue could be well tolerated following treatment with a 220 kVp beam, and that with 5 mg g\(^{-1}\) Gd delivered to the tumour, produces corresponding treatment plans with excellent dose-volume histograms with sparing of organs at risk and dose delivered to 100% of the tumour volume for 72 Gy.

6.2. Proton and ion radiation

Modeling studies of incident protons radiation effects with AuNPs were carried out by Lin et al (2014, 2015). Using TOPAS (TOol for PArticle Simulation, Perl et al 2012), a software platform employing Geant4 as the MC
engine. They predicted significant dose enhancement in the immediate vicinity of the surface of AuNPs activated by incident protons, at a level comparable to the nanoscale dose enhancement produced for incident kV and MV photons. However, they found that unlike incident photons, which can also produce photoelectrons with a much longer range (typically a few microns), incident protons only produce short-range secondary electrons from collisions with AuNPs and thus, cannot generate dose enhancement on larger scales (Lin et al 2014). This was confirmed by another study using TOPAS together with experimental measurements (Cho et al 2016). This implies that AuNPs would be required to internalise inside cells and ideally, inside the nucleus to maximize radio-enhancement of protons. In their follow-up radiobiological modelling study, Lin et al (2015) predicted that if AuNPs cannot be internalised into tumour cells, then no sensitisation will be observed for proton therapy treatment.

Wälzlein et al (2014) were the first to study the relative radiation enhancement effects of different high-Z elements, namely gold, platinum, gadolinium, silver and iron. They used the track structure MC code TRAX to evaluate the participation of Auger electrons when NPs of different sizes (r = 22 and 2 nm) are activated by monoenergetic proton beams with energies of therapeutic interest (2, 80 and 300 MeV). They concluded that high-Z metallic nanoparticles may be of great interest to improve proton and heavy ion therapy. Verkhovtsev et al (2015a, 2015b) then demonstrated, with theoretical and quantum chemistry calculations using time-dependent density functional theory (DFT), that 1 MeV protons interacting with metallic clusters (3–40 atoms) may induce giant electronic excitations in the metallic atoms due to the ionisation of 5d electrons from outer shells, but also due to excitation of plasmons (collective modes of conduction band electrons). Hence, by evaluating the contribution of each mechanism they found that radio-enhancement by platinum is more efficient than gold, and both are expected to be an order of magnitude more efficient than silver and gadolinium (figure 13). Thus, they established that collective excitation followed by plasmon damping makes a non-negligible contribution to low energy electron emission in the vicinity of metallic nanoparticles (this is relevant for NPs composed of a metallic core, such as platinum and gold NPs, but not for oxides such as Gd-based complexes).

McKinnon et al (2016) used Geant4-DNA to investigate local dose enhancement from 100 nm ceramic oxide NPs (Ta2O5 and CeO2) irradiated by 5 MeV and 50 MeV monoenergetic protons. They found that although Auger electrons make a non-negligible contribution to radio-enhancement, it is minor compared to delta electrons produced by impact ionization. They also found a smaller dose enhancement for ceramic oxide NPs (≈1.15) compared to AuNPs (≈1.25) for the same simulation set-up, which they attributed to lower densities. Martinez-Rovira and Prezado (2015) used Geant-DNA to simulate the effects of Au and Gd NPs of sizes ranging from 4 to 50 nm activated by different proton beam configurations (with various distances between the source and nanoparticle and using different source sizes) and found that geometrical parameters (source-target distance in particular) strongly influence the radio-enhancement properties of nanoparticles. In particular, they found a maximum amplification of 1.7 when the distance between the beam and the nanoparticle is zero (which corresponds to the configuration used in the studies of Verkhovtsev et al (2015a, 2015b) and Wälzlein et al (2014) cited above). On the basis of their Geant4-DNA results, Martinez-Rovira and Prezado concluded that physical processes play a minor role in nanoparticle radio-enhancement in a realistic proton therapy treatment scenario and that consequently, NRE effects observed in experimental studies must instead be largely due to chemical and biological effects (i.e. sensitisation).
However, MC studies to date have not explicitly considered LET effects, which in vitro studies have shown to be important (see section 5). Simulations are needed to compare NP activation by heavy ions (e.g. helium and carbon) to NP activation by protons within the Bragg peak. Additionally, simulation studies have not modelled electron transfer effects (Stumpf et al 2013) that can potentially contribute to NRE effects beyond that predicted by Auger electron decay and impact ionisation (see section 2.3). MC studies are also needed to simulate the effects of nanoparticle clustering observed in in vitro studies. It is likely that strong nonlinear amplification of nanoscale radio-enhancement may result from multi-NP activation by electrons and radicals (see figure 10), yet this remains to be quantified.

7. Opportunities

Emerging opportunities in nanoparticle-enhanced radiation therapy include: (i) image-guided external beam radiotherapy; (ii) internal radionuclide targeted therapy and diagnostic imaging (theranostics); and (iii) concomitant therapies.

7.1. Image-guided external beam radiotherapy

Image guidance is now regarded as essential in external beam radiotherapy. In particular the use of MRI to gain anatomical information without exposure to x-ray CT is a growing trend (Chetty et al 2015). Hence, an increasing number of studies focus on GdNPs, which offer the advantage of providing both MRI bright contrast enhancement and radio-enhancement (Le Duc et al 2011, Sancey et al 2014, Lux et al 2015, Taupin et al 2015, Detappe et al 2015, 2016, Dufort et al 2016). An additional advantage is that gadolinium is able to pass the blood–brain barrier and thus, the potential to treat brain tumours with highly targeted radiotherapy is a particularly attractive clinical goal (Kotb et al 2016).

Studies to date have demonstrated radio-enhancement effects for a range of incident photons, including kV beams and synchrotron microbeams, as well as clinical MV beams. Similarly, more recent studies have also begun to investigate the radio-enhancement properties of superparamagnetic iron oxide nanoparticles (SPIONs), which are commonly used to enhance dark contrast in MRI (e.g. Huang et al (2010) and Khoei et al (2014)). While most of these studies have also confirmed the MRI contrast enhancement by Gd-based nano-complexes or SPIONs, to date none have demonstrated enhanced MRI contrast and radio-enhancement simultaneously. This is a unique opportunity for MRI-guided external beam radiotherapy.

NREs that can also be imaged provide the added advantage of monitoring their biodistribution and delivering radiotherapy only when peak uptake in the tumour is observed. As mentioned in section 3, pre-clinical animal studies using NREs to enhance CT or MRI contrast in conjunction with radiotherapy has already been demonstrated. X-ray fluorescence (XRF) computed tomography is another modality that has been demonstrated to be useful for imaging AuNP biodistribution in small animals (e.g. Manohar et al (2016)). For clinical applications, however, the biodistribution of NREs in the body is needed. While high-Z NREs (e.g. AuNPs) can be monitored by CT imaging, the concentrations that are needed make this modality clinically impractical. Magnetic NREs (e.g. GdNPs, SPIONs), on the other hand, provide MRI contrast even at relatively low concentrations. The potential for theranostic NREs to improve the performance of particle therapy (proton and hadron therapy) remains to be explored.

7.2. Internal radionuclide theranostics

A rapidly emerging branch of nanomedicine is ‘radio-nanomedicine’, where radio-labeled nanoparticles deliver diagnostic and therapeutic isotopes directly to a tumour. Systemic radiation treatment offers a promising new window of opportunity for tumour targeting, with a minimal exposure of normal tissue that cannot be matched by existing external beam radiotherapy techniques (Zhang et al 2010, Lee et al 2015).

Initial studies explored labeling nanoparticles with radio-tracers for biodistribution imaging studies with PET or SPECT (de Barros et al 2012, Thorek et al 2014, Normandin et al 2015). This has inevitably led to labeling nanoparticles with radiotherapeutic isotopes. Yook et al (2015), for example, conjugated AuNPs with the β−-emitter 177Lu and with the human monoclonal antibody panitumumab specific to the epidermal growth factor receptor (EGFR) that is over-expressed in breast cancer. Their in vitro studies showed that even though the antibody targeting agent was effective in promoting cellular internalization of the AuNPs and delivering radiation dose to the nucleus, cross-fire of the β−-particles (which have a range ≈2 mm in tissue) also contributes to radiotoxicity in neighboring tumour cells, even without active targeting. This demonstrates an important advantage of internal radiotherapeutic nano-delivery over chemotherapeutic nano-delivery, which requires the drug to be delivered across both the plasma membrane and ideally also the nuclear membrane to achieve maximal efficacy. Radio-enhancement due to the interaction of the emitted particles (alphas, betas, gammas) with AuNPs (or other high-Z NPs) has not been investigated.
7.3. Concomitant therapies

In current clinical practice, radiotherapy treatment is often delivered concurrently with chemotherapy as this has been demonstrated in many cases to be more effective than single-modality treatment (Seiwer et al 2007). Considerable research effort has been invested in developing nanoparticle carriers of systemically applied chemotherapeutic drugs to improve targeted delivery to the tumour, both spatially (tumour accumulation, tumour-to-organ ratio) and temporally (circulation time, tumour residence time) but arguably the most promising results have been demonstrated when combined with radiotherapy (Lammers et al 2008). Recently, Li et al (2016) investigated pegylated AuNPs co-loaded into a hydrogel with doxorubicin (DOX) to achieve combined nanoparticle radio-enhancement and controlled DOX delivery in chemoradiotherapy. They showed, with in vitro studies, that tumour cell viability was substantially compromised following 6 MV irradiation when both AuNP and DOX were administered compared to irradiation only or irradiation with AuNPs. With an in vivo study, they found enhanced inhibition of tumour cell growth and proliferation after 6 MV radiotherapy treatment with both AuNP and DOX, compared to radiotherapy only, radiotherapy with DOX or radiotherapy with AuNPs. These results suggest that AuNPs play a key role in the synergistic enhancement of chemoradiotherapy treatment efficacy.

An important advantage of concomitant therapies is that they can reduce the amount of NREs needed to achieve improved therapeutic outcomes. In Hainfeld et al’s original study in 2004, 2.7 g Au/kg body mass was needed for radio-enhancement. This is not clinically viable for human use and alternate strategies such as combined therapies need to be explored before AuNPs can be clinically translated (Cooper et al 2014). In a later study, Hainfeld and co-workers investigated the synergistic therapeutic effects of combined hyperthermia and radiotherapy using AuNP aggregates engineered for infrared absorption (Hainfeld et al 2014). They found that the dose required to control 50% of the tumours in mice could be reduced from $\approx 55$ Gy down to $< 15$ Gy using 100 kVp x-rays when combined with hyperthermia.

Radioimmunotherapy is another emerging synergistic approach that is showing tremendous promise. In particular, pre-clinical animal studies using checkpoint inhibitors that activate and harness the body’s own immune system to fight against tumour cells have shown remarkable improvements in survival when combined with external beam radiotherapy. Importantly, the observed strong response to radioimmunotherapy was replicated in a clinical trial involving patients with different cancer types (Twyman-Saint Victor et al 2015). The role of nanoparticle radio-enhancement in radioimmunotherapy remains unexplored.

8. Conclusions and outlook

Several conclusions can be drawn from the results obtained with different high-\textit{Z} NREs (AuNPs, GdNPs, PtNPs, HfNPs and SPIONs). Firstly, in vivo small animal studies have provided unequivocal evidence for nanoparticle-mediated enhancement of radiation damage initiated by photons. For incident keV x-rays and MeV gamma rays, radio-enhancement effects generally translate to amplification of radiation damage by a factor of $\approx 1.5$–$2$ for injected doses $< 1$ g of high-\textit{Z} element per kg body weight. Corresponding in vitro studies have produced similar results. For clinically relevant MV photon beams, however, more in vivo data (especially statistical survival data) is needed to better quantify possible radio-enhancement effects from NREs. From the perspective of clinical translation, NRE treatment strategies should be evaluated not only in terms of radio-enhancement efficiency, but also in terms of reproducibility and mode of administration, as well as targeting and image guidance capability (to optimise therapeutic gain from NREs by monitoring nanoparticle biodistribution and delivering radiotherapy only when tumour uptake peaks).

The experimental results performed to date verify the underlying principle that NREs have the capacity to transfer energy from incident photons to the surrounding biological medium much more effectively than water molecules, and thus to increase lethality of radiation effects. However, the evidence for NREs activated by protons and heavy ions is considerably weaker; to date, only two in vivo studies, conducted by the same group, have been reported for proton beams (and none for ion beams). Additional in vivo studies are thus urgently needed to evaluate the relative enhancement of high-LET over low-LET particles, as well as the overall enhancement relative to NREs activated by photons. Here, complementary MC simulations will be invaluable for quantifying relative NRE effects and elucidating the contribution from ion tracks interacting with high-\textit{Z} nanoparticles.

From the in vitro studies, we can conclude that nuclear DNA is not an exclusive radiation target and that NRE effects are concomitant with enhanced free radical production when radiation is delivered under normoxic conditions. Here, additional studies are needed to investigate the influence of oxygen concentration on the capacity for NREs to enhance radiation damage in radioresistant hypoxic tumours.

Future in silico studies should be carefully designed to complement in vivo, in vitro and/or molecular studies and simulate experimental observations (e.g. nanoparticle clustering). Additional physical and chemical processes (e.g. plasmon decay, charge transfer, radical production, oxygenation, recombination and diffusion) also
need to be modelled to clearly identify the key physico-chemical processes, which are otherwise difficult to disentangle and isolate from the biological processes in real experiments.

Lastly, we highlight two emerging paradigms as an outlook for future research. The first is radio-nanomedicine, where radiolabelled high-Z nanoparticles are used to deliver internal radiation therapy. The potential to amplify radiation effects with this approach, in direct analogy to external beam radiotherapy, has not yet been explored. The second emerging opportunity is the development of ultra-high dose rate radiation sources, which demonstrate superior therapeutic index over conventional radiotherapy (Favaudon et al 2014, Fouillade et al 2017, Montay-Gruel et al 2017). To this end, the combination of NREs with new radiation modalities (internal and ultra-high dose rate radiation) opens up a wider range of possibilities for novel nano-enhanced treatment paradigms.

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Conflicts of interest

None.

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