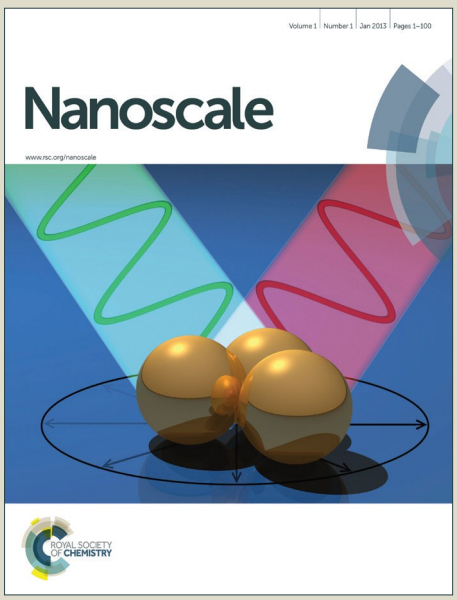
Nanoscale

Accepted Manuscript



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Opinion

Scientific and industrial challenges of developing nanoparticle-based theranostics and multiple-modality contrast agents for clinical application.

Received 12th June 2015,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000

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Abstract: Designing of theranostics and dual or multi-modality contrast agents are currently two of the hottest topics in biotechnology and biomaterial science. However, for single entity theranostics, a right ratio of their diagnostic component and their therapeutic component may not always be realized in a composite suitable for clinical application. For dual/multiple modality molecular imaging agents, after *in vivo* administration, there is an optimal time window for imaging, when an agent is imaged by one modality, the pharmacokinetics of this agent does not allow imaging by another modality. Due to reticuloendothelial system clearance, efficient *in vivo* delivery of nanoparticles to lesion site is sometimes difficult. The toxicity of these entities also remains poorly understood. While the medical need of theranostics is admitted, the business models remain to be established. There is an urgent need for a global and internationally harmonized re-evaluation of the approval and marketing processes of theranostics. However, a reasonable expectation exists that, in the near future, the current obstacles will be removed, thus allowing the wide use of these very promising agents.

Personalized medicine is generally considered as the precise use of drug(s) that can specifically target a patient's diseased tissue, and molecular imaging is considered one of the cornerstones of personalized therapy. [1,2] The designing of theranostics and dual or multi-modality contrast agents is among the hottest topics in biotechnology and biomaterial science. [3-10] Recently, numerous published research articles on developing theranostics and dual/multiple modality imaging agents for the ultimate aim of clinical application demonstrated the considerable efforts of the concept. It was suggested that nanoparticle-based approach might be able to play a crucial role in cancer diagnosis and treatment, in terms of molecular imaging of the tumour microenvironment and image-guided interventions including drug delivery, surgery and ablation therapy. Nanoparticles can offer multifunctional nanoplatforms with specific *in vivo* delivery of drugs without systemic toxicity, the dose delivered as well as the therapeutic efficacy can be accurately measured by bioimaging non-invasively over time. [4, 5, 9, 10] Hereby we discuss the concept of theranostics, and the scientific and industrial challenges of developing nanoparticle based theranostics and multiple-modality contrast agents for clinical application.

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Theranostics: a fashionable and sometimes overused concept

The concept of "theranostics" was coined by the US consultant John Funkhouser, in August 1998, to describe a material that allows the combined diagnosis, treatment and follow up of a disease.[7] Therefore, theranostics refers to the development of molecular diagnostic tests and targeted therapeutics in an interdependent, collaborative manner with the goals of individualizing treatment by targeting therapy to an individual's' specific disease subtype and/or genetic profile. This strategy is expected to optimize drug efficacy and safety and to assist in streamlining the drug development process.

The current usage theranostics term is quite broad and sometimes confusing. Recently, Nicolaides and coworkers suggested distinguishing three categories of theranostics based on the association or not of the biomarker, the diagnostic tool and the therapeutic drug: [11]

a) The non-targeted theranostics: the biomarker, the therapeutic agent and the biological target are all distinct entities. One example is insulin: blood glucose is the biomarker, insulin is the therapeutic agent and the target is insulin receptor. [11]

b) In targeted theranostics, the therapeutic target and the biomarker are the same entity, while the diagnostic tool is distinct. The combination of Genentech's trastuzumab (Herceptin®) with DakoCytomation's immunohistochemistry HercepTest® is probably the best-known, and very successful, example of a commercialized theranostics.[12] Trastuzumab targets the HER2 protein, which is overexpressed in 25% to 30% of breast cancers. Physicians use the HercepTest® to detect susceptible tumours, which enables targeting of the treatment to patients that are likely to benefit from the drug. The FDA approved trastuzumab as a biological product in September 1998; at the same time, the FDA's Center for Devices and Radiological Health reviewed and approved DakoCytomation's HercepTest® diagnostic kit for HER2 expression. The drug and the diagnostic came to market at the same time, with the drug's labeling specifying the requisite diagnostic test. In this case of example, the therapeutic agent, i.e. trastuzumab, and the diagnostic tool, i.e. HercepTest® are two different entities.

c) In "leveraged" theranostics, the diagnostic and therapeutic agents share at least one moiety. Radioimmunotherapy is an example of leveraged theranostics. The anti-CD20 monoclonal antibody ibritumomab is covalently conjugated to the ligand tiuxetan which chelates the β-emitting isotope ^{90}Y or the γ-emitting ^{111}In (Zevalin®). Ibritumomab tiuxetan is approved for the treatment of non-Hodgkin lymphoma patients. The ^{111}In--labeled monoclonal antibody allows patient selection and confirms selective distribution while the ^{90}Y- labelled antibody specifically kills CD20+ malignant B lymphocytes.[13] Another interesting example of this approach is the vintafolide/etarfolatide couple, with both compounds developed by the same company Endocyte Inc. Vintafolide (EC145, Vynfinit®) is a conjugate of folic acid and the vinca alkaloid desacetylvinblastine hydrazide. It targets the folate receptor-α (FR-α), a major membrane marker for ovarian cancer (and other solid cancer types of epithelial origin). The radiolabelled contrast agent etarfolatide (^{99m}Tc-EC20, FolateScan®) is its companion imaging marker.[14] Unfortunately, in FR-α -positive (i.e. etarfolide-labeled) ovarian cancer patients, vintafolide in combination with pegylated liposomal doxorubicin (PLD) *versus* PLD alone did not meet the pre-specified criteria for progression-free survival. This led to withdrawal of conditional marketing authorization applications from the European Medicines Agency (EMA) for this drug and its companion biomarker. However, vintafolide in combination with docetaxel was reported to extend overall survival in patients with FR-positive recurrent non-small cell lung cancer compared to patients receiving monotherapy docetaxel in the TARGET Phase 2b clinical trial. [15]

Furthermore, interventional radiology is evolving to theranostic approach. Liver transarterial chemoembolization (TACE) is probably the first example of theranostics. Conventional oily chemoembolization consists in the intra-arterial administration of a mixture associating a cytotoxic drug and Lipiodol®, is the standard of care for the treatment of patients with non-invasive, multinodular asymptomatic hepatocellular carcinoma without vascular invasion.[16] Lipiodol® induces a transitory and plastic embolic effect that slows or even stops tumour blood flow, thus allowing the local delivery of the cytotoxic drug.[17] Its radio-opaque properties are crucial in helping the clinician to perform the procedure and that the early degree of Lipiodol® labelling of the tumour has been found to be an independent prognostic parameter for patient survival.[18-20]

However, none of the above successful agents involves nanoparticles.

Scientific challenges

The combination of imaging and therapeutic agents is usually not a natural fit. The dosage is low for imaging, while it is usually much higher for agents to execute its therapeutic function. A right ratio of their diagnostic component and their therapeutic component may not always be realized in a composite suitable for *in vivo* application. Radioisotope probes enabling single-photon emission computed tomography (SPECT) and positron- emission tomography (PET) offer preferable features for theranostic agent clinical translation. Due to the high sensitivity of radioisotope probes, patients can be treated with nanocarriers loaded with high therapeutic doses, yet labelled with rather trace amounts of isotopes. However, the short half-life of most clinically-suitable radionuclides has generally led to their placement on the surface of therapeutic nanoparticles. The short time window for the labeling and usage in patients create obstacles for clinical application. In addition, stability of the radiolabeling should be ensured. For example, if radioisotope radiometal is lost from the nanoparticle, acquired images will lead to misleading results.

Dual- or multi-modality molecular imaging is the synergistic combination of two or more detection techniques, an approach made possible by multi-modal probes and imaging agents. There are various dual/multiple modality molecular imaging agents being devised, including nanoparticles combining fluorescence and magnetic resonance imaging, gadolinium chelates and organic fluorophores, gadolinium chelates and nanoparticles, quantum dots and an magnetic resonance imaging contrast agent, iron oxide nanoparticles and an optical probe, and radionuclear imaging/optical imaging agents, magnetic resonance imaging/positron emission tomography probes, and dual contrast agent for magnetic resonance imaging/x-ray computed tomography (CT).[3-6, 9, 10, 21] However, for the practical application of these dual/multiple modality molecular imaging agents, there are a few fundamental challenges. First is the pharmacokinetics, there is usually an optimal time window when the administrated agent is at the right concentration for imaging. As a way of example, to assess liver tumour, a triphasic approach is usually taken. Following the injection of contrast agent, images are acquired during the arterial (ca. 25 seconds), portal (ca. 60 seconds) and equilibrium (ca. 180 seconds) phases.[22, 23] In many cases when an agent is administered and imaged by one modality, the pharmacokinetics of this agent does not allow imaging by another modality. Another consideration is that, in many circumstances, dual/multiple modality is not used in the same day for lesion assessment. For example, when a brain tumour is assessed with contrast enhanced magnetic resonance imaging, it is very unlikely it will be assessed again with contrast enhanced CT in the same day. For the combination of optical and magnetic resonance imaging probe, the limited penetration depth of luminescent or fluorescence contrast agent would be a significant obstacle. To quantify signal strength quantification of luminescent or fluorescence are also known to be problematic. [24, 25] Optimal instrument suitable for small animal will not be suitable for human imaging except for small superficially located lesion.

Efficient *in vivo* delivery of nanoparticles to lesion site can be extremely difficult. In theory, through the enhanced permeability and retention (EPR) effect, i.e. when intravenously administrated nanoparticles can accumulate preferentially at tumour sites as tumour neovessels are highly permeable, thus allowing the leakage of circulating nanoparticles into the tumour interstitium, and also many tumours lack an effective lymphatic drainage, leading to subsequent nanoparticle accumulation.[26,27] However, size of intravenously injected nanoparticles greatly affects their *in vivo* biodistribution, e.g. particles from 60 to 150 nm in size are taken up by the reticuloendothelial system leading to rapid uptake in the liver and spleen. Intravenously injected nanoparticles with diameters of 10–40 nm allow longer blood circulation and can cross capillary walls, and they are often phagocytosed by macrophages which traffic to lymph nodes and bone marrow[28]. The EPR effect is not commonly observed in some types of cancers.[29] In other cases, the tumour core may not be well-perfused. Small metastases (<100 mm^3) are poorly vascularized and do not evoke EPR. [30] Imaging-guided directly injected nanoparticles will not diffuse evenly to the whole lesion; instead nanoparticles will aggregate with high concentration locally in the injection site due to the tissue network resistance. Though theoretically attractive, magnetic targeting, i.e. using external magnetic field to locate iron containing magnetic nanoparticle to the desired site is difficult because the force of blood flow can be stronger than external magnetic field.[31] While it has been more than 30 years since the concept of receptor-specific

targeted nanoparticles for cancer imaging or treatment was introduced,[32] but to date none have been clinically approved. Molecular drug targeting of cancer requires extraordinary sensitivity because concentrations of biological molecules abnormally expressed in tumour tissues are very low. Also not all cancer cell types overexpress the same unique receptors and often, overexpressed receptors are also present on normal tissue. An absolute specificity of a target molecule for cancer cells (or any other targets) may not exist. In fact, many "tumour specific" target molecules including receptors for folate, integrins and transferrin exist in non-tumour cells and pose the danger of off-target binding and effects. To execute therapeutic effect, nanoparticles must also overcome high intratumoural fluid pressure and penetrate tumour extracellular matrix, adding a targeting moiety may hamper this process by increasing the nanoparticle size. The use of targeted nanoparticles with may elicit a "binding site barrier" wherein binding to target cells paradoxically reduces penetration in deep layers of the tumours. [33, 34] This may be particularly problematic for nanoparticles due to higher diffusion limitations. As a way of example, intravenous application of antibody-conjugated magnetoliposomes does not lead to sufficient concentration of magnetic iron nanoparticle to be applicable for local hyperthermia. [35]

For many published theranostics composites or multi-modality molecular imaging nanoparticles, the toxicity of these entities remains poorly understood. For example, silica is a common choice to encompass the nanoparticles due to perceived biocompatibility and easy manipulation. [36-38] However, silica can be toxic which has been noted in some studies. [39, 40] In addition, long term exclusion of silica from human body remains unknown.

In addition to that clinical development cost of the single entity composite theranostics or multi-modality molecular imaging agents is formidable[8], another point of consideration is that the proposed theranostics should have better efficacy or more cost-effective than the current standard therapy, such as surgery removal or radiation therapy for cancer. These standard therapies can actually be superior in efficacy compared with recently proposed novel treatments such as photothermal therapy and thermotherapy in many cases. For example, in 2005 Johannsen *et al*. described the first clinical application of magnetite-nanoparticle-mediated hyperthermia in prostate cancer.[41] However, till now androgen deprivation, prostatectomy, or radiation therapy remain the treatments of choice for prostate cancer.

Industrial challenges

If not marketed, the most exciting theranostic agents, even if clinically-validated, will not ultimately benefit to patients. Substantial chemical, biological, economical and regulatory approval barriers must be overcome for single entity theranostics and multiple-modality contrast probes to realize clinical translation. Indeed, these barriers are not prohibitive for the availability of theranostic innovations for the patients. A better mutual knowledge and understanding between academic and industry researchers and "developers" is crucial. For the safety concern, Choi and Frangioni proposed three criteria to guide clinical translation of nanoparticles: degradability (complete clearance), surface charge (minimal non-specific tissue uptake) and size/shape (smaller than 5 nm with enhanced renal clearance).[42] Health authorities not only request the rigorous demonstration of the proof of concept, but also of improved patient outcome.[43] This requirement is not only determinant for the theranostic solution approval by health authorities, but often for the insurance coverage as well.

The long history of marketing efforts for small dextran-coated superparamagnetic iron oxide (SPIO) nanoparticles provides an illustration of the difficulties in developing nanoparticles for clinical application. These magnetic resonance imaging contrast agents have been extensively studied, both experimentally and clinically.[44-48] From the copious literature, it is obvious that these nanoparticles are of considerable clinical value[28]. However, although several SPIO have been developed as contrast agents because of their unique ability to target macrophage or to allow blood pool imaging, we are reluctantly forced to admit that these efforts were not conclusive. So far, the only available SPIO (ferumoxytol, Ferahaeme®) is marketed for the treatment of iron deficiency anaemia in adult patients with chronic kidney diseases. There are several causes for what must be considered, for now, as a failure: a) the requirement by health authorities of central reading procedure for all images acquired during clinical studies, i.e. in conditions that are very different from the routine practice where the clinical setting of the imaging procedure is well known by the radiologists[43]; b) the

exorbitant cost of development, c) the expected low return on investment. Of note, it should be underlined that, in general, the manufacturing process of SPIO was not a major matter of concern and did not negatively impact the development process. The selection of clinical indications is also an important topic to consider. For example, the low return on investment/regulatory requirements ratio anticipated for the imaging of stem cell migration and immune cell trafficking indication probably explains why little industrial efforts have been made in this field. The weight of budget investment needed for the development of iron oxide nanoparticles for cell tracking is far too high when compared to the risk of therapeutic medicine failure.

If validated, theranostics will be of major interest in that it allows to identify patients eligible to expensive and sometimes poorly tolerated treatments and to follow up these patients with objective and quantitative criteria[49]. Well-designed pharmacoeconomic studies are also warranted for an objective evaluation of the cost/benefits ratio for the community.

Another key issue is the current lack of coordinated regulatory guidance, from health authorities worldwide, for the co-development of diagnostic agents and therapeutic drugs when associated in theranostics solutions.[50] Interestingly, a similar challenge was raised several years ago in the case of nanobiotechnology and environmental protection and it might be helpful to benefit from the experience of academic and industrial players in this field. [51] It would make sense that, when a companion imaging biomarker includes the same pharmacophore (i.e. the molecular entity that binds the biological target of interest), synergistic development be considered. This issue makes mandatory a very close collaboration between the companies involved in the project. The above-mentioned US biopharmaceutical company Endocyte Inc. is a good example of early management of both the therapeutical and companion biomarker issues by the same entity. The commercial future of a qualified companion biomarker whose associated therapy failed is another interesting issue. In addition to the regulatory issues that may arise in the case of a combined development, the nature of the biological target itself may play a role in the future of the compound.

Unfortunately, it seems that the pharmacology of contrast agents as a distinct class of drugs is seldom taught in universities. Therefore, the specificities of these agents are not well known among the medical and pharmaceutical communities worldwide, making the “nostics” suffix of “theranostics” sometimes less attracting for researchers than the “thera” prefix. Last, this may also slow down the desirable networking on nanoparticle-based theranostics.

Nanoparticles for the sole use as imaging agents would have a much lower return on investment than theranostic combinations.[8] Each new functionality elevates complexity (e.g. multi-step syntheses, purification and characterization) and cost (e.g. lower yields, more costly materials), and regulatory barriers arise (e.g. owing to multi-component, heterogeneous formulations). Industry can only invest nanotechnology when nanomaterials meet medical needs and also the development hurdles are manageable. The business models associated with theranostics associating contrast agents need to be completely reconsidered since it cannot be extrapolated from the theranostics solutions including biomarkers derived from molecular biology. Frequently, the therapeutic drug and its companion contrast agent are not developed by the same companies. This raises the question of risk sharing between pharmas and companies developing contrast agents which have a much lower return on investment.[50] Development of theranostics is a multi-technological issue, the complete value chain must be involved, including academics, pharmas, regulatory authorities, insurances, imaging equipment manufacturers, clinicians, etc. This is the only way theranostics involving imaging agents will be economically viable and eventually reach routine practice and, benefit patients. Indeed, this analysis is not incompatible with enthusiasm of both academic and researchers in the private sector as regards nanoparticle-based theranostic agents. Nanoparticle-based theranostics lies at the crossroad of almost all the current therapeutic methods and all the imaging approaches (MRI, X-ray, optical imaging, CT, photoacoustic imaging, etc.)[51,52]. Once the initial enthusiasm phase has died down, most benefits and scientific weaknesses of this approach are now well-known. Furthermore, major health agencies such as the US Food and Drug Agency and EMA have established initiatives to facilitate the approval process[53]. A reasonable expectation exists that, in the near future, the current obstacles will be removed following an efficient networking

between all stakeholders, thus allowing the wide use of these very promising agents into routine healthcare.

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