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Multimodal nanoparticle imaging agents: Design and Applications

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Summary

Molecular imaging, where the location of molecules or nanoscale constructs can be tracked in the body to report on disease or biochemical processes, is rapidly expanding to include combined modality or multimodal imaging. No single imaging technique can offer the optimum combination of properties (e.g. resolution, sensitivity cost, availability).

The rapid technological advances in hardware to scan patients, and software to process and fuse images, is pushing the boundaries of novel medical imaging approaches, and hand-in-hand with this is the requirement for advanced and specific multimodal imaging agents. These agents can be detected using a selection from radioisotope, magnetic resonance and optical imaging, amongst others. Nanoparticles offer great scope in this area as they lend themselves, via facile modification procedures, to act as multifunctional constructs. They have relevance as therapeutics and drug delivery agents that can be tracked by molecular imaging techniques with the particular development of applications in optically guided surgery and as radiosensitisers.

There has been a huge amount of research work to produce nanoconstructs for imaging and the parameters for successful clinical translation and validation of therapeutic applications are now becoming much better understood. It is an exciting time of progress for these agents as their potential is closer to being realised with translation into the clinic. The coming 5-10 years will be critical, as we will see if the predicted improvement in clinical outcomes becomes a reality. Some of the latest advances in combination modality agents are selected and the progression pathway to clinical trials analysed.

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1. Multimodal imaging – general introduction and applications of multimodal imaging nanoparticle agents

Molecular imaging has developed over the past 30 years in parallel with the advances of medical imaging technologies. PET/CT scanners arrived into clinical use in the early 1990s; combining the molecular detection component with an anatomical technique to provide improved data sets to the clinicians and demonstrating the potential for multimodal imaging. Many radiology/ nuclear medicine departments are now using PET/MRI as the next generation technology, however, there is still much work to be done to use combined molecular imaging techniques to their full potential. There are also complications as to how they fit into clinical pathways, the consistency of data production and imaging protocols, but the potential is clear.

Whilst the technology for combined modality scanners or the fusing of image data from separately collected multiple image modalities has advanced, there have been more limited advances in the multimodality imaging tools in clinical use to allow the full potential of multimodal imaging to be reached. Their development is complicated by the fact that optimisation for a single imaging technique is not appropriate, it must be for the combination, and the chemical standards/ parameters have not been set in terms of isotopes, wavelengths for optical or MRI pulse sequences.

The driver behind the production of multimodal scanners and agents is to balance the advantages and disadvantages of different imaging techniques i.e. sensitivity of detection vs. resolution of the image. Table 1 shows many of the key molecular imaging techniques and their properties. The key applications are in diagnosis of disease, patient stratification and the therapy response. Mulitmodal imaging is also an excellent tool for drug development and radiolabelling of constructs can be used to obtain ADME and PK characteristics of novel molecules or constructs that are in development as therapeutics

Nanoparticles are ideal for these applications with large surface areas that can be functionalised to introduce multiple modality reporters and targeting vectors to modify localisation properties. The particle size, morphology and surface chemistry can also be modified to influence their behaviour *in vivo*.[1] The assessment of *in vivo* characteristics including biodistribution, clearance and targeting are essential for successful translation.[2]

No single molecular imaging modality has the capability to offer all of the required data to fully characterise the properties of an administered agent.[3] Optical techniques have limited tissue penetration *in vivo*, MRI has high resolution but low sensitivity whilst radioisotope imaging techniques offer much improved sensitivity but relatively poor resolution. Multimodal imaging validation can expedite progress of nanomaterials to clinical trials and also provides key design/ development data at the preclinical stage. This can streamline development and, clinically, can offer a secondary mode of analysis in excised tissue or be used to guide surgery.[4]

2. Multimodal imaging agents – formation of and key example applications

2.1. PET or SPECT with MR

The combination of nuclear imaging techniques (PET or SPECT) with magnetic nanoparticles has been an active area of research over the last decade. The design of radioisotope/MR imaging agents is generally carried out by starting with a magnetic nanoparticle core and either attaching radioactive isotopes to the surface, or incorporating them into the core. Using magnetic nanoparticle contrast agents in MRI is relatively low sensitivity and whilst variable contrast can be seen in high uptake tissues, quantified biodistribution is not possible. The radiolabelling of MR contrast agents for PET (or SPECT) imaging can allow them to be assessed at tracer doses in 'phase 0' clinical trials, and to demonstrate that MRI is appropriate for routine clinical procedures at required higher doses of the agent. Nuclear imaging also allows the tumour targeting and drug delivery properties to be rapidly determined non-invasively and with minimal animal use.[5]

The simplest way to radiolabel a nanoparticle is *via* the direct interaction of the radioisotope with the surface of a pre-formed nanoconstruct. The term 'chelator free' generally refers to the use of radioactive metals that form stable interactions directly with the surface or core of nanoparticles. This methodology allows the direct labelling of constructs without significant alteration of surface properties, making it attractive for use when the parent nanoparticle has already been optimised for various applications, e.g. targeting, magnetism or drug delivery. For example, Voulgari *et al.* developed a PMAA-graft-PEG copolymer-derived iron oxide nanoparticle system for the delivery of cisplatin to tumours which showed improved therapeutic effect *in vivo* compared to free cisplatin, particularly in the presence of an external magnetic field.[6] Final step chelator-free gallium-68 radiolabelling and dynamic PET imaging indicated the increased potency in the presence of a magnetic field was likely to be caused by an increase in internalisation of the cisplatin-loaded carrier rather than a direct increase in nanoparticle (and therefore drug) concentration in the tumour. Chelator free methodologies can also be used to directly understand clinically approved nanoparticles. Ferumoxytol, an FDA approved nanoparticle, has been successfully radiolabelled with a range of radiometals by a heat-induced method to allow highly sensitive evaluation,[7, 8] although this method is only feasible when nanoconstructs are stable with respect to heat. Loudos and co-workers have used chelator-free methods for imaging of ^{99m}Tc labelled magnetic nanoparticles using a planar gamma camera,[9-11] comparing the biodistribution of cobalt ferrite and magnetite nanoparticles[9, 11] and quantifying the increase in tumour uptake with actively targeted nanoparticles.[10] Cheng *et al.* radiolabelled $FeSe_2$ doped Bi_2Se_3 nanosheets with copper-64 to form a stable system,[12] PET imaging was used to offer dynamic information on tumour accumulation over 24 hours. In a similar manner, Lee *et al.* used ^{99m}Tc radiolabelled SPIONs to quantify increased tumour localisation when using Lipiodol as a carrier.[13]

Radiometal isotopes for PET (or SPECT) can be attached on the nanoparticle surface using a chelator, either final-step or two-step. The majority of reported examples of final step chelator based radiolabelling use the combination of copper-64 and DOTA,[14, 15] likely based on the availability of chelator precursors rather than designed compatability.[16] An alternative method for forming chelator based radiolabelled nanoparticles is the initial radiolabelling of a bifunctional chelator (BFC, chelating agent with a reactive group) followed by conjugation of the radiometal complex to the nanoparticle. The advantage of this method is that the radiometal is stably bound to the chelate before attachment to the

nanoparticle, hence other surface coordination interactions are not possible. Chelate/metal stability can be assessed prior to conjugation and so only the stability of chelator attachment would need to be assessed for the formed conjugate with the nanoparticle. Louie and co-workers developed one of the first radiolabelled magnetic nanoparticle systems using this methodology.[17] Copper-64 radiolabelling of an amine functionalised DOTA derivative was performed before conjugation to a dextran sulfate nanoparticle. An alternative approach is to use a BFC which can bind to the nanoparticle core. Torres and co-workers have developed a bisphosphonate bifunctional system which, by modification of the chelating unit, can bind to either ^{99m}Tc for SPECT imaging or ^{64}Cu for PET imaging (see Figure 1).[18, 19] These units can then coordinate directly to the metal oxide core surface *via* strong bisphosphonate interactions with the iron atoms.[20]

In an interesting study, Wang *et al.* introduced multiple radioactive isotopes in the same construct at different positions to track the *in vivo* behaviour and metabolism of the nanoparticle.[21] ^{59}Fe was used to label the core, ^{111}In chelated to DTPA was added to the lipid (DMPE) coating along with ^{14}C-oleic acid as a stabiliser. *In vivo* biodistribution studies were used to see how the different components were processed. As expected,[22] the oleic acid used as a surfactant does not remain attached *in vivo* after only 10 minutes, whereas ^{59}Fe and ^{111}In largely correlate, assuming initially that the DTPA-DMPE remains attached to the nanoparticle. However, significant differences were noticed in kidney and robust control experiments (administration of ^{111}In-DTPA-DMPE alone) showed a similar biodistribution potentially caused by micelle formation, suggesting instability of chelator attachment. This type of multi-isotope study where different components can be separately tracked is complex to set up but can give useful and accurate information about the processing of nanoparticles *in vivo*.

2.2. Optical/MR

Optical imaging is often used for *in vitro* biological experiments as, compared to other imaging techniques, there are many practical advantages; simplicity of use with a live readout, open timeframe, availability of (and ability to design) a range of fluorophores with tuned properties, and lack of ionising radiation.[23] Limited light penetration prevents non-invasive deep tissue *in vivo* use, but it is clinically important in applications such as fluorescence guided surgery. Optical imaging of MR contrast agents overcomes the low sensitivity of MR imaging and allows the collection of real-time information during *in vitro* assessment, allowing for rapid screening and identification of key characteristics for iterative design. In addition, there is the potential for one agent to be used for non-invasive MR imaging to determine tumour localisation and characteristics, followed by intraoperative optical imaging to ensure complete tissue removal.

The first and most successful approach to the formation of optical/MR imaging agents is via conjugation of an organic dye on the surface of an iron oxide nanoparticle. This allows for retention of the core properties of the previously designed nanotechnologies with only limited chemical modification. Josephson and co-workers labelled cross-linked iron oxide nanoparticles (CLIOs) with a Cy5.5 dye for gliosarcoma imaging, allowing for both non-invasive and intraoperative imaging pre-clinically (see Figure 2).[24] Surface functionalisation of magnetic nanoparticles with organic dyes has limitations, often caused by low photostability. Increasing sensitivity and utility of the optical properties has been a focus of many studies, for example, by introducing additional multifunctionality to the system. This has recently been demonstrated using multifunctional gold and iron oxide composites which are conjugated with both a fluorescein organic dye and a europium(III) complex which could be targeted to the folate receptor and a calcium sensor.[25] This highly complex functionality allowed for high sensitivity cell counting, however, increased nanoparticle complexity significantly limits potential clinical translation. An alternate route to increased sensitivity is via surface encapsulation of the organic dyes instead of conjugation, leading to reduced photobleaching and the potential to increase dye loading content.[26-28] With the development of organic dye functionalised nanoparticles, there is also a significant recent move towards near exclusive use of near infra-red (NIR) dyes to allow for increased tissue penetration of the signal.[29]

An alternate strategy for the formation of optical/MR multimodal agents is through the use of quantum dots (QDs). Using QDs ensures high sensitivity fluorescence due to their high photostability, quantum yield and extinction coefficients. There are a range of methods for the formation of QD based optical/MR probes including using gadolinium(III) chelates and iron-oxide nanoparticles, amongst others.[30, 31] A recent interesting example describes the formation of a graphene QD which can be conjugated to iron oxide nanoparticles as MRI contrast agents, after which the nanoprobe can be both functionalised with folic acid for cancer targeting and loaded with doxorubicin for therapy.[32] This agent allowed *in situ* monitoring of cellular uptake and drug release via FRET.

The most recent major addition to the library of optical/MR imaging agents are based on upconversion nanoparticles (UCNPs) in which high energy visible light can be emitted when excited by NIR light. UCNPs have the advantage of significant stability, photobleaching resistance and very high sensitivity, with single particle imaging having been demonstrated. Significant materials chemistry research efforts have been focused on methodologies for the formation of UCNPs coupled with magnetic nanoparticles without disrupting the optical or magnetic properties respectively.[28, 33, 34] Additionally, UCNPs often contain lanthanide cores, which allow for direct replacement with MR responsive gadolinium(III) ions, without causing significant structural change.[35, 36]

2.3. PET or SPECT with Optical

The combination of optical with nuclear imaging allows for additional information to be gathered on a single construct. Nuclear imaging is used for high sensitivity data to give dynamic tracer information at short time-points, optical imaging allows for longitudinal imaging to assess biodistribution at further time points. A radioisotope is often added to an optical imaging nanoparticle to give high sensitivity biodistribution data in a similar manner to that which is done with MR agents. A key application for PET(SPECT)/optical imaging agents is for image guided surgery in which nuclear imaging is carried out to determine localisation and the optical imaging is used intraoperatively to mark diseased regions to aid in surgical removal.

Early examples of radiolabelling of inorganic nanoparticles, including optical imaging agents, focused on chelator based radiolabelling and surface modification, for example, Chen *et al*. labelled quantum dots (QDs) with ^{64}Cu using surface functionalised DOTA chelators.[37] However, more recently, focus has moved away from chelator based approaches, with the aim of imaging the inherent nanoparticle without having to make structural modifications which could affect its properties. Hu *et al.* and subsequently Sun *et al.* synthesised radiolabelled gold nanoclusters and QDs respectively by doping ^{64}Cu via a cation-exchange reaction in a chelator-free protocol.[38, 39] Introduction of the radioactive isotope also causes Cerenkov resonance energy transfer, providing self-illuminating nanoparticles and avoiding the usually required external excitation photon. Direct replacement of elements can also be carried out to form radiolabelled nanoparticles, for example by the introduction of ^{198}Au in to gold nanoparticles without modification of the chemical structure.[40] Radiolabelling has allowed for imaging biodistribution assessment of various shaped nanoparticles (nanospheres, nanodisks, nanorods, and cubic nanocages) to determine the optimal shape for tumour targeting.[41] Analogously, Zhou *et al*. radiolabelled CuS nanoparticles with ^{64}Cu to form the identical structure, forming a self-illuminating an optical/PET imaging agent which could be used for photothermal ablation therapy.[42]

The alternative approach for forming PET or SPECT with optical multimodal imaging agents is by dual modification on non-optical based nanoparticles. Lin *et al.* formed an optical/PET agent by surface attachment of chelator based ^{64}Cu and a Cy5.5 dye to ferritin nanocages.[43] Similarly, Blanco *et al.* developed a SapC-DOPS nanoparticle functionalised with an organic dye and radiolabelled with $^{124/127}$I.[44] PET imaging was used to quantify targeting to glioblastoma *in vivo*, with the optical dye potentially acting as a surgical marker to ensure all cancerous tissue is removed during invasive brain surgery.

2.4. Trimodal imaging agents

Imaging agents which combine an optical/MR agent with a radioactive isotope for PET or SPECT are often referred to as trimodal and are often designed due to their complementary features or to use nuclear imaging to understand previously designed agents.[45] For example, using a trimodal probe, a biological system can be rapidly screened using high throughput optical imaging, if an interesting system or disease model is selected for non-invasive imaging, it can be radiolabelled for dynamic high sensitive short-timeframe imaging, followed by longitudinal MR imaging. However, it must be noted that this increased complexity presents significant chemical design challenges and subsequent regulatory hurdles.

One of the simplest, and earliest, methods designed for the formation of trimodal imaging agents is decoration of the surface of iron oxide nanoparticles with both an organic dye and a radioactive isotope, often a using a radiometal /chelator approach. Xie et al. designed a system for PET/MRI/optical imaging which uses final step ^{64}Cu radiolabelling of DOTA and a cy5.5 dye functionalised iron oxide nanoparticles which have also been modified for active tumour targeting using human serum albumin (HSA).[46] Having a trimodal system allows for simple assessment of signal overlap to give an indication of material stability, *ex vivo* organ analysis showed little correlation between near infrared fluorescence (NIRF) imaging and the PET image and whilst this was attributed to the high background of NIRF imaging, the data is also consistent with radiolabel chelation instability (which was not assessed in the study). By changing any single parameter, magnetic core, dye, radioisotope, or active targeting agent, an entirely different system can be designed with similar target properties. For example, Hwang *et al.* used a nucleolin targeted cobalt ferrite nanoparticle which was radiolabelled *via* NOTA based ^{67}Ga chemistry for SPECT imaging and conjugated with rhodamine for optical imaging (see Figure 3).[47] One conclusion from the studies is that, from a simplicity perspective, when radiolabelling, a chelator-free approach is preferred, as, for example, demonstrated by Stelter *et al.* in their formation of trimodal agents for both PET and SPECT with ^{68}Ga and ^{111}In respectively.[48]

Alternatively, the nanoparticle core can be replaced with an UCNP derivative from which a trimodal agent can be built. Lee *et al.* designed an erbium(III) and ytterbium(III) doped $NaGdF_4$ UCNP which could be both radiolabelled with ^{124}I for PET imaging and also integrin targeted using surface RGD peptides for angiogenesis imaging.[49] In this study, the PET imaging was used to quantify tumour uptake and were therefore able to demonstrate that selectivity *via* blocking. It is also possible to design a trimodal imaging agent in which the core serves no imaging function, with imaging agents grafted on the surface. Li *et al.* designed a trimodal theranostic liposome system in which both gadolinium(III) DOTA agents and a NIR dye were attached on the surface, whilst ^{99m}Tc for SPECT imaging was internally encapsulated along with doxorubicin, alternatively, a PET imaging version could be produced using surface conjugated ^{64}Cu DOTA based radiolabelling.[50] Multimodal *in vivo* imaging studies demonstrated the potential of this system in squamous cell carcinoma imaging of head and neck (SCCHN) tumour xenografts, however, this was only achieved via *intra tumoural* administration of the imaging agent in the reported study.

3. Translation to clinical trials

Despite slow progress,[51, 52] the parameters for successful translation to clinical trials are now becoming better understood for nanoparticle constructs. Unsurprisingly the most rapidly translated constructs are either modifications of nanoparticles that are in current clinical use (or have previously had approval) or encapsulated formulations of known therapeutic drug molecules.[53] The latter category, which includes a variety of drug delivery agents, many of them passively targeted, in liposomal formulations. Doxil (a doxorubicin delivery agent) was approved in 1995, and more recently Onivyde (an irinotecan delivery agent) was approved in 2015. This is the main area of clinical trial activity. However, inorganic and multimodal nanoparticles are coming to the fore in some recent advances.[54]

3.1 Recent and ongoing clinical trials

In terms of inorganic nanoparticles, the iron oxide nanoparticles have the greatest wealth of clinical data. A key benefit for this approach is the well characterised toxicological profiles and the compatibility of iron oxides with metabolic processing due to the requirement for iron in the human body.[55] These agents have long established safety profiles going back over 20 years, with uses as iron replacement therapies, and hence toxicological issues and potential complications are well understood. The iron oxide nanoparticles (Endorem and Resovist), that were used as T_2 contrast agents did not enter into widespread routine use, leading to them being discontinued as products. Ferumoxytol was originally approved for the treatment of iron deficiency but has subsequently been used in cancer imaging studies (MR T_2 contrast agent) and is now being investigated as a multimodal imaging agent through a variety of studies to radiolabel the construct.

As well as MR/PET advances (many of which are linked to the increasing availability of PET MRI integrated clinical scanners) there are also many developments in the combination of optical imaging with radioisotopes. The Cornell Dots are the most obvious success in this area of research, in terms of translation to clinical trials.[56] The initial study showed no toxic or adverse events, prompting an expansion of this work in 2014 to the currently ongoing clinical trials. These agents combine the use of targeting peptides, radioisotopes and an optical dye (cyanine 5 dye) into a silica nanoparticle. There are many lessons to be learnt by examining the initial research approach that the researchers followed and the modifications that were made to ensure translational work was viable. A key issue was the modification of the size of the particles- the group started off with particles in the 30-50 nm range but later reduced this to the 5-7 nm particle size range to modify the excretion and tissue retention profile, ensuring both safety and effective targeting. The iodine-124 isotope was used as a long-lived PET emitter (although as the decay is only 25.6% positron emission, future protocols are more likely to utilise the now widely available zirconium-89 isotope), see Figure 4. These compounds are not quantum dots which have raised concerns in the past with the toxic materials, such as cadmium, that were included as components in the first generation of these materials.[4] Although quantum dots have high stability, it is an unnecessary risk for clinical use, hence less toxic elements such as zinc are now being used in the next generation QDs to allow the excellent optical properties to be exploited.[57]

An important trial that is ongoing is the AGUiX trial for radiosensitisation using a silica nanoparticle (ca. 5 nm diameter) with chelated gadolinium on the surface using DOTA chelators that offer contrast in MR imaging studies.[58] The developmental process is an interesting one to follow as it is an excellent example of the tools that are now becoming more recognised in the approval process for nanomedicines, with multimodal imaging a key component. Clinical trials started in mid-2016 for applications in radiosensitisation of multiple brain metastases.[59-61] The Tillement group and their collaborators have been working over the past five years to develop radiolabelled derivatives of this construct, both to provide data on the constructs for the trials and to further develop applications such as tagging large biomolecules with the ultrasmall nanoparticles for multimodal imaging.[62, 63] There are two key criteria that must be considered when utilising a radiosensitiser; what is the timescale for optimal localisation/ tissue concentration and how should the radiation beam be applied (clinical beam parameters and dose administered).[59] Investigation of these parameters is possible using zirconium-89 labelled constructs.[63]

In terms of other clinical trials, thermal ablation (hyperthermia) has also been a key development area and may point to some of the other types of particles that may be repurposed for imaging and targeting. The use of silica/ gold nanoparticles (Aurolase) in lung tumours and iron oxide nanoparticles (Magnablate) in prostate tumours are examples of this application.[55] As clinical trial processes can be started many years in advance, we do not always see rapid response, but the trends to small actively targeted particles that can be applied vascularised tumours are likely to be observed in future developments with the concurrent use of MR / radionuclide imaging as key components in the developmental pathway.

3.2 Factors to consider for clinical translation

Although nanoparticles and nanotechnology have caught the public interest, both in fact and in fiction, there are valid concerns about their use which need to be considered.[64] Regulatory and public bodies are particularly concerned about the toxicity issues, that could cause some different problems to small molecules and biologic as drugs, and the impact on the environment of such formulations.[65] Any planned clinical translational project will need to mitigate these issues and provide the data required to ensure both that the concerns of public bodies are fully addressed but also that the system has been appropriately optimised in the preclinical setting.[66] At this stage the particle size effects and biodistribution can be determined using imaging techniques. It is also becoming much clearer to regulators what they should be looking for in a data set. Imaging is the key technology that will facilitate rapid translation by providing much of the ADME data in animal models and informing early phase (“phase 0”) trials It is also worth considering that there are challenges in the production and manufacturing of nanomedicines[51], including the subtle effects of physical conditions on the variability of the particle properties and there are few facilities appropriately tooled for the production of such materials in bulk to GMP standards.

The low mass modifications from radiolabelling approaches are appealing and give the required sensitivity of detection. The key reasons why this is attractive are:

(1) The particles can be treated as essentially the same construct with a non-disruptive radiolabel for tracking

(2) Tracer level studies can reduce safety concerns and subsequently be used facilitate patient selection for trials.
(3) Assessment of PK and ADME parameters is possible.
(4) Dosing level can be determined (and time to treatment for radiotherapies)

There are other opportunities including low cost fast fail screening of constructs in small scale human trials and a move away from rodent models to either large animal studies (e.g. pigs) with fewer animals required or potentially the use in veterinary clinical trials in companion animals.[67-69] Overall, it is clear that accurate and effective *in vivo* tracking of nanoconstructs using radionuclide labelling can be a facilitating technique for progression to clinical trials, and this is even more effective when combined with optical imaging techniques for *ex vivo* tissue analysis and MR for longitudinal imaging.[5, 70]

4. New applications and developments

The predicted explosion in the use of nanoparticles for clinical imaging and therapy has not yet occurred. This is mainly due to the unanticipated challenges in terms of optimisation of the systems. In retrospect is seems obvious that a more complex system should take a longer time to have impact, particularly in a crowded preclinical scientific development space.[55, 71] The combination of novel targeting approaches and therapy is compelling and has now been implemented in approaches that have transitioned to trials in man.

A lot of the information to inform the best methodology for clinical development is now available in the literature (although not always considered in as much detail by researchers as it should be). The message is clear for any particles that require circulation and active targeting; a size less than 10 nm with a renal clearance pathway is the approach that has been validated. Particles that localise passively by enhanced permeability and retention (EPR), target the liver/lungs or are delivered by direct injection will have larger optimal size profiles and different shapes. Also, an under considered issues is the formation of protein corona on the surface of the nanoparticles, this influences the circulation time and the overall *in vivo* properties (and can vary between species/ particle types).

The progression to clinical trials of modified Ferumoxytol, gold nanoparticle constructs, the silica particles (such as the Cornell dots) and the multi-Gd AGuIX systems shows the pathways that can be adopted effectively. Applications, beyond drug delivery with liposomal and micellar formulations, that are most active in current clinical trials are hyperthermia (thermal ablation), optically guided surgery and radiation sensitisation.[72-74] The approach of validating delivery using nuclear medicine techniques is very useful and may become a prerequisite for trial approval in the future. A good example of how this can work is seen in the chelator free labelling methods for radiolabelling of Ferumoxytol with a variety of radionuclides.[7] Ferumoxytol is an example that shows minimal disruption of the construct on addition of the radiolabel and hence a potentially rapid pathway to approval for trials and it may have applications in tumour imaging.[75, 76] Some of the most exciting new opportunities are in optically or radiation guided surgical interventions. This allows

excision of the maximal amount of tumour tissue and minimises removal of healthy tissue to ensure the best outcome. Radiation sensitisation is a key area for development with the gadolinium and the gold nanoparticles (as already discussed). There are also opportunities to use such labelling strategies to validate novel administration methods for drugs such as inhalation or direct injection which can be validated, characterised and optimised using this strategy.[77, 78]

5. Future perspective

It has been stated that nanotechnology has underperformed in its translation from laboratory bench to clinical impact. As with many scientific advances, the discovery is only a first step along the route to understanding and optimising these materials for the applications. Nanomaterials have inherent complicating factors in comparison to small molecule drugs which have to be understood and analysed throughout the development process.

The selected research discussed herein shows that understanding has significantly increased of the impact of particle size/shape, coating and functionalisation on the material properties. Also the analysis methods *in vivo* are now more clearly defined to allow better selection/ optimisation process for the nanoconstructs. Multimodal imaging provides the information that underpins this and sits centrally to the process of translating nanoparticles into a variety of clinical applications from diagnostic imaging to therapeutic drug delivery.

It is likely that further imaging combinations will be developed and a greater degree of multi-functionality will be introduced for new nanoparticles in the future. Even if the imaging aspects are only used for validation at an early developmental stage, rather than in the medical use of the final construct, they are of high value to justify progression. The additional information offered from the combination of imaging techniques could also be used at the clinical stage in patient stratification or even therapy response introducing greater flexibility in the development pathway, and minimal alteration of the nanoconstructs to repurpose them.

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Table 1 – Comparison of the key characteristics of the most common imaging techniques used in multi-modal imaging.

Modality	**Energy measured**	**Spatial Resolution (mm)**	**Relative Sensitivity**	**Preclinical Use**	**Clinical Use**	**Advantages**	**Limitations**
PET	γ-rays	1-2 (pre-clinical) 6-10 (clinical)	Excellent	Yes	Yes	High sensitivity Quantitative Dynamic	Cost Availability
SPECT	γ-rays	0.5-2 (pre-clinical) 7-15 (clinical)	Good	Yes	Yes	Established clinical facilities Good sensitivity	Targeted imaging tracer availability Semi-quantitative
MRI	Radio waves	0.01-0.1 (pre-clinical) 0.5-1.5 (clinical)	Poor	Yes	Yes	Established clinical facilities High spatial resolution	Targeted imaging tracer availability Low sensitivity
Optical	Visible to infrared light	1-5	Good	Yes	No	High throughput Low cost	Limited tissue penetration

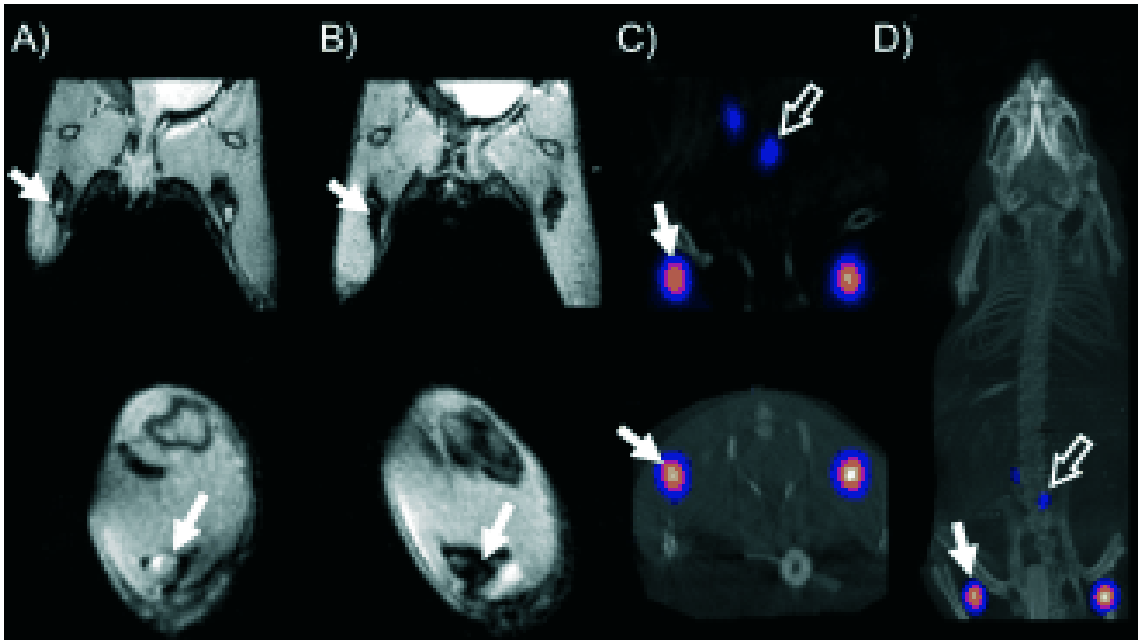

Figure 1 – PET/MR images of a copper-64 radiolabelled iron oxide nanoparticle demonstrating co-registration of lymph node uptake.[18]

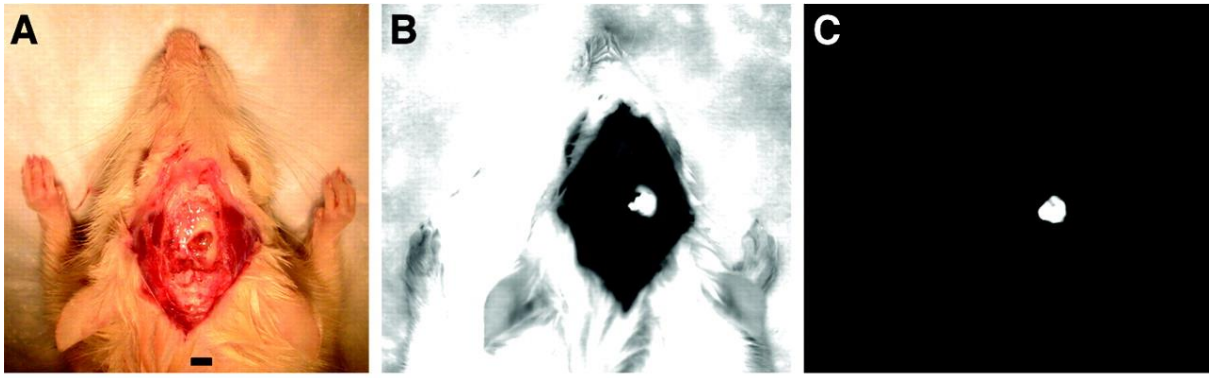

Figure 2 – Uptake of Cy5.5 tagged optical/MR multimodal nanoparticles in GFP-expressing 9L glioma. (A) White light image, (B) GFP channel and (C) Cy5.5 channel.[24]

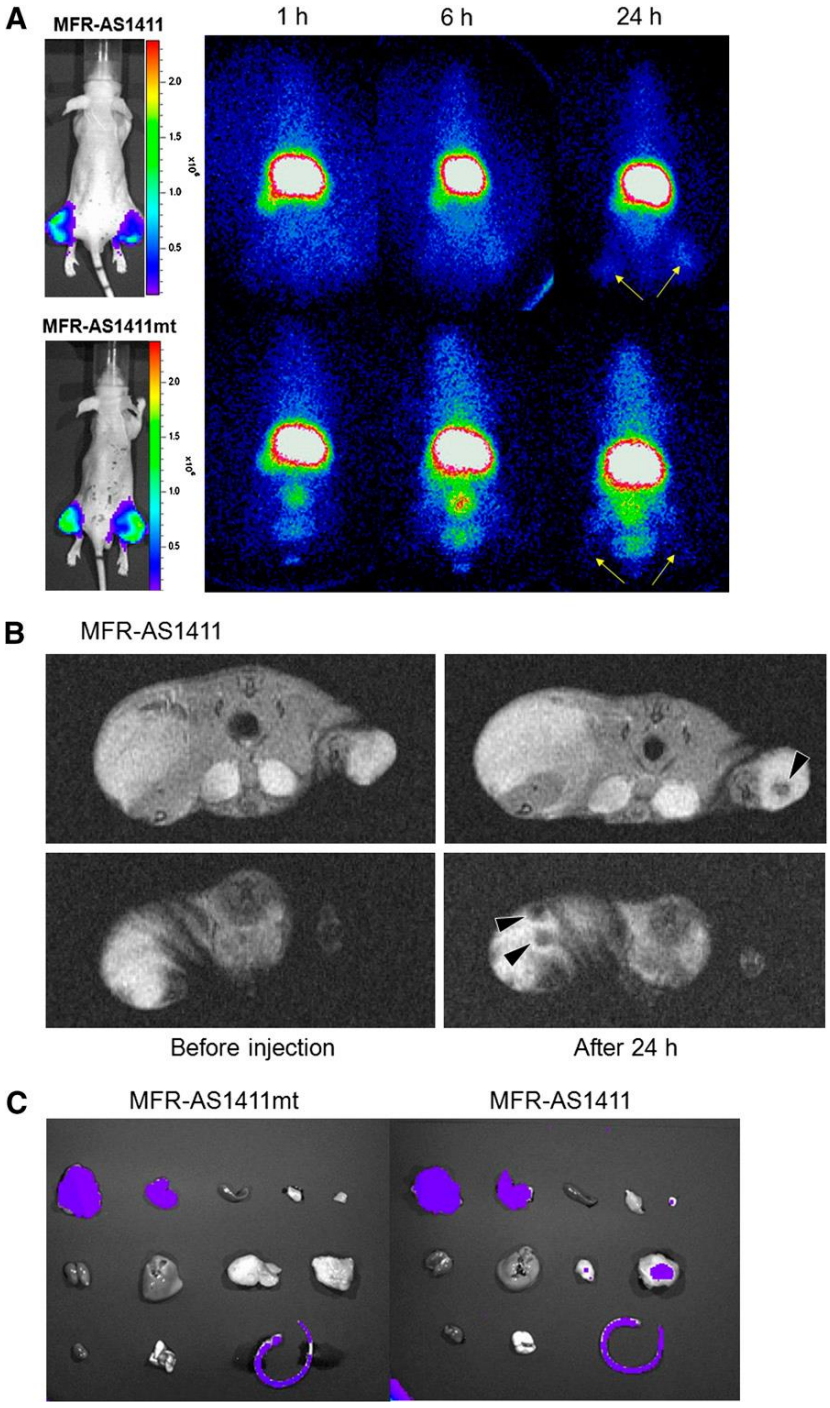

Figure 3 – Multimodal *in vivo* and *ex vivo* imaging using ^{67}Ga labelled cancer targeting cobalt ferrite nanoparticle. (A) SPECT, (B) MR and (C) optical imaging.[47]

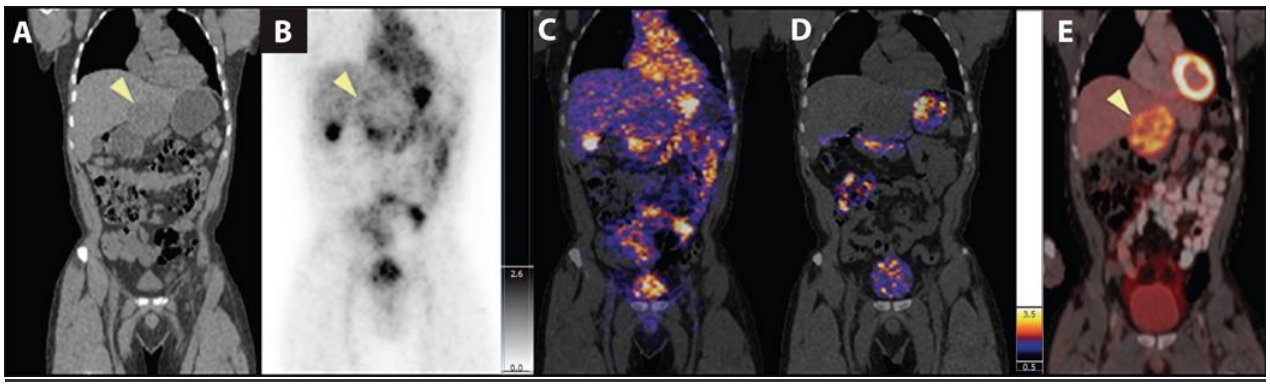

Figure 4 – PET imaging of particle distribution and tumour uptake after systemic injection of the ^{124}I-cRGDY–PEG–C dots (A) CT scan with the metastasised tumour indicated by the arrow (B) PET image 4 hours after injection showing the localisation of the particles (including at the tumour margin) (C) co-registered PET-CT at 4 hours (D) co-registered PET-CT at 24 hours (E) ^{18}F-FDG PET-CT image to show the metastasised tumour.[56]