Targeted nanoparticles for tumour radiotherapy enhancement—the long dawn of a golden era?

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Comment on: Popovtzer A, Mizrachi A, Motiei M, *et al.* Actively targeted gold nanoparticles as novel radiosensitizer agents: an in vivo head and neck cancer model. Nanoscale 2016;8:2678-85.

Abstract: Despite considerable progress in (I) our understanding of the aetiopathology of head and neck cancer and (II) the precise delivery of radiotherapy, long-term survival rates for many patients with head and neck cancer remain disappointingly low. Over the past years, gold nanoparticles (NP) have emerged as promising radiation dose enhancers. In a recent study published in *Nanoscale*, Popovtzer *et al*. have used gold NP coated with an antibody against the epidermal growth factor receptor (EGFR) in an attempt to enhance radiation-induced tumour cell killing in a head and neck cancer xenograft model. They report a significant impact of the combined treatment with radiation and gold NP on tumour growth and suggest an involvement of apoptosis, inhibition of angiogenesis and diminished tissue repair. In this perspective, we illustrate the underlying radiobiophysical concepts and discuss some of the challenges associated with this and related nanoparticle-radiotherapy studies from a physics, chemistry, biology and therapy angle. We conclude that strong interdisciplinary collaborations spanning all these areas are crucially important to proceed towards effective cancer treatment with gold NP "from bench to bedside".

Keywords: Head and neck cancer; radiotherapy; nanomaterials; dose enhancement; epidermal growth factor receptor (EGFR)

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Introduction

In a recent study, Popovtzer *et al*. (1) investigated the effects of actively targeted nanoparticles (NP) as radiosensitizers in an experimental *in vivo* head and neck squamous cell carcinoma (HNSCC) model. Utilising the observation that the epidermal growth factor receptor (EGFR) is frequently overexpressed in HNSCC they coated gold NP with the EGFR-blocking antibody cetuximab to increase their binding to tumour cells. The authors showed that these NP are safe for intravenous injection and enhance the effect of irradiation without increasing side effects after radiotherapy. They suggest that earlier and greater apoptosis, inhibition of angiogenesis and diminished repair may contribute to the observed tumour response. Here we want to illuminate some of the underlying radiation biophysical aspects and discuss the relevance and implications of the findings of that study.

The unmet clinical need

Radiotherapy is fundamental in multimodal cancer treatment with over 50% of all patients receiving irradiation at some time during their disease. Aiming for the complete elimination of all tumour cells, the curative effect of radiotherapy is local tumour control and is directly

attributed to the dose administered to the tumour. However, it is limited by the risk of damage to the surrounding normal tissue. Although recent advances in radiation technologies, treatment planning and image guidance now enable better sparing of normal tissue, the risk of acute and late toxicity is still the major dose-limiting factor.

Great progress has been made in our understanding of some of the genetic, physiological and biochemical profiles of head and neck cancers that govern their vast biological diversity. One major milestone has been the categorisation of HNSCC into human papillomavirus (HPV) positive and negative ones. Whilst the former respond very well to current standard treatment regimens of surgery and cisplatin-based radiochemotherapy and are associated with a good prognosis, frequent local recurrences cause long-term survival rates for patients with HPV-negative HNSCC to remain disappointingly low. Recurrences are believed to be caused by resistant cancer cells that have escaped surgery and survived the harsh radiochemotherapy treatment. Furthermore, due to the multitude of functionally important normal tissues in the head and neck region, precise radiation delivery remains a major challenge, despite all the recent advances in irradiation and imaging techniques. This, in consequence, severely limits the total radiation dose that can be given to the tumour site to inactivate those remaining cancer cells whilst avoiding severe adverse effects.

For this reason, several strategies have been and are being devised to enhance treatment efficacy for HPV-negative head and neck cancers. One of these involves the selective radiosensitisation of tumour cells by targeting biochemical pathways that are frequently deregulated in HNSCC and confer treatment resistance, such as the EGFR pathway (2). A survival benefit observed in a phase III study of EGFR blockade by the monoclonal anti-EGFR-antibody cetuximab in combination with radiotherapy compared to sole radiotherapy treatment caused great initial excitement and resulted in the approval of cetuximab (3,4); however, subsequent clinical trials with various anti-EGFR antibodies have produced rather disappointing results (5-9). Other potential biological targets for the radiosensitization of HNSCC include poly(ADP-ribose) polymerase (PARP), Chk1, Wee1 and ATR (clinical trials NCT02308072, NCT02555644, NCT02585973, NCT02567422).

Gold NP as a microenvironmental radiation booster

An alternative approach aimed at improving radiotherapy treatment efficacy seeks to enhance the radiation dose received by the tumour. Electron-dense materials such as gold were proven to be suited for this purpose in a series of *in vitro* and *in vivo* studies [e.g., (10-15)]. It is important to point out here that gold NP do not *per se* alter the cellular radiosensitivity of a tumour. Instead, upon irradiation, they produce an intense shower of secondary electrons that cause additional ionisations and thereby boost the radiation damage induced in the immediate vicinity of those particles, resulting in greater cell killing. A selective targeting of these particles to the tumour is therefore of paramount importance.

The experimental *in vitro* studies published so far confirm the dose enhancing effect of NPs. Size, concentration and surface coating of NP were identified as variables affecting dose modification as well as beam quality. However, a wide range of enhancement factors have been reported [reviewed in (10) and (16)] which might be a result of the diverse experimental methods and endpoints applied.

Among the physical mechanisms responsible for the enhancement of radiation damage, Butterworth *et al*. (10) discussed an increased photo-electron production in gold when irradiating with photon beams with energy in the keV range (mostly used in *in vitro* experiments) and the production of a higher number of Auger electrons if the photon source has therapeutic energies (MeV range). However, up to now, a clear correspondence between increased cell killing and the physical effects that contribute to a dose enhancement could not be established.

The vagaries of dose enhancement calculations

A clear correspondence between the interactions of photons and the DNA damage enhancement around gold NP is necessary for a correct dose calculation during treatment planning. A number of Monte-Carlo simulations of possible clinical scenarios were carried out in the last few years to this purpose [see, for example, (17-22)]. Such simulations are based on the fact that the transport of ionizing radiation in matter is of random nature and can be described using probabilistic models. Therefore, if all the interactions experienced by an ionizing particle are simulated in chronological succession, the energy deposition (or other relevant physical quantities) in a given volume of interest can be calculated with high accuracy at the micrometre and nanometre level. In this way, an alteration of the particle tracks in the presence of gold NP and the effects of this alteration at the nanometric scale

can be investigated. The existing Monte Carlo studies simulate the transport of photons and electrons in water around a single nanoparticle or in a geometry that simulates a simplified spatial distribution of several particles at a micrometric or nanometric scale [see Subiel *et al.* (23) for a review on this subject]. These simple studies can therefore help in confirming or discarding some hypotheses about the mechanisms involved in the enhancement of cell killing after irradiation (such as those mentioned by Popovtzer *et al.*). Unfortunately, the calculated dose around gold NP varies strongly among publications reporting on similar set-ups. A computational study from Tsiamas *et al.*, for example, reports dose enhancements in water of a factor of about 1.7 at a distance of 1 μm from a 100-nm gold NP irradiated with megavoltage photons (18), while Jones *et al.* calculated an enhancement of a factor of about 8 for a similar geometry and photon spectrum (19). McMahon *et al.*, moreover, carried out *in vitro* experiments in therapeutic photon fields and corresponding Monte-Carlo simulations (20). They showed that, while the relative biological effectiveness of gold NP for the investigated cell line is approximately of a factor of two, the calculated absorbed dose increases dramatically in the proximity of a gold NP (up to a factor of 10,000 within 100 nm).

These examples demonstrate that, for the use of gold NP as sensitizers in radiotherapy, the absorbed dose alone is not the appropriate physical quantity for assessing the biological effectiveness of gold NP and for treatment dose planning.

The quantification of a true dose enhancement requires the assessment of dose response curves over a range of doses both for control and combined treatment in which their displacement correlates with the extent of dose modification which is defined as the ratio of doses for a given level of cell kill. As recently stated by Subiel *et al.* (23) the literature survey concerning the dose enhancing effect of NPs is hindered by the fact that terminology and methodology is not consistent. Dose-modification is determined at various levels of cell kill, dose enhancement is named as sensitizer enhancement ratio (SER), dose enhancement ratio (DER) or dose modification ratio (DMR) irrespective of radiobiological definitions.

Biological pathways of treatment response

Popovtzer *et al.* have used cetuximab to target gold NP to the tumour, which should result in their initial accumulation at the membrane of tumour cells expressing high levels of EGFR. EGFR-mediated endocytosis would then be expected to result in the internalisation and accumulation of these nanoparticle-antibody complexes in the cytoplasm. While one of the limitations of this study is that it does not include any data on the macroscopic or cellular localisation of the cetuximab-coated NP, other studies targeting EGFR using fluorescent NP support this notion [e.g., (24)]. As proximity is such an important parameter in determining the radiation dose enhancement factor of gold NP, precise analysis of their intracellular distribution will be required to help us assess the extent to which pure radiation physics can explain the observed biological effectiveness of gold NP.

Although irradiation causes lethal DNA-damage, the affected cell will undergo a limited number of cell divisions before it suffers loss of reproductive ability. This so-called mitotic death is the prominent route of radiation-induced cell inactivation (25), whereas apoptosis is not generally considered the main mechanism for the death of cancer cells in response to radiation (26). Therefore permanent tumour control is the most relevant endpoint for preclinical testing of potential curative radiotherapeutic approaches (27), whereas tumour growth delay is considered to correlate with the palliative effect of radiotherapy on tumour volume and does not correlate well with inactivation of clonogenic tumour cells (28). However, the mechanism of action leading to increased radiation-induced cell death mediated by NP is not yet fully understood. An increased generation of reactive oxygen species by secondary water radiolysis (29) as well as a potential role of mitochondria as biological mechanisms contributing to NP-mediated enhancement of radiation treatment (30) have been discussed. A direct functional proof for enhanced dose delivery could be the analysis of initial DNA damage foci, such as gamma-H2AX or 53BP1, in tumour and neighbouring normal tissue of an experimental model system.

Standard care for HNSCC patients involves fractionated radiotherapy, giving 2 Gy per day, based on the empirical finding that such a course of treatment enables normal tissues to recover more efficiently than most tumours. In contrast, Popovtzer *et al.* delivered 25 Gy in a single fraction. This discrepancy should be kept in mind when considering the biological mechanisms underlying the reported effects in this study. In addition to reduced tumour growth, Popovtzer *et al.* reported reduced vascularisation (CD34 staining), proliferation (Ki67), tissue repair (PCNA) and increased apoptosis (TUNEL assay) 1 week after treatment. However, it remains unclear to what extent these processes contribute to tumour control.

Tumour targeting: functionalisation of nanomaterials

The study by Popovtzer *et al*. underlines once more the potential—but also the challenges—of AuNP tumour targeting in general and with cetuximab specifically (31-33). The research focus in general, however, is shifting from promising proof-of-concept studies to the feasibility of translation to the clinic (34,35). In this regard, the recent analysis presented by Chan's group has stimulated considerable discussion (36). They pointed out that, considering the research efforts and resources spent during the past decade, the tumour delivery efficiencies are disappointingly low. This aspect is not really discussed by the authors.

The surface chemistry used by the authors is reasonable and well established (37). PEG-based ligand layers are commonly used in nanomedicines because PEG is well-known to improve the pharmacokinetic properties and stability of nanomaterials (38,39). Also, the use of antibodies for tumour targeting is a popular strategy (40,41). An overview of according preclinical and clinical studies with gold NP/AuNP can be found, e.g., in ref (42). Considering the major challenges for translation to clinic, however, it is worth to look at the role of surface chemistry in more detail. Major challenges are the reproducibility, both in the syntheses of the nanomedicines and in their effects, the scalability of their production and their stability at high concentrations, under storage and in complex biological environments. Reproducibility, stability and safe application are intertwined and require well-defined formulations and a profound understanding of their mechanism of action. Antibodies might not be the optimum choice for several reasons: (I) they are not easily produced in large amounts and at low cost; (II) limited stability and half-life can be problematic and (III) their controlled binding (because they bear multiple functional groups) to and regiospecific presentation on NP is challenging. In the presented study, the amount of bound cetuximab is not quantified and consequently targeting efficiency and relative doses cannot be calculated. The UV/vis spectra shown in Figure 2 of Popovtzer *et al.* (1) suggest some aggregation of the antibody-coated gold NP (CTX-GNP), noticeable from the broadening of the plasmon resonance band and increased absorption in the range 600–800 nm (43). In general, it is not uncommon that binding antibodies to NP results in increased adhesion of the NP to vessel walls and some extent of aggregation during concentration and purification. It should also be considered that bound antibodies significantly increase the size of the nanoconjugate and, even more important, completely change their interface. While this is the desired effect for targeting, it can also render the beneficial effects of the PEG coating ineffective, affecting e.g., immune response, stability and pharmacokinetic profiles. In terms of reproducibility, stability and therefore safety and potential for successful clinical translation the design rationale should therefore be to keep the nanomaterial as simple as possible. In this sense, small binding molecules such as the small molecule EGFR inhibitor IRESSA, ligands such as EGF, functional peptides, carbohydrates, aptamers etc. may be preferable. They are typically cheaper and available in sufficiently large quantities; they can be bound to the nanoparticle in a controlled manner; and with state-of-the-art surface chemistries and ligand layer design a very controlled and even stimuli-responsive presentation can be envisaged.

Considering the heterogeneity and variability of tumours and the biological barriers to overcome during systemic delivery it is standing to reason that highly defined, complex and controlled surface chemistries will be needed, most probably including more than one type of functional molecule. At the same time the nanosystem has to meet the criteria discussed above, and thorough preliminary studies are needed to understand the physical, chemical and biological mechanisms of action and potential side effects. To proceed towards effective cancer treatment with gold NP "from bench to bedside", strong interdisciplinary collaborations seem therefore inevitable.

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Footnote

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References

1. Popovtzer A, Mizrachi A, Motiei M, et al. Actively targeted gold nanoparticles as novel radiosensitizer agents: an in vivo head and neck cancer model. Nanoscale 2016;8:2678-85.

2. Ang KK, Andratschke NH, Milas L. Epidermal growth factor receptor and response of head-and-neck carcinoma to therapy. Int J Radiat Oncol Biol Phys 2004;58:959-65.
3. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567-78.
4. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 2010;11:21-8.
5. Ang KK, Zhang Q, Rosenthal DI, et al. Randomized phase III trial of concurrent accelerated radiation plus cisplatin with or without cetuximab for stage III to IV head and neck carcinoma: RTOG 0522. J Clin Oncol 2014;32:2940-50.
6. Eriksen JG, Maare C, Johansen J, et al. OC-009: Update of the randomised phase III trial DAHANCA 19: Primary C-RT or RT and zalutumumab for squamous cell carcinomas of head and neck. Radiother Oncol 2015;114:10.
7. Giralt J, Trigo J, Nuyts S, et al. Panitumumab plus radiotherapy versus chemoradiotherapy in patients with unresected, locally advanced squamous-cell carcinoma of the head and neck (CONCERT-2): a randomised, controlled, open-label phase 2 trial. Lancet Oncol 2015;16:221-32.
8. Magrini SM, Buglione M, Corvò R, et al. Cetuximab and Radiotherapy Versus Cisplatin and Radiotherapy for Locally Advanced Head and Neck Cancer: A Randomized Phase II Trial. J Clin Oncol 2016;34:427-35.
9. Mesía R, Henke M, Fortin A, et al. Chemoradiotherapy with or without panitumumab in patients with unresected, locally advanced squamous-cell carcinoma of the head and neck (CONCERT-1): a randomised, controlled, open-label phase 2 trial. Lancet Oncol 2015;16:208-20.
10. Butterworth KT, McMahon SJ, Currell FJ, et al. Physical basis and biological mechanisms of gold nanoparticle radiosensitization. Nanoscale 2012;4:4830-8.
11. Hainfeld JF, Slatkin DN, Smilowitz HM. The use of gold nanoparticles to enhance radiotherapy in mice. Phys Med Biol 2004;49:N309-15.
12. Hainfeld JF, Dilmanian FA, Zhong Z, et al. Gold nanoparticles enhance the radiation therapy of a murine squamous cell carcinoma. Phys Med Biol 2010;55:3045-59.
13. Hainfeld JF, Smilowitz HM, O'Connor MJ, et al. Gold nanoparticle imaging and radiotherapy of brain tumors in mice. Nanomedicine (Lond) 2013;8:1601-9.
14. Joh DY, Kao GD, Murty S, et al. Theranostic gold nanoparticles modified for durable systemic circulation effectively and safely enhance the radiation therapy of human sarcoma cells and tumors. Transl Oncol 2013;6:722-31.
15. McQuade C, Al Zaki A, Desai Y, et al. A multifunctional nanoplatform for imaging, radiotherapy, and the prediction of therapeutic response. Small 2015;11:834-43.
16. Coulter JA, Hyland WB, Nicol J, et al. Radiosensitising nanoparticles as novel cancer therapeutics--pipe dream or realistic prospect? Clin Oncol (R Coll Radiol) 2013;25:593-603.
17. Lechtman E, Chattopadhyay N, Cai Z, et al. Implications on clinical scenario of gold nanoparticle radiosensitization in regards to photon energy, nanoparticle size, concentration and location. Phys Med Biol 2011;56:4631-47.
18. Tsiamas P, Liu B, Cifter F, et al. Impact of beam quality on megavoltage radiotherapy treatment techniques utilizing gold nanoparticles for dose enhancement. Phys Med Biol 2013;58:451-64.
19. Jones BL, Krishnan S, Cho SH. Estimation of microscopic dose enhancement factor around gold nanoparticles by Monte Carlo calculations. Med Phys 2010;37:3809-16.
20. McMahon SJ, Hyland WB, Muir MF, et al. Nanodosimetric effects of gold nanoparticles in megavoltage radiation therapy. Radiother Oncol 2011;100:412-6.
21. Xie WZ, Friedland W, Li WB, et al. Simulation on the molecular radiosensitization effect of gold nanoparticles in cells irradiated by x-rays. Phys Med Biol 2015;60:6195-212.
22. He C, Chow JC. Gold nanoparticle DNA damage in radiotherapy: A Monte Carlo study. AIMS Bioengineering 2016;3:352-61.
23. Subiel A, Ashmore R, Schettino G. Standards and Methodologies for Characterizing Radiobiological Impact of High-Z Nanoparticles. Theranostics 2016;6:1651-71.
24. Gao M, Su H, Lin G, et al. Targeted imaging of EGFR overexpressed cancer cells by brightly fluorescent nanoparticles conjugated with cetuximab. Nanoscale 2016;8:15027-32.
25. Baumann M, Krause M, Dikomey E, et al. EGFR-targeted anti-cancer drugs in radiotherapy: preclinical evaluation of mechanisms. Radiother Oncol 2007;83:238-48.
26. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer 2005;5:231-7.

27. Zips D, Thames HD, Baumann M. New anticancer agents: in vitro and in vivo evaluation. In Vivo 2005;19:1-7.
28. Krause M, Zips D, Thames HD, et al. Preclinical evaluation of molecular-targeted anticancer agents for radiotherapy. Radiother Oncol 2006;80:112-22.
29. Misawa M, Takahashi J. Generation of reactive oxygen species induced by gold nanoparticles under x-ray and UV Irradiations. Nanomedicine 2011;7:604-14.
30. Taggart LE, McMahon SJ, Currell FJ, et al. The role of mitochondrial function in gold nanoparticle mediated radiosensitisation. Cancer Nanotechnol 2014;5:5.
31. Qian Y, Qiu M, Wu Q, et al. Enhanced cytotoxic activity of cetuximab in EGFR-positive lung cancer by conjugating with gold nanoparticles. Sci Rep 2014;4:7490.
32. Karmani L, Labar D, Valembois V, et al. Antibody-functionalized nanoparticles for imaging cancer: influence of conjugation to gold nanoparticles on the biodistribution of 89Zr-labeled cetuximab in mice. Contrast Media Mol Imaging 2013;8:402-8.
33. Conde J, Bao C, Cui D, et al. Antibody-drug gold nanoantennas with Raman spectroscopic fingerprints for in vivo tumour theranostics. J Control Release 2014;183:87-93.
34. Min Y, Caster JM, Eblan MJ, et al. Clinical Translation of Nanomedicine. Chem Rev 2015;115:11147-90.
35. Hare JI, Lammers T, Ashford MB, et al. Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. Adv Drug Deliv Rev 2016. [Epub ahead of print].
36. Wilhelm S, Tavares AJ, Dai Q, et al. Analysis of nanoparticle delivery to tumours. Nat Rev Mater 2016;1:16014.
37. Sapsford KE, Algar WR, Berti L, et al. Functionalizing nanoparticles with biological molecules: developing chemistries that facilitate nanotechnology. Chem Rev 2013;113:1904-2074.
38. Jokerst JV, Lobovkina T, Zare RN, et al. Nanoparticle PEGylation for imaging and therapy. Nanomedicine (Lond) 2011;6:715-28.
39. Karakoti AS, Das S, Thevuthasan S, et al. PEGylated inorganic nanoparticles. Angew Chem Int Ed Engl 2011;50:1980-94.
40. Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Deliv Rev 2004;56:1649-59.
41. Peer D, Karp JM, Hong S, et al. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2007;2:751-60.
42. Pedrosa P, Vinhas R, Fernandes A, et al. Gold nanotheranostics: proof-of-concept or clinical tool? Nanomaterials 2015;5:1853-79.
43. Ghosh SK, Pal T. Interparticle coupling effect on the surface plasmon resonance of gold nanoparticles: from theory to applications. Chem Rev 2007;107:4797-862.

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