

# Radiosensitising Nanoparticles as Novel Cancer Therapeutics — Pipe Dream or Realistic Prospect?

Coulter, J. A., Hyland, W. B., Nicol, J., & Currell, F. J. (2013). Radiosensitising Nanoparticles as Novel Cancer Therapeutics — Pipe Dream or Realistic Prospect? *Clinical Oncology*, *25*(10), 593-603. https://doi.org/10.1016/j.clon.2013.06.011

#### Published in: Clinical Oncology

Document Version: Peer reviewed version

#### **Queen's University Belfast - Research Portal:** Link to publication record in Queen's University Belfast Research Portal

#### Publisher rights © 2013 Elsevier.

Licensed under the Creative Commons Attribution -NonCommercial-NoDerivs License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

#### General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

#### Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

#### **Open Access**

This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: http://go.qub.ac.uk/oa-feedback

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

**Authors:** Jonathan A Coulter<sup>1</sup>, Wendy B Hyland<sup>1,4</sup>, James Nicol<sup>1</sup>, Fred J Currell<sup>2</sup>

**Affiliation:** <sup>1</sup>School of Pharmacy, McClay Research Centre, Queen's University Belfast. <sup>2</sup>School of Mathematics and Physics, Queens University Belfast. <sup>4</sup>Belfast Health and Social Care Trust.

## **Corresponding author:**

Dr. Jonathan A Coulter

School of Pharmacy

Queen's University Belfast

97 Lisburn Road, Belfast

BT9 7BL

UK Phone: +44(0) 28 90972253; Fax +44(0) 28 90247794

Email: j.coulter@qub.ac.uk

Word count: 4194

### 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 IC<sub>50</sub> concentration of 350  $\pm$  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<sup>+</sup> 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 gilosarcoma (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 ( $\sim$ 25-100  $\mu$ m) 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

(27).

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 colocalisation 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  $\gamma$ -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 intratumoural 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  $\mu$ g/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 greaterthan-predicted effect with the same GNP preparation. Used to pre-treat bovine aortic endothelial cells at a concentration of 41  $\mu$ g/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  $\mu$ g/mL GNPs, 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 goldabsorbed 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 human glioblastoma cancer. In this instance aggressive nonfunctionalised 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 bloodtumour barrier to accumulate within the tumour at a 19:1 tumour-tonormal 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 y-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  $\alpha$ -emitters is particularly attractive in terms of future targeted cancer therapeutics. However, the excessive production of radioactive daughters following  $\alpha$ -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  $\alpha$ -emitter <sup>225</sup>Ac located at the core of the structure (52)(Figure 4). The  $\alpha$ -emitter was then wrapped with a composite material composed of equimolar quantities of Lanthanum and Gadolinium orthophosphate {La<sub>0.5</sub>Gd<sub>0.5</sub>}(<sup>225</sup>Ac)PO4, utilising the radiation resistant properties of Lanthanum to act as shield

preventing the release of low energy radioactive daughters such as <sup>221</sup>FR. This generates a 4 nm central nanoparticle to which four additional layers of Gadolinium orthophosphate (GdPO4) 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 <sup>225</sup>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 thrombomodilin 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 reticuloendiothelial 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/m<sup>-2</sup> to 600 μg/m<sup>-2</sup> 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 posttreatment 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<sup>®</sup> 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<sup>®</sup>. 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 is 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

1. Lee SJ, Atala A. Scaffold technologies for controlling cell behavior in tissue engineering. Biomed Mater. 2013 Jan 25;8(1):010201.

Siccardi M, Martin P, McDonald TO, Liptrott NJ, Giardiello M, Rannard
s, et al. Research spotlight: Nanomedicines for HIV therapy. Ther Deliv.
2013 Feb;4(2):153-6.

3. Ali HM, Urbinati G, Raouane M, Massaad-Massade L. Significance and applications of nanoparticles in siRNA delivery for cancer therapy. Expert Rev Clin Pharmacol. 2012 Jul;5(4):403-12.

4. Kumar P, Gulbake A, Jain SK. Liposomes a vesicular nanocarrier: Potential advancements in cancer chemotherapy. Crit Rev Ther Drug Carrier Syst. 2012;29(5):355-419.

5. Jain S, Coulter JA, Hounsell AR, Butterworth KT, McMahon SJ, Hyland WB, et al. Cell-specific radiosensitization by gold nanoparticles at megavoltage radiation energies. Int J Radiat Oncol Biol Phys. 2011 Feb 1;79(2):531-9.

6. Shukla R, Bansal V, Chaudhary M, Basu A, Bhonde RR, Sastry M. Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: A microscopic overview. Langmuir. 2005 Nov 8;21(23):10644-54.

7. Espinoza-Castaneda M, de la Escosura-Muniz A, Gonzalez-Ortiz G, Martin-Orue SM, Perez JF, Merkoci A. Casein modified gold nanoparticles for future theranostic applications. Biosens Bioelectron. 2013 Feb 15;40(1):271-6.

8. Miladi I, Le Duc G, Kryza D, Berniard A, Mowat P, Roux S, et al. Biodistribution of ultra small gadolinium-based nanoparticles as theranostic agent: Application to brain tumors. J Biomater Appl. 2012 Jul 24.

9. Meesungnoen J, Jay-Gerin JP, Filali-Mouhim A, Mankhetkorn S. Lowenergy electron penetration range in liquid water. Radiat Res. 2002 Nov;158(5):657-60.

10. McMahon SJ, Hyland WB, Muir MF, Coulter JA, Jain S, Butterworth KT, et al. Biological consequences of nanoscale energy deposition near irradiated heavy atom nanoparticles. Sci Rep. 2011;1:18. 11. McMahon SJ, Hyland WB, Muir MF, Coulter JA, Jain S, Butterworth KT, et al. Nanodosimetric effects of gold nanoparticles in megavoltage radiation therapy. Radiother Oncol. 2011 Sep;100(3):412-6.

12. Azizzadeh B, Yip HT, Blackwell KE, Horvath S, Calcaterra TC, Buga GM, et al. Nitric oxide improves cisplatin cytotoxicity in head and neck squamous cell carcinoma. Laryngoscope. 2001 Nov;111(11 Pt 1):1896-900.

 Butterworth KT, McMahon SJ, Currell FJ, Prise KM. Physical basis and biological mechanisms of gold nanoparticle radiosensitization. Nanoscale.
2012 Aug 21;4(16):4830-8.

14. Zhao D, Sun X, Tong J, Ma J, Bu X, Xu R, et al. A novel multifunctional nanocomposite C225-conjugated Fe3O4/ag enhances the sensitivity of nasopharyngeal carcinoma cells to radiotherapy. Acta Biochim Biophys Sin (Shanghai). 2012 Aug;44(8):678-84.

15. Pan J, Kong L, Lin S, Chen G, Chen Q, Lu JJ. The clinical significance of coexpression of cyclooxygenases-2, vascular endothelial growth factors, and epidermal growth factor receptor in nasopharyngeal carcinoma. Laryngoscope. 2008 Nov;118(11):1970-5.

16. Ruan L, Li XH, Wan XX, Yi H, Li C, Li MY, et al. Analysis of EGFR signaling pathway in nasopharyngeal carcinoma cells by quantitative phosphoproteomics. Proteome Sci. 2011 Jun 28;9:35,5956-9-35.

17. Kang Y, Park MA, Heo SW, Park SY, Kang KW, Park PH, et al. The radiosensitizing effect of xanthohumol is mediated by STAT3 and EGFR suppression in doxorubicin-resistant MCF-7 human breast cancer cells. Biochim Biophys Acta. 2012 Dec 16;1830(3):2638-48.

18. Skvortsova I, Skvortsov S, Stasyk T, Raju U, Popper BA, Schiestl B, et al. Intracellular signaling pathways regulating radioresistance of human prostate carcinoma cells. Proteomics. 2008 Nov;8(21):4521-33.

19. Xu R, Ma J, Sun X, Chen Z, Jiang X, Guo Z, et al. Ag nanoparticles sensitize IR-induced killing of cancer cells. Cell Res. 2009 Aug;19(8):1031-4.

20. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009 Feb 24;3(2):279-90. 21. Lim HK, Asharani PV, Hande MP. Enhanced genotoxicity of silver nanoparticles in DNA repair deficient mammalian cells. Front Genet. 2012;3:104.

22. Bentle MS, Reinicke KE, Dong Y, Bey EA, Boothman DA. Nonhomologous end joining is essential for cellular resistance to the novel antitumor agent, beta-lapachone. Cancer Res. 2007 Jul 15;67(14):6936-45.

23. Groselj B, Sharma NL, Hamdy FC, Kerr M, Kiltie AE. Histone deacetylase inhibitors as radiosensitisers: Effects on DNA damage signalling and repair. Br J Cancer. 2013 Jan 29.

24. Le Duc G, Miladi I, Alric C, Mowat P, Brauer-Krisch E, Bouchet A, et al. Toward an image-guided microbeam radiation therapy using gadoliniumbased nanoparticles. ACS Nano. 2011 Dec 27;5(12):9566-74.

25. Maeda H, Sawa T, Konno T. Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J Control Release. 2001 Jul 6;74(1-3):47-61.

26. Iyer AK, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. Drug Discov Today. 2006 Sep;11(17-18):812-8.

27. Jo J, Schiff D, Purow B. Angiogenic inhibition in high-grade gliomas: Past, present and future. Expert Rev Neurother. 2012 Jun;12(6):733-47.

28. Young SW, Qing F, Harriman A, Sessler JL, Dow WC, Mody TD, et al. Gadolinium(III) texaphyrin: A tumor selective radiation sensitizer that is detectable by MRI. Proc Natl Acad Sci U S A. 1996 Jun 25;93(13):6610-5.

29. Khuntia D, Mehta M. Motexafin gadolinium: A clinical review of a novel radioenhancer for brain tumors. Expert Rev Anticancer Ther. 2004 Dec;4(6):981-9.

30. Chang JE, Khuntia D, Robins HI, Mehta MP. Radiotherapy and radiosensitizers in the treatment of glioblastoma multiforme. Clin Adv Hematol Oncol. 2007 Nov;5(11):894,902, 907-15.

31. Hashemy SI, Ungerstedt JS, Zahedi Avval F, Holmgren A. Motexafin gadolinium, a tumor-selective drug targeting thioredoxin reductase and ribonucleotide reductase. J Biol Chem. 2006 Apr 21;281(16):10691-7.

32. Edelman MJ, Otterson G, Leach J, Malpass T, Salgia R, Jones D, et al. Multicenter phase II trial of motexafin gadolinium and pemetrexed for second-line treatment in patients with non-small cell lung cancer. J Thorac Oncol. 2011 Apr;6(4):786-9.

33. Carde P, Timmerman R, Mehta MP, Koprowski CD, Ford J, Tishler RB, et al. Multicenter phase ib/II trial of the radiation enhancer motexafin gadolinium in patients with brain metastases. J Clin Oncol. 2001 Apr 1;19(7):2074-83.

34. Bradley KA, Pollack IF, Reid JM, Adamson PC, Ames MM, Vezina G, et al. Motexafin gadolinium and involved field radiation therapy for intrinsic pontine glioma of childhood: A children's oncology group phase I study. Neuro Oncol. 2008 Oct;10(5):752-8.

35. Aaseth J, Haugen M, Forre O. Rheumatoid arthritis and metal compounds--perspectives on the role of oxygen radical detoxification. Analyst. 1998 Jan;123(1):3-6.

36. Regulla DF, Hieber LB, Seidenbusch M. Physical and biological interface dose effects in tissue due to X-ray-induced release of secondary radiation from metallic gold surfaces. Radiat Res. 1998 Jul;150(1):92-100.

37. Herold DM, Das IJ, Stobbe CC, Iyer RV, Chapman JD. Gold microspheres: A selective technique for producing biologically effective dose enhancement. Int J Radiat Biol. 2000 Oct;76(10):1357-64.

38. Hainfeld JF, Slatkin DN, Smilowitz HM. The use of gold nanoparticles to enhance radiotherapy in mice. Phys Med Biol. 2004 Sep 21;49(18):N309-15.

39. Coulter JA, Jain S, Butterworth KT, Taggart LE, Dickson GR, McMahon SJ, et al. Cell type-dependent uptake, localization, and cytotoxicity of 1.9 nm gold nanoparticles. Int J Nanomedicine. 2012;7:2673-85.

40. Roeske JC, Nunez L, Hoggarth M, Labay E, Weichselbaum RR. Characterization of the theorectical radiation dose enhancement from nanoparticles. Technol Cancer Res Treat. 2007 Oct;6(5):395-401.

41. Kong T, Zeng J, Wang X, Yang X, Yang J, McQuarrie S, et al. Enhancement of radiation cytotoxicity in breast-cancer cells by localized attachment of gold nanoparticles. Small. 2008 Sep;4(9):1537-43.

42. Rahman WN, Bishara N, Ackerly T, He CF, Jackson P, Wong C, et al. Enhancement of radiation effects by gold nanoparticles for superficial radiation therapy. Nanomedicine. 2009 Jun;5(2):136-42. 43. Chithrani DB, Jelveh S, Jalali F, van Prooijen M, Allen C, Bristow RG, et al. Gold nanoparticles as radiation sensitizers in cancer therapy. Radiat Res. 2010 Jun;173(6):719-28.

44. Liu CJ, Wang CH, Chen ST, Chen HH, Leng WH, Chien CC, et al. Enhancement of cell radiation sensitivity by pegylated gold nanoparticles. Phys Med Biol. 2010 Feb 21;55(4):931-45.

45. Geng F, Song K, Xing JZ, Yuan C, Yan S, Yang Q, et al. Thio-glucose bound gold nanoparticles enhance radio-cytotoxic targeting of ovarian cancer. Nanotechnology. 2011 Jul 15;22(28):285101,4484/22/28/285101. Epub 2011 Jun 8.

46. Hainfeld JF, Dilmanian FA, Zhong Z, Slatkin DN, Kalef-Ezra JA, Smilowitz HM. Gold nanoparticles enhance the radiation therapy of a murine squamous cell carcinoma. Phys Med Biol. 2010 Jun 7;55(11):3045-59.

47. Hainfeld JF, Smilowitz HM, O'Connor MJ, Dilmanian FA, Slatkin DN. Gold nanoparticle imaging and radiotherapy of brain tumors in mice. Nanomedicine (Lond). 2012 Dec 24. 48. van Vlerken LE, Vyas TK, Amiji MM. Poly(ethylene glycol)-modified nanocarriers for tumor-targeted and intracellular delivery. Pharm Res. 2007 Aug;24(8):1405-14.

49. Kumar D, Meenan BJ, Dixon D. Glutathione-mediated release of bodipy(R) from PEG cofunctionalized gold nanoparticles. Int J Nanomedicine. 2012;7:4007-22.

50. Chattopadhyay N, Cai Z, Kwon YL, Lechtman E, Pignol JP, Reilly RM. Molecularly targeted gold nanoparticles enhance the radiation response of breast cancer cells and tumor xenografts to X-radiation. Breast Cancer Res Treat. 2013 Jan;137(1):81-91.

51. Woodward J, Kennel SJ, Stuckey A, Osborne D, Wall J, Rondinone AJ, et al. LaPO4 nanoparticles doped with actinium-225 that partially sequester daughter radionuclides. Bioconjug Chem. 2011 Apr 20;22(4):766-76.

52. McLaughlin MF, Woodward J, Boll RA, Wall JS, Rondinone AJ, Kennel SJ, et al. Gold coated lanthanide phosphate nanoparticles for targeted alpha generator radiotherapy. PLoS One. 2013;8(1):e54531.

53. Kimura K, Taguchi T, Urushizaki I, Ohno R, Abe O, Furue H, et al. Phase I study of recombinant human tumor necrosis factor. Cancer Chemother Pharmacol. 1987;20(3):223-9.

54. Taguchi T. Phase I study of recombinant human tumor necrosis factor (rHu-TNF:PT-050). Cancer Detect Prev. 1988;12(1-6):561-72.

55. Heim ME, Siegmund R, Illiger HJ, Klee M, Rieche K, Berdel WE, et al. Tumor necrosis factor in advanced colorectal cancer: A phase II study. A trial of the phase I/II study group of the association for medical oncology of the german cancer society. Onkologie. 1990 Dec;13(6):444-7.

56. Whitehead RP, Fleming T, Macdonald JS, Goodman PJ, Neefe J, Braun TJ, et al. A phase II trial of recombinant tumor necrosis factor in patients with metastatic colorectal adenocarcinoma: A southwest oncology group study. J Biol Response Mod. 1990 Dec;9(6):588-91.

57. Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, et al. Colloidal gold: A novel nanoparticle vector for tumor directed drug delivery. Drug Deliv. 2004 May-Jun;11(3):169-83.

58. Powell AC, Paciotti GF, Libutti SK. Colloidal gold: A novel nanoparticle for targeted cancer therapeutics. Methods Mol Biol. 2010;624:375-84.

59. Libutti SK, Paciotti GF, Byrnes AA, Alexander HR,Jr, Gannon WE, Walker M, et al. Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine. Clin Cancer Res. 2010 Dec 15;16(24):6139-49.

60. Gad SC, Sharp KL, Montgomery C, Payne JD, Goodrich GP. Evaluation of the toxicity of intravenous delivery of auroshell particles (gold-silica nanoshells). Int J Toxicol. 2012 Nov-Dec;31(6):584-94.

61. El-Sayed IH. Nanotechnology in head and neck cancer: The race is on. Curr Oncol Rep. 2010 Mar;12(2):121-8.

## **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  $\gamma$ -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  $\{La_{0.5}Gd_{0.5}\}PO_4 \alpha$ -emitter. This is surrounded by four GdPO<sub>4</sub> 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 1.



Figure 2.



Figure 3.



Figure 4.

![](_page_41_Figure_0.jpeg)

Table 1.

Author	Voor	GND Size	Concentration	Surface	Cell Line	Photon	Observed	Dredicted
Addion	i cai	(nm)	Concentration	Conting	Centine	Enorm	DEE/Effect	DEE
		(1811)		coaung	DULTAS	160 Ma	0.02"	160 May 1 05
					DU-145	100 KVP	0.92 1.13 <sup>4</sup>	6 MV-1 0005
<b>Jain <i>et al</i>(Jain,</b> Coulter et al. 2010)	2011	1.9 nm (AuroVist <sup>m</sup> )	12 μM (500 μg/mL; 0.05%*)	Proprietary thiol		160 kVn	1.41 (n = 0.005)	15 MV:
					MDA-MB-231	6 MV	1.29 (p = 0.002)	1.0005
						15 MV	1.16 (p = 0.19)	
					L132	160 kVp	1.05	
						6 MV	1.08	
			15 nM	Glu-GNPs	MCF7		1.63	
Kong et			7.05 - 14	ALT CHID-	MACHT	200 kVp	1 77	~1.01
<b>al</b> (Kong, Zeng	2009	10.8 nm	3.85 NM	ALT-GNPS	MCE 10A		1.00 (5-20.05)	1.01
et al. 2008)	2.005		15 nM/3.85 nM	Glu-GNPs/	MICT-10A	662 keV	~ 1.13	1,00008
			(115/29.5 µg/mL;	AET-GNPs	MCF7	DDL HET		
			0.012/0.003%*)			1.2 MV	~ 1.13	1.00001
<b>Chithrani et</b> <b>a/</b> (Chithrani, Jelveh et al. 2010)	2010	14 nm	1nM				1.20	
		50 nm	(14/ 50/ 74 nm =		Hela	220 kVp	1.43	~ 1.002 - 1.22
		74 nm	17/ 760/ 2465	Citrate			1.26	
		50 nm	µg/mL; 0.002/0.076/0.25 \$/\$1			105 kVp	1.66	1.36
		50 nm				662 keV	1.18	1.0006
		50 nm	76.7			6 MV	1.17	1.0008
<b>Rahman et</b> <b>a</b> /(Rahman, Bishara et al. 2009)	2009	1.9 nm (AuroVist <sup>™</sup> )	0.25 mM	Proprietary thiol	BAEC	80 kVp	4	3.3
			1 mM	(1mM =			24.6	13.2
			0.5 mM	0.04g/mL;		150kVp	14	52
			1 mM	4%*)			2.2	10.4
<b>Butterworth et</b> a/(Butterworth, Coulter et al. 2010)	2010	1_9 nm (AuroVist <sup>TM</sup> )	0.24 µM (10 µg/ml; 0.001%*)	Proprietary thiol	AGO-1552B		1.16	1.001
					Astro		1.04	
					DU-145	160 kVp	0.98	
					L-132		0.86	
					MCE-7		1.41	
					PC-3		1.67	
					T98G		1.30	1.01
			2.4 μM (100 μg/ml; 0.01%*)		AGO-1552B		1.97	
					Astro		0.96	
					DU-145		0.81	
					L-132		0.87	
					MCF-7		1.09	
					MDA-MB-231		1.11	
					PL-3		1.02	-
livet of tiv			500 uM/0.69		1300	160 kVn	~1.2	~45
Wang et al. 2010)/	2010	6.1 nm	g/mL; 69%*)	PEG	EMT-6/ CT26	6 keV	~ 7	~ 12
						6 MV	~1.25	~16
Per et el(Per			15 nM					
Then g et al	2009	10.8 nm	(115 µg/ml.; 0.01%*)	Glu-GNPs	DU-145	662 keV	>1.5	1.0001
Znang et al.								
2009)			E - M			001-14	13	1.000
Geng et	2011	14 nm	5 nM /83	Glu	SK-OV-3	элкир	13	LUUZ
all Geng, Song			µg/mL:0.008%*)			6 MV	12	1.00009
et al. 2011)								
Chang et	2009	12	LUNM (133 padrat	Citrate	B16510	6 MV o	~1.02	1.00
al(Chang, Shiau			;0.01%*)	ciude	110.10	<b>D</b>		1140
et al. 2008)								
Chien et	2007	20	0.125 - 2 mM	Commenter	(T.Y.	C MV	N 1 10 (A 1	1.00
<b>al</b> (Chien, Wang	2007	20 nm	10-97 g/m1; 600 - 97/0192+1	urate	U1-20	owve	mMi	1.00
et al. 2007)			5,, 60, 6 1					
Zhang et				Thio-			> 1.46	
al(Zhang, Xing	2008	30 nm	15 nM	glucose-	DU-145	200 kVp		~1.20
et al. 2008)			(2464	capped			. 1 71	
			µg/mi;v.25%*)	Neutral (TIGS_CND-)			> 1.31	
	I		1	fire-ours)				1