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Radiosensitising nanoparticles as novel cancer therapeutics - Pipe dream or realistic prospect?

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Abstract

The field of high atomic number nanoparticle radiosensitising agents is reviewed. After a brief discussion of the new mode of physicochemical action implied by irradiation of high atomic number nanoparticles embedded in biological systems, a series of exemplars are discussed. Silver-, gallium-and gold-based nanoparticles are discussed in order of increasing atomic number with functionalization strategies being outlined. *In-vitro* and *in-vivo* evidence for radioenhancement and the mechanisms attributed to the increased biological effect are discussed.

Introduction

An interdisciplinary approach to the development of future therapeutics has helped fuel the nanoscale revolution of the past decade. This has led to the production of a multiple of nanoparticle preparations for the treatment of a variety of pathological conditions ranging from novel scaffolds for tissue engineering, to new HIV therapeutics and perhaps most commonly for the development of new anti-cancer agents (1-4). This review specifically details various nanoparticle preparations that have
been created to augment the efficacy of current radiotherapy treatment plans.

By far the most common radiosensitising approach is to exploit the increased photon absorption of high atomic number (Z) materials at kilovoltage (kVp) photon energies (5). Adopting this approach, therapeutic nanoparticles have been produced using silver (Z=47), gadolinium (Z=64) and most extensively gold (Z=79). In addition to the high atomic number of these materials, the unique physicochemical properties of these nanomaterials permit relatively simplistic functionalisation through the binding of amine and thiol subgroups (6). Furthermore, increasingly complex multifunctionalisation strategies have resulted in new terminology such as theranostics, where a particle is designed for both therapeutic and diagnostic purposes (7, 8).

**Physicochemical mode of action**

In contrast to the low-atomic number species found predominantly in living systems, the presence of the high-Z species interacting with highly ionising radiation implies a new physicochemical mode of action. Due to the photoelectric effect, these high atomic number species can undergo inner-shell ionization – where one of the deeply bound electrons is removed – with high efficiency. The result is a highly unstable atomic
system that stabilizes by emission of lower energy photons (fluorescence) and electrons (Auger emission). Several Auger emissions can occur effectively simultaneously from a single inner shell ionization in a process called an Auger cascade. The electrons produced by this Auger cascade typically have energies of a few keV or less and hence they have penetrations of typically 10 to 100 nm (9). As a result, these electrons deposit their energy very locally. This highly localized deposition of energy is akin to that found in ion therapy and indeed the biological effect has been well described using the local effect model first developed to relate ion-induced radiation track structure to biological effect (10, 11). This highly localized deposition of energy is one of the attractive features of nanoparticles clinically – if appropriately localized, they offer the promise of performance usually associated only with heavy ion facilities but using conventional clinical linacs. However, a full description of the mode of action following from the localized energy deposition brought about through the Auger cascade is still not available. For example, the energy dependence for DNA damage by gold nanoparticles in the photon energy range (20 – 80 keV) shows an unexplained double maximum (12). This is significant because this energy range is the range over which photoelectric interactions with gold nanoparticles (e.g. from shower
particles) are most likely. This unexplained energy dependence in even such a simple system shows the difficulty in connecting this collective physical mode of action, via physicochemical processes to biological effects. The physical basis of radiosensitization and the resultant biological mechanisms have been reviewed elsewhere, for the specific case of gold nanoparticles (13).

**Silver Nanoparticles (Z=47)**

Zhao *et al* (2012) developed a multifunctional magnetic iron/silver nanocomposite particle functionalised, with Cetuximab, a monoclonal antibody designed to target the epidermal growth factor receptor (EGFR) (14). This is an attractive target as upregulation of EGFR is commonly observed in many cancers including nasopharyngeal carcinomas and is strongly associated with tumour metastasis, recurrence and poor overall survival (15, 16). The Fe(3)O(4)/Ag–cetuximab nanoparticle evoked dose dependent cytotoxicity with an IC₅₀ concentration of 350 ± 3.14 μg/L. However, when used in combination with radiation at 30 μg/L (~10% of the IC₅₀ concentration) significant radiosensitisation was achieved producing an impressive dose enhancement factor (DEF) of 2.26. In addition, to high Z radiosensitation caused by the Ag nanoparticles,
additional functionality was conferred by the conjugation of Cetuximab resulting in the attenuation of EGFR by approximately 50% (14). This reduction is particularly relevant as EGFR signalling through downstream pathways such as Ras-mitogen-activated protein kinase (MAPK), phosphatidyl inositol 3-kinase (PI3K)-Akt and Jak-STAT have been associated with solid tumour radio-resistance (17, 18). An earlier study investigating silver nanoparticles as sensitisers for the treatment of radioresistant glioblastoma tumors reported variable efficacy dependent upon nanoparticle size and concentration (19). The extent of the sensitising capability was further enhanced using higher concentrations of the nanoparticles, while maximizing the surface area to volume ratio by opting for smaller sized particles. In this instance the authors propose that the mode of sensitisation is due to the release of Ag° cations, which subsequently capture free electrons generating an oxidative agent, which further reduced ATP production and increased the production of intercellular reactive oxygen species (ROS) (20). In addition to enhancing the generation of potentially damaging radicals, Ag nanoparticles have also been shown to negatively regulate the activity of DNA dependent protein kinase (DNA-PK), a key enzyme involved in DNA damage repair via
Non-homologous End Joining (NHEJ) (21). This finding is particularly relevant, as the primary mode of radiation-induced cytotoxicity is the generation of potentially lethal double strand breaks (DSB), therefore novel therapeutics that augment the radiation induced damage profile while inhibiting the repair process have attracted much attention (22, 23).

**Gadolinium Nanoparticles (Z=64)**

Other high Z materials such as Gadolinium (Gd) have been investigated on the nanoscale as potential radiosensitising agents. Le Duc *et al* have developed a 2 nm gadolinium nanoparticle (GdNp) as a positive contrast agent to enhance magnetic resonance imaging and as a novel radiosensitiser (24). A rat intracerebral 9 L gliosarcoma (9LGS) model was chosen to demonstrate the theranostic properties of this nanoparticle preparation. The authors describe both preferential accumulation within the tumors, attributed to the enhanced permeability and retention effect (EPR) caused by the tortuous and leaky tumour vasculature and an increased radiosensitising capacity (24-26). This effect was neatly demonstrated by the difference in clearance rates between the two hemispheres of the brain. In the normal left hemisphere of the rat brain
the GdNps signal increased for up to 5 min, after which point the intracerebral concentration rapidly decreased by approximately 53%, 20 min post administration of the nanoparticles. However, in the right hemisphere and in particular within the 9LGS tumour, GdNps were retained and cleared much slower with 88% of the maximal GdNps retained 20 min post administration (24) **Figure 1**. On this occasion image guided microbeam (~25-100 μm) radiation therapy, designed to spare normal brain tissue was administered, using the positive MRI signal conferred by the GdNps, as a treatment delivery guide. Due to the extremely aggressive and invasive nature of gliosarcoma tumours, the median survival time (MeST) for untreated animals was 19 days. This was significantly improved upon with the administration of microbeam radiation therapy extending the MeST to 47 days, an increase in survival time by 147%. However, the radiosensitising effect conferred by the GdNps profoundly augmented the therapeutic benefit of radiotherapy, extending the MeST to 90 days, an increase in overall survival time of 373% compared to untreated animals and 191% over radiation therapy alone (24). This could have a significant clinical impact, as the long-term prognosis for many patients developing glioblastoma tumours is particularly bleak, with a median survival rate of 1-year post diagnosis
Gadolinium-chemotherapeutic conjugates have also been used with radiotherapy, a number of which have entered clinical trials. One of the most widely used is Motexafin Gadolinium ((MGd) – trade name-Xcytrin), a redox-active porphyrin-like texaphyrin licensed by the FDA for the treatment of non-small cell lung cancer with secondary brain metastases (28). With this novel therapeutic, radiosensitisation is though to be less modulated by the high atomic number of MGd, but more directly associated with an imbalance in the radical scavenging capability of the target cells. MGd promotes both the generation of ROS through the oxidation of various intracellular metabolites such as ascorbate, NADPH and glutathione. This continual cycle of ROS production effectively depletes the cells of the reducing agents required to repair cytotoxic damage, thus permitting the accumulation of potentially lethal radiation induced DNA double strand breaks (29, 30). Furthermore, in addition to elevating the production of intracellular ROS, MGd also negatively regulates the ability of the cell to eliminate ROS. This is primarily modulated through the impaired activity of thioredoxin reductase (TrxR1) an important enzyme of the antioxidant system, thus permitting the accumulation of potent reactive oxygen intermediates such as superoxide
and hydrogen peroxide (31, 32). Finally, MGd has also been shown to inhibit the DNA synthesis/repair processes by suppressing the activity of the enzyme ribonucleotide reductase (RAR), the primary role of which is to maintain the reserves of dNTPs required for efficient DNA repair and replication. Detailed confocal microscopy studies have identified co-localisation between MGd and the R1 subunit of the RAR enzyme, which is associated with impaired S-phase DNA synthesis. This multimodal action has produced impressive anti-cancer effects in both pre-clinical models and in the clinical setting (31, 33). Clinically, MGd has primarily been used as a treatment for brain tumours with a phase I study in children to determine the maximum tolerated dose. In total, 44 children received increasing doses of MGd ranging from 1.7 mg/kg to 9.2 mg/kg per day prior to radiation therapy for up to 6 weeks. The maximum tolerated dose within the approved ethical parameters of the study was 4.4 mg/kg with the primary reported toxicity being grade 3-4 hypertension. This relatively low toxicity profile and the pre-clinical studies supporting clinical efficacy have ensured progression to phase II clinical trials (34).

Gold nanoparticles (Z=79)
Undoubtedly gold has received the majority of attention in relation to its potential as a radiosensitiser. This is due to gold’s presumed biocompatibility, supported by its historical use in the treatment of rheumatoid arthritis and its superior absorption of X-rays over soft tissue (13, 35). Early work by Regulla et al (1998) clearly demonstrated the radiosensitising potential of gold. In particular, an enhanced biological effect was observed in vitro when monolayers of mouse embryo fibroblasts were irradiated in close contact to thin metallic gold foil. Analysis of the survival curves of cells irradiated in the absence and presence of gold, revealed dose enhancement factors (DEFs) of up to 50 (36). The dose enhancement property of gold was further illustrated by Herold et al. using 3 μm gold microspheres irradiated with various kilovoltage energy X-ray beams (37). DEFs of 1.42 were reported following irradiation of rodent cells with 200 kVp X-rays in the presence of 1% gold. In addition, the significance of incident photon energy was demonstrated when no radiosensitisation was observed using a Cs-137 source, emitting 662 keV γ-rays. This strongly highlights the importance of the differential in mass absorption coefficient between soft tissue and high Z materials over the kilovoltage energy range. However, attempts to deliver the microspheres to in vivo subcutaneous tumours by intra-
tumoural injection resulted in little radiosensitisation. The lack of effect was attributed to the fact that the microspheres were found to accumulate at the injection sites and were not homogeneously distributed throughout the tumour volume (37).

The surge of interest in nanotechnology and specifically nanomedicine accelerated the development of gold nanoparticles (GNPs) as novel radiosensitising therapeutics. These smaller particles circumvented many of the delivery issues associated with larger bulk gold, while apparently retaining its attractive biologically inert properties. Initial in vivo proof of concept experiments where subcutaneous EMT-6 mammary carcinomas were administered with 1.35 mg Au/g, produced no discernable impact on tumour growth rates as compared with the untreated control animals, highlighting the lack of biological activity. However, when the same dose of GNPs were delivered 2 min prior to radiation treatment, potent radiosensitisation, cumulating in tumour regression was observed out to 1 year post treatment (38). While this experimental setup clearly demonstrates the efficacy of GNPs as in vivo radiosensitisers, there are fundamental concerns in that the sheer quantity of GNPs administered to achieve this therapeutic effect would prove prohibitive on a cost basis if scaled for therapeutic purposes.
Despite these potential issues, numerous groups have performed detailed investigations of the effects of multiple variables including particle size, shape, surface coating, concentration and photon energy on the radiosensitising potential, the key findings of which are summarised in Table 1. Given the sheer number of variables presented it is difficult to draw direct comparisons between studies, however, taken together the results highlight a number of fundamentally important findings which require further discussion.

One such example is the apparent disparity between the radiation sensitivity of different tumour cell lines, despite continuity between all other variables (5). Although GNP uptake was found to occur in all three cell lines investigated, it was greatest in the MDA-MB-231 cells; suggesting that both intracellular gold concentration and localisation strongly influence the potential radiosensitisation (39). Also of significant interest is the disconnect between the observed experimental data and the predicted magnitude of dose enhancement. Roeske et al. performed a comprehensive Monte Carlo modelling study investigating the dose enhancing properties of various high Z materials over a range of kV X-ray energies, from which the take home message was that a GNP concentration of >0.1% by weight (i.e. 1 mg/g tumour) is necessary to
generate radiosensitisation using low energy x-rays (40). However, significant radiosensitisation has been reported in vitro using considerably lower concentrations of GNPs (5, 41). Specifically, a dose enhancement of 1.05 was theoretically predicted to occur for a GNP concentration of 0.05% by mass (i.e. 500 μg/mL) in combination with 160 kVp X-rays, however, the observed experimental data produced a maximal DEF of 1.4 in MDA-MB-231 cells treated with 1.9 nm GNPs, 24 h prior to irradiation (5). Rahamen et al. also demonstrated this greater-than-predicted effect with the same GNP preparation. Used to pre-treat bovine aortic endothelial cells at a concentration of 41 μg/mL, a DEF of 1.24 was achieved, 11% greater that the predicted dose enhancement (42). These results suggest that overly simplistic models, which make assumptions based purely on physical dose enhancement, homogeneous nanoparticle distribution and whole cell systems fail to account for the contribution of nanoscale energy deposition and complex biological interactions (10).

One of the most clinically significant findings in relation to the development of GNPs as radiosensitisers was the evidence that sensitisation using MV X-rays was achievable. These finding appear to contradict the physical similarities between the mass energy absorption
coefficients of gold and soft tissue using MV radiation sources. Despite
this numerous groups have reported significant DEFs using clinical
radiation sources delivering MV X-rays (5, 43-45). The data presented by
Chithrani et al clearly demonstrate this effect. Although a maximum
radiosensitising effect was found for cells irradiated with 105 kVp X-rays
in the presence of 760 μg/mL GNP
s, a DEF of 1.17 ± 0.02 was reported in
combination with unmodified LINAC-generated 6 MV X-rays (43). This
result is in clear disagreement with a theoretically predicted value of
1.008, again highlighting the complex nature of nanoparticle-induced
radiosensitisation and the need to consider the shower spectrum (11).

**Pre-clinical evaluation**

Recently, several pre-clinical *in vivo* models for multiple cancer types have
demonstrated efficacy in relation to GNP radiosensitisation. Utilising 1.9
nm GNPs, Hainfeld et al. investigated how radiation dose, beam energy
and hyperthermia influenced the potential radiosensitising effect of mice
bearing SCCVII squamous cell carcinoma tumours (46). Briefly, mice were
pre-treated using GNPs at a concentration of 1.9 g/kg body weight just
prior to radiation treatment. Experimental efficacy was determined by
the increase in tumour doubling time. On average tumours pre-treated
with GNPs and irradiated with 42 Gy using 68 keV X-rays took 43% longer
to double in volume compared with radiation only treated mice. In addition, an X-ray energy dependency was demonstrated as the same approximate dose delivered using 157 keV X-rays yielded a mere 7% extension in tumour growth delay in the presence of GNPs. This was attributed to the fact that for 68 keV X-rays, almost 100% of the gold-absorbed photon energy is deposited inside the tumour volume, given the range of the ejected photoelectrons and low energy secondary and Auger electrons that are subsequently emitted (46). The same group recently published impressive tumour growth delay data in a model mimicking aggressive human glioblastoma cancer. In this instance non-functionalised 11 nm GNPs were administered by i.v. injection 15 h prior to radiation treatment. Interestingly, GNPs did not have the ability to cross the normal blood-brain barrier, but efficiently crossed the blood-tumour barrier to accumulate within the tumour at a 19:1 tumour-to-normal brain ratio. These glioblastoma tumours proved imminently lethal in the absence of any therapeutic intervention, with 0% (n=10) of the animals surviving beyond 23 days. Unsurprisingly, irradiation treatment extended survival, although all animals had succumbed by day 150 post treatment. However, the combination of pre-administration of 4g Au/kg prior to radiation resulted in 50% long term (>1 year) survival, markedly
increasing the therapeutic efficacy of radiation therapy alone (47) (Figure 2).

A new generation of molecularly targeted GNP preparations are being developed to avoid over dependence on passive targeting strategies such as the EPR effect. This is particularly relevant to nanoparticles with an outer gold shell due to the established surface chemistry of GNPs for the attachment of targeting agents (48, 49). One such example is the development of a functionalised GNP designed to target the Human Epidermal Growth Factor Receptor-2 (HER-2), by conjugating the monoclonal antibody trastuzumab to a 30 nm GNP, generating Au-T (Au-P – a control non targeted 30 nm GNP) (50). Using an MDA-MB-361 HER-2 positive model of breast cancer, the authors demonstrated an in vitro dose enhancement factor of 1.6 using 100 kVp X-rays, delivered 24 h after GNP administration. Furthermore, treatment of established MDA-MB-361 sub-cutaneous tumours with Au-T and 11 Gy, translated into a 46% reduction of tumour burden over a 120-day time period. This compared favourably to radiation treatment alone, which produced an overall 16% increase in tumour burden. The authors of this study attribute the mechanism by which Au-T exerts its sensitising effects to Auger electron induced double strand break damage (DSB). The in vitro γ-H2AX foci
formation data supports this conclusion indicating a 1.7 and 3.3 fold increase in DSB damage in cells pre-treated with Au-P and Au-T respectively (50) (**Figure 3**). Perhaps the most interesting finding was the additional efficacy conferred by the addition of trastuzumab to the GNP. A reasonable assumption would be that conjugation of the antibody would enhance systemic targeting ability, however, the Au-T nanoparticle preparation also appears to promote cellular uptake following direct application.

Increasingly complex nanoparticle formulations illustrate the potential which nanotechnology holds. The high relative biological effectiveness (RBE) produced by α-emitters is particularly attractive in terms of future targeted cancer therapeutics. However, the excessive production of radioactive daughters following α-particle decay severely restricts their therapeutic potential due radiation-induced toxicity to healthy, non-target tissue (51). To circumvent this issue, McLaughlin *et al.* have developed a multi-layered nanoparticle with the α-emitter 225Ac located at the core of the structure (52) (**Figure 4**). The α-emitter was then wrapped with a composite material composed of equimolar quantities of Lanthanum and Gadolinium orthophosphate \( \{La_{0.5}Gd_{0.5}\}^{225}\text{Ac} \text{PO}_4 \), utilising the radiation resistant properties of Lanthanum to act as shield
preventing the release of low energy radioactive daughters such as $^{221}$Fr.

This generates a 4 nm central nanoparticle to which four additional layers of Gadolinium orthophosphate (GdPO$_4$) are added providing additional protection against radioactive decay by-products and conferring the magnetic properties of Gadolinium, thereby producing an effective contrast agent for systemic MRI scanning. The final layer involves the addition of an outer gold shell, permitting functionalisation using targeting ligands and conferring the biocompatibility properties associated with gold. The decay process for $^{225}$Ac produces 4 $\alpha$-particles with a mean energy of 6.2 MeV, meaning layered encapsulation attenuates less than 0.2% of their energy as they exit the centre of the nanoparticle. Conversely, 99.99% of the low energy radioactive daughters ($<100$ keV) are effectively retained within the layered nanoparticle preventing non-target tissue damage, a key concern of many $\alpha$-emitting therapeutics. In addition, to this the authors also presented convincing evidence of effective systemic targeting. Conjugation of the monoclonal antibody 201b to the outer gold surface of the nanoparticle enabled targeting of the thrombomodulin receptor, which is highly expressed in lung endothelium. This was confirmed by whole animal SPECT/CT imaging 1 h post administration of the nanoparticle, with high accumulations
localised to the lung in the mAb 201b targeted NP, while the non-targeted equivalent nanoparticles accrued preferentially within the liver and spleen. Currently, targeted alpha therapy experiments in tumour model systems are underway to directly assess the therapeutic efficacy of these nanoparticles (52).

**Gold nanoparticle clinical trials**

Despite the wealth of proof-of-concept and pre-clinical data supporting GNPs as effective radiosensitisers, only two GNP based preparations have proceeded into clinical trials. CYT-6091 is a novel nanomedicine, which is composed of a 27 nm colloidal GNP, surface functionalised with thiolyated polyethylene glycol (PEG) and recombinant human tumour necrosis factor alpha (rhTNF). TNF as an anti-cancer therapeutic has been extensively investigated producing minimal clinical benefit with dose limiting side effects including hepatotoxicity, malaise and hypotension (53-56). This was thought to be, in part, due to the inability to adequately target the drug to the site of disease. In the development and preclinical evaluation of CYT-6091, the inclusion of PEG along with rhTNF restricted rapid uptake by the reticuloendothelial system as well as limiting toxicity associated with rhTNF (57). Development of CTY-6091 progressed to a phase I dose escalation study in advanced stage cancer patients. Doses
ranging from 50 μg/m² to 600 μg/m² were administered with no major
dose limiting toxicity reported, while the maximum tolerated dose was
not obtained. Interestingly, the dose of GNP bound rhTNF was three times
higher than the maximum tolerated dose of free rhTNF. Tumour specific
targeting of the rhTNF-GNP was passively achieved by the EPR effect. This
was confirmed by transmission electron microscopy analysis of post-
treatment core biopsies, which indicated the presence of GNPs
specifically within the tumour tissue while remaining undetectable in the
healthy tissue (58). Although, this trial did not investigate the potential
efficacy of a combined radiotherapy treatment plan, the target site
accumulation of rhTNF resulted in one partial response and three patients
achieving stable disease states (59).

An ongoing pilot study due for completion in December 2013, is
investigating the potential for adverse side effects and any therapeutic
benefit conferred to targeted tumours following a course of AuroLase®
therapy. This is a photothermal therapy designed to confer anti-tumour
efficacy by the conversion of near infrared laser energy to heat using small
gold coated nanoparticles called Auroshells®. Auroshells combine the
biologically inert properties of GNPs with a non-conducting, dielectric
silica core designed to act as an exogenous absorber of near infrared laser
energy, thus promoting tumour ablation by hyperthermia. As before, the
GNPs are designed to passively accumulate within the tumour by the EPR
effect. Extensive preclinical evaluations of Auroshells reported that the
particles were well tolerated when injected i.v. with no toxicities or
bioincompatibilities observed (60). The study aims to deliver a single dose
of Auroshells followed by one or more interstitial illuminations with an
808 nm laser, with tumour nanoparticle uptake estimated by neutron-
activated analysis from post-treatment biopsies (61).

Conclusions
The sheer diversity and complexity of nanoparticle preparations that have
been developed hold significant potential for the treatment of a plethora
of disease states including cancer. However, there remain many
uncertainties, not least those based around the predictions that high Z
radiosensitisation is solely attributed to the differential mass absorption
coefficient of high Z materials and soft tissue. This was born out through
numerous reports of significant disparities between the biological
responses and the predicated outcome based on physical interactions.
Moreover, the assumption that nanomaterials will exhibit the same
degree of biological and chemical inactivity to bulk material appears to
have been overly simplistic, with contradictory reports of toxicity for
presumed inert materials such as gold in the nanoscale range. Due to the extensive variability of base material, size, charge, shape, surface coating and functionalisation, relative comparisons of radiosensitisation capabilities are impossible, therefore requiring each nanoparticle preparation to be extensively evaluated on its own merit. This exhaustive process will inevitably delay progression towards the clinic. This delay is best illustrated by the disparity in complexity of the advanced particles investigated in pre-clinical models and the relative simplicity of the targeting and therapeutic approaches for nanoparticle preparations which have entered clinical trials. Furthermore, there is a significant lack of pre-clinical in vivo data demonstrating therapeutic efficacy using clinically relevant MV photon energies, despite numerous in vitro models suggesting radiosensitising efficacy using high Z materials.

There is little doubt that nanotechnology has a huge potential to significantly augment the therapeutic benefit of current treatment modalities such as chemo- and radiotherapy. However, without rigorous quality control to limit internal production variability and detailed systematic evaluations of efficacy, which currently appear to be lacking, the true potential of radiosensitising nanoparticles may not be realised for some time.
Conflict of interest

The authors report no conflict of interest

References


Figure Legends

**Figure 1.** Enhanced contract properties conferred by GNP accumulation within the brain of a 9LGS-bearing rat before and 5, 20, and 45 min after intravenous injection of GBNs.
**Figure 2.** Kaplan–Meier survival curves of mice bearing Tu-2449 highly malignant tumours indicative of human gliomas. Only animals receiving the combination therapy of GNPs plus radiation treatment survived in excess of 1-year post treatment.

**Figure 3.** Induction of DSB damage in MDA-MB-361 cells pre-treated with trastuzumab functionalised GNPs. DSB damage was quantified by γ-H2AX foci formation. The presence of the HER-2 monoclonal antibody significantly increased the DNA damage profiles following radiation treatment.

**Figure 4.** A schematic representation of a gold-coated lanthanide phosphate nanoparticle. Located in the center of the nanoparticle is the \( \{\text{La}_{0.5}\text{Gd}_{0.5}\}\text{PO}_4 \) α-emitter. This is surrounded by four GdPO\(_4\) layers that retain radioactive decay products, and a gold shell, which increases biocompatibility and provides a surface for functionalisation.

**Table 1.** A summary of the findings of *in vitro* studies investigating the effect of GNPs in combination with ionising radiation. Predicted dose enhancement factors were calculated based on the mass fraction of GNP used, beam energy and the mass attenuation coefficient.
Figure 2.
Figure 3.
Figure 4.
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<th>Author</th>
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<th>Cell Line</th>
<th>Photon Energy</th>
<th>Observed DEF/Eff</th>
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<td>2008</td>
<td>15 nm</td>
<td>5 μM (0.05 μg/mL, 0.01% w/v)</td>
<td>C677</td>
<td>MEM-1900</td>
<td>750 nm</td>
<td>1.13 x 10^7</td>
<td>1.5 x 10^7</td>
</tr>
</tbody>
</table>