Gadolinium theranostics for the diagnosis and treatment of cancer

Amy G. Robertson and Louis M. Rendina

According to the World Health Organization (WHO), there were 18.1 million new cancer cases and 9.6 million cancer deaths reported worldwide in 2018. These numbers are expected to rise over the next decade, and the development of new and effective cancer treatments and diagnostic tools is urgently required, particularly for aggressive and intractable malignant cancers such as those of the brain. An exciting field of cancer research involves combining therapeutic and diagnostic tools into a single ‘theranostic’ platform. The role of theranostics in the personalized management of oncology patients is increasing, as is the demand for new types of theranostic agents. Some of the most promising cancer theranostics exploit the lanthanoid metal gadolinium, an element possessing favourable therapeutic and imaging properties.

Key learning points
(1) Gadolinium has long been used in medicine as a contrast agent in MRI but its therapeutic applications are less well-developed.
(2) Gadolinium theranostics have already made an important impact in cancer medicine, with selected Gd agents entering clinical trials.
(3) Gadolinium theranostics can be categorized into two distinctive categories: molecular theranostics and nanotheranostics (nanoparticles).
(4) A variety of tumour-targeting strategies have been employed for gadolinium theranostics in order to maximize their clinical benefit.
(5) Gadolinium theranostics potentially offer a viable option in the diagnosis and treatment of aggressive and intractable malignant cancers such as those of the brain.

1. Introduction

According to the World Health Organization (WHO), there were 18.1 million new cases of cancer and 9.6 million deaths reported worldwide in 2018. These figures are expected to rise in the next decade owing to several factors including increased population growth and an increasingly aging population, as well as increases in the prevalence of those cancers that are linked to socioeconomic factors. With this increase in cancer cases, new and effective treatment and diagnostic tools will be required. One exciting route involves a combination of therapeutic and diagnostic agents into a single ‘theranostic’ platform. The origin of the term ‘theranostic’ has largely been attributed to John Funkhouser, president and CEO of PharmaNetics, Inc. However, the origin of the term was used more along the lines of personalized medicine whereby disease diagnosis and the application of appropriate therapies are closely linked rather than constituting part of a single (nano)molecular agent. One of the first successful examples of a cancer theranostic used in the clinic was trastuzumab (Herceptin®), a monoclonal antibody that targets malignant breast cancer that specifically overexpresses the HER-2 (human epidermal growth factor receptor 2) protein, and is found in approximately 15–30% of all breast cancers. This treatment was coupled with the HercepTest® immunohistochemical assay which assessed the degree of HER-2 protein overexpression. This combination of diagnostic and therapeutic tools led to a remarkable 37% increase in the overall survival rate of patients when compared to the use of chemotherapy alone.

In subsequent years, the term ‘theranostic’ has evolved to mean a single agent in which the diagnostic and therapeutic tools are combined. By combining both the diagnostic and therapeutic aspects, it is possible to reduce both the number of agents administered to patients, their dose, and the number of invasive treatments they must undergo. One important example in theranostic development involves the lanthanoid metal gadolinium (Gd). Gd theranostics can be categorized into two distinctive categories: molecular theranostics and nanotheranostics (nanoparticles). Each category possesses its own unique advantages and disadvantages. Molecular theranostics...
tend to be small in terms of overall size and molecular weight. Many such entities can be classed as cytotoxic agents, whereby a known cancer chemotherapeutic is linked to a diagnostic Gd agent. Alternatively, binary molecular agents are largely non-toxic and can circumvent some toxicity issues associated with cytotoxic agents as the therapeutic effect is initiated by an external radiation (X-ray or thermal neutron) source, rather than via a specific cytotoxic interaction with a key cancer protein or DNA.

Provided molecular theranostics are sufficiently lipophilic, they can readily pass through cancer cell membranes. Their application as theranostics can sometimes be limited when a compound is too hydrophilic with only limited membrane permeability and thus rapid excretion from the host is observed when these agents are administered in vivo. These compounds can also often show non-specific biodistribution which can lead to adverse side-effects. Nanoparticles on the other hand are taken up by tumours and can carry hydrophilic payloads. However, when these entities enter a tumour cell via an endocytic pathway they are often trapped within endosomes which results in their eventual degradation in lysosomes which can limit their therapeutic effectiveness as many chemotherapeutics require effective delivery to specific sub-cellular components leading to highly-selective cancer cell death. In this review, there will be a focus on the use each of the two approaches outlined above in the design and development of Gd theranostics.

2. Small molecule theranostics

The majority of existing cancer chemotherapeutics, including those currently undergoing US Food and Drug Administration (FDA) approval, are small molecules. There is an average of 30–35 new molecular entities approved by the FDA each year and ca. 90% of existing therapeutics are small molecules. As such, there is already a variety of cancer therapeutics that can be further functionalized to include a diagnostic component, or which already contain a diagnostic component but have not been considered for experimental therapeutic applications such as gadolinium neutron capture therapy (GdNCT). When looking into the design of new small molecule theranostics, one common approach is to have the therapeutic component covalently linked to the diagnostic component. These compounds can be cytotoxic in their own right or can act as pro-drugs whereby the therapeutic component is cleaved from the theranostic agent and/or released upon entry into cancer cells. As outlined above, these small molecular compounds have certain limitations in that their hydrophilicity and lipophilicity must be carefully managed. This often means that small molecule theranostics will need to exploit active transport mechanisms to increase tumour cell uptake. These transport mechanisms can include targeting those receptors which are overexpressed by many types of cancer cells, or exploiting the morphological differences typically found in tumours (e.g. leaky vasculature or high cell proliferation rates). Improved tumour uptake can also be achieved by using endogenous biological molecules which are usually taken up by normal, healthy cells to actively transport the theranostic agent into the cancer cell.

2.1 Platinum anti-cancer drugs

Cisplatin and related platinum-based chemotherapeutics are some of the most effective chemotherapeutics used in the treatment of certain solid cancers such as those of the genitourinary region and head and neck. Over the past few decades, cisplatin has been incorporated in a range of different therapeutics in order to increase its efficacy and also reduce its cytotoxicity. Most of the work in the development of hybrid Gd–Pt theranostic agents has involved the incorporation of the platinum agent into micelles or other types of nanoparticles.

Amy Robertson obtained her Bachelor of Science (Hons I) from the University of Sydney (Australia) in 2016. She is currently a doctoral candidate at the University of Sydney (Australia) under the supervision of Prof. Louis M. Rendina. Her research interests involve the use of fluorescent delocalised lipophilic cations as targeting agents for multimodal theranostic agents in the diagnosis and treatment of intractable brain tumours such as glioblastoma multiforme.

Prof. Louis M. Rendina FRSC FRACI C. Chem. leads the Synthesis and Inorganic Drug Discovery group in the School of Chemistry at The University of Sydney, Australia (@GroupRendina). His research is primarily focused on boron and gadolinium, with a strong interest in the application of their compounds in drug discovery and cancer theranostics, respectively. Prof. Rendina is the recipient of several research awards and international fellowships, including two national awards from the Royal Australian Chemical Institute (RACI) for his original contributions to the areas of Medicinal Chemistry and Organometallic Chemistry, the only individual to have ever received both awards.
The rationale here is that platinum drugs are very cytotoxic and incorporation into these larger systems is known to reduce their host toxicity. Crossley et al.\textsuperscript{7} reported a Gd–Pt agent whereby Gd–DTPA (DTPA = diethylenetriaminepentaacetic acid) was functionalized with two Pt\textsuperscript{II}(terpy) (terpy = 2,2':6',2''-terpyridine) units (1, Fig. 1). This compound was shown to target the nuclei of lung tumour cells and functioned as a potent DNA intercalator, with high uptake of both the Pt and Gd within the tumour cell nuclei, as determined by highly-sensitive synchrotron X-ray fluorescence techniques. This example was the first to unequivocally demonstrate the delivery of Gd to a tumour cell nucleus. As the elemental localization maps of Pt and Gd were the found to be almost identical, this result confirmed that the complex remained intact \textit{in vitro}. However, this Gd–Pt complex was found to be quite cytotoxic towards both normal and tumour cells which made it unsuitable for use as a potential theranostic agent. Following on from this work, Fenton et al.\textsuperscript{8} sought to address the issues with the ‘first generation’ Gd–Pt complexes by the use of only a single Pt\textsuperscript{II}(terpy) unit which decreased the overall charge of the complex from +2 to +1 and incorporated a macrocyclic DOTA ligand instead of the acyclic DTPA for Gd\textsuperscript{III} complexation in order to minimise the loss of highly toxic Gd\textsuperscript{3+} ions into solution (2, Fig. 1). This complex was found to possess a significantly reduced cytotoxicity when compared to the di-Pt\textsuperscript{II} complex discussed above while still maintaining excellent DNA targeting ability. Zhu et al.\textsuperscript{9} also employed this strategy in order to synthesize Gd–Pt agents as a way to identify and quantify Pt metabolism in cisplatin-like anticancer drugs (3, Fig. 1). In these complexes, the Pt\textsuperscript{II} complexes were linked to the Gd\textsuperscript{III} centre by means of pyridin-2-ylmethanamine and pyridin-2-ylenamine ligands. These Gd–Pt complexes were found to undergo partial ligand substitution \textit{in vitro}, leading to the formation of a mixture of active Pt therapeutics, residual Gd complexes and unmodified drugs. This combination of features meant that the complexes showed a similar cytotoxicity profile to cisplatin at MR-suitable concentrations, with the advantage that the Gd component had a longer retention time and could thus be tracked by means of MRI methods.

### 2.2 Pro-drugs

Another approach for the generation of Gd theranostic agents involves the use of pro-drugs. With these compounds, the pro-drug is only activated once it reaches the desired location within the tumour. This can usually be achieved by means of an acid-labile covalent bond, which is cleaved upon entry to the acidic environment within the tumour. The main advantage of these agents over other types of molecular theranostics is that, with appropriate design, it is possible to control several aspects of the drug delivery process. These factors include, but are not limited to, coupling the cytotoxic component to either a tumour-targeting moiety or an imaging component, or synthesizing a fluorescent ‘on/off’ system whereby, for example, the theranostic becomes fluorescent once it is released from the targeting moiety.

Yang et al.\textsuperscript{10} reported a pro-drug containing Gd–DOTA (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), biotin and camptothecin (CTP) (4, Fig. 2). The active CTP drug was linked to the scaffold by means of a labile disulfide bond which was cleaved selectively within the reducing environment of cancer cells. This compound also had the added advantage that CTP is non-fluorescent when linked to the Gd–DOTA moiety but becomes fluorescent upon its cleavage and release. This important feature allows for confocal imaging to track the release of the active drug \textit{in vitro}, and the MRI component of the Gd–DOTA may be used to track the drug \textit{in vivo}. The authors reported that the pro-drug was selective for cancer cells as it was able to effectively target the biotin-receptor which is overexpressed by many types of cancers. The CTP was also selectively cleaved in the reducing environment of the cells which led to a considerable improvement in tumour treatment \textit{in vivo}, with only minimal tumour growth found in an animal model after 25 days.

### 2.3 Binary agents

Binary agents have the potential to completely transform Gd theranostics. These agents contain two relatively innocuous
paramagnetism, all Gd(III) complexes developed for potential use in MRI contrast agents owing to its high photon, processes accompany the expulsion of a prompt gamma photon with an energy of approximately 7.94 MeV. A number of parallel excited and short-lived $^{158}$Gd nucleus which relaxes to a stable $^/C_2$ any naturally occurring element (2.57 barns). When $^{157}$Gd possesses the highest neutron capture cross-section of any naturally occurring element ($2.57 \times 10^5$ barns). When $^{157}$Gd captures a low energy (thermal) neutron it forms the excited and short-lived $^{158*}$Gd nucleus which relaxes to a stable $^{158}$Gd nucleus by releasing a prompt gamma photon with an energy of approximately 7.94 MeV. A number of parallel processes accompany the expulsion of a prompt gamma photon, e.g. the emission of Auger Coster-Kronig (ACK) electrons with very low energies. ACK electrons can only travel a very short distance (ca. 12.5 nm), and thus cause highly localized cell death due to their highly ionizing properties and limited path-length.\textsuperscript{11,12}

Photon activation therapy (PAT) shares many parallels with NCT, however, any high-Z element can in principle be used in this particular cancer therapy as it is not a nuclear process restricted by its neutron capture cross-section or, indeed, the nature of the isotope. In PAT, a photoemissive element is irradiated with an X-ray beam that is tuned to a higher energy than the binding energy of the inner-most electrons ($K$-edge). This process ejects the inner-core electrons, creating an orbital vacancy or ‘hole’. The subsequent filling of this vacancy by higher-energy electrons results in the release of energy along with the emission of ACK electrons which causes the cytotoxic effect, as outlined above for NCT.\textsuperscript{13} A subset of PAT known as synchrotron stereotactic radiotherapy (SSR) is where the X-ray photons are produced by a synchrotron source. The patient is then rotated about the tumour site in order to increase the relative radiation dose.

In photodynamic therapy (PDT), a photosensitizer is incorporated into the Gd complex. This photosensitizer is typically a rigid, highly-conjugated ring system which can absorb light of a given wavelength, leading to excitation involving several excited states, although the medically-relevant one is generally a long-lived triplet excited state. The triplet state can then directly initiate photochemical reactions which lead to the production of free radicals. Energy can be transferred from the excited photosensitizer to ground-state triplet O$_2$ molecules, resulting in the formation of highly-reactive singlet oxygen ($^1O_2$) and other reactive oxygen species (ROS). These ROS can effect efficient radical bond scission and/or oxidation reactions in sub-cellular components such as proteins and nucleic acids which can ultimately lead to cell death.\textsuperscript{14,15}

2.3.1 Porphyrins. Porphyrins have attracted a large amount of interest in recent years due to their versatility in therapeutic applications. Their rigid conjugated system allows for the absorption of light which is then transferred to dioxygen to produce ROS which have many useful therapeutic and diagnostic applications regardless of the metal ion that is chelated. These ROS are highly cytotoxic and are the main cytotoxic products of PDT. Porphyrins can also convert this absorbed energy into molecular vibrations. When this occurs, a photothermal therapeutic (PTT) effect occurs which leads to cellular hyperthermia, a condition that adversely affects cancer cells.\textsuperscript{16} PTT has some advantages over PDT in that it does not require oxygen and will thus can still remain effective in hypoxic tumour environments. Porphyrins are not only used as therapeutics but also have an equally large diagnostic range. The process of PDT can trigger thermoelastic expansion, which converts to molecular vibrations and can be detected as sound in the diagnostic technique known as photoacoustic imaging (PAI). As mentioned earlier, the absorption of light by PDT agents results in the excitation of atoms to multiple excited states. Whilst the triplet state is primarily responsible for cytotoxicity, there are also singlet states formed which can

![Fig. 2](image-url) A Gd(II)–DOTA–CTP based pro-drug.
radiatively decay and undergo near-infrared fluorescence (NIRF) which is very useful for biological imaging. Both of these imaging techniques, whilst useful, are limited by low tissue penetration of the incident light. This problem is easily circumvented in porphyrins by the incorporation of a metal ion that can be used in deep tissue imaging modalities. Examples include $^{64}$Cu for PET imaging, $^{99m}$Tc for SPECT imaging and Gd(III) for MRI. Despite the many clinical advantages in the use of porphyrins, these entities are largely limited by poor aqueous solubility and their tendency to form non-covalent aggregates in aqueous solution which limits their bioavailability. They also have limited tumour selectivity which can lead to high phototoxicity in the skin of patients. The most well-established example of a Gd(III)–porphyrin theranostic is Motexafin-Gd (MGo, 5). First developed by Sessler et al. and Pharmacyclics, Motexafin is based on a texaphyrin macrocycle, which is a class of porphyrins that contains five nitrogen donor atoms rather than the four found in a typical porphyrin. This expanded macrocycle allows larger lanthanoid ions, such as Gd(III), to coordinate strongly to the texaphyrin ring. Motexafin initially showed great promise as a radiosensitizer and proceeded to Phase II clinical trials, where a dosage of 10 mg kg$^{-1}$ was well tolerated, but it was ultimately rejected by the FDA in 2007. Some work, however, has been subsequently done toward utilizing Motexafin as a carrier for selected chemotherapeutic agents. This research has largely involved the anticancer drug doxorubicin, a frontline chemotherapeutic that is also highly fluorescent, and platinum-based chemotherapeutics such as cisplatin in order to decrease non-specific cytotoxicity. One of the initial attempts was performed by the Sessler group which led to the synthesis of two generations of cancer chemotherapeutics. The first generation compounds involved the functionalization of Motexafin with existing platinum(II)-based chemotherapeutics (Fig. 3), namely, cisplatin and oxaliplatin (6). Whilst these compounds showed some promise and were found to result in an increase in platinum localization within cancer cells, they did not overcome all of the platinum resistance that is well-established for cancer cells, and also showed instability in aqueous environments which led to the loss of Pt(II) ions. To combat these issues, second-generation complexes were synthesized. These complexes consisted of a Pt(IV) center rather than the Pt(II) center of the previous generation. Pt(IV) compounds have been shown to be somewhat unstable when exposed to ambient light leading to a reduction of the metal center and the release of Pt(II), allowing these researchers to design a compound in which the release of the metal ion could be regulated. These complexes also showed good cytotoxicity towards both cancer cells and cisplatin-resistant cell lines, with all compounds displaying a lower cell resistance than cisplatin alone.

Lee et al. synthesized a number of complexes based on a Motexafin unit that is functionalized with doxorubicin by
means of a series of labile bonds. In the first of these examples, two units of doxorubicin were connected to the texaphyrin core via a hydrazone linker as it was expected that in the acidic environment of a tumour, the acid-labile imine bond would readily cleave. This compound showed enhanced selectivity for cancer cell lines over healthy controls. It was also found to successfully release doxorubicin in the acidic lysosomes which caused enhanced cell death. In the second of these compounds, Motexafin was conjugated to doxorubicin by means of a labile disulfide bond which was designed to be cleaved by reducing agents such as glutathione (GSH) which is upregulated in cancer cells. The conjugate was administered to animals using a folate receptor-targeting liposome to further increase tumour uptake and selectivity. This compound showed excellent tumour selectivity, enhanced imaging (both MR and fluorescence) and it also possessed antiproliferative properties.

While the texaphyrins allow the coordination of a large Gd(III) ion to form a macrocyclic complex with high stability, it is still possible to use conventional porphyrins for the complexation of this lanthanoid ion. However, there is evidence that the stability of these complexes is limited and can lead to the loss of Gd^{3+} ions in solution. This is not ideal as the loss of Gd^{3+} ions from the complex not only reduces its overall effectiveness as a MRI agent, but free Gd^{3+} ions are highly toxic particularly towards patients with renal failure. Complex stability can be improved by functionalization of the porphyrin side chains. Recently, Yuzhakova et al. synthesized two Gd(III)–porphyrin chelates with different side chains. Both complexes showed improved MRI relaxivity when compared with the standard MRI contrast agent Magnevist\(^{16}\). However, only one of the compounds (GdPz2) showed significant potential as a PDT agent, indicating that the functionalization of the porphyrins has a significant effect on the PDT properties of the compound.

Another method of increasing both the aqueous solubility and also the cytotoxicity of porphyrins is to functionalize the macrocycle with a known MRI contrast agent such as Gd(III)–DOTA, rather than using the porphyrin itself as the MRI contrast agent. This strategy not only increases the aqueous solubility of the porphyrins, as the MRI contrast agents are themselves highly water-soluble, but it also helps to improve their stability as these chelated macrocycles are generally more stable than their parent porphyrins. Gros et al.\(^{21}\) synthesized one of the first examples of these types of complexes, i.e. a Gd(III)–DO3A functionalized porphyrin chelated with Cu(II). This complex showed improved relaxivity over free Gd(III)–DOTA, most likely due to the large size of the molecule which reduced its tumbling rate in solution, although no further work was done toward investigating its biological activity. In principle, this compound could be used as a combined MRI and PET agent (due to the possible inclusion of the positron-emitting \(^{64}\)Cu isotope) with the possibility of further application in PDT.

One of the limitations involving many porphyrin-PDT agents is that they rely on visible light, with poor tissue penetration, in order to produce ROS. One recent solution to this problem is to exploit two-photon PDT. This process utilizes compounds that are capable of absorbing two photons with approximately half the energy of those used in conventional PDT. This allows for near-IR light to be used which has greater tissue penetration. Schmitt et al.\(^{22}\) synthesized a water soluble, two-photon photosensitizer conjugated with Gd(III)–DOTA, and this complex showed good relaxivity and phototoxicity using both single and two-photon irradiation. The ease of functionalization of porphyrins means that researchers are not limited to one Gd(III) centre per porphyrin unit. Two related compounds were reported by Sour et al.\(^{23}\) and Luo et al.\(^{24}\). Both of these compounds consisted of a central porphyrin macrocycle decorated with four Gd(III)–DOTA moieties (8). The two compounds differed only in the choice of linker unit. Where one used a simple xylyl linker (Fig. 4), the other used an extended amide linker. Both Gd(III) complexes displayed good relaxivity, but it became apparent that the rigid xylyl linker provided improved relaxivity over the more flexible amide linker. The xylyl linker also appeared to show an improvement in the fluorescence and \(^{1}O_{2}\) quantum yields which resulted in high cytotoxicity upon irradiation.

Porphyrins are also found in Nature and so these types of molecules can inspire the design of new Gd theranostics. The most prevalent of these molecules are derivatives of pyropheophorbide-a, a product of the breakdown of chlorophyll A, for example, 3-(1’-hexyloxyethyl)pyropheophorbide-a (HPPH). These derivatives are attractive as potential Gd theranostics as they possess high fluorescence quantum yields and a relatively long absorption wavelength (665 nm) which helps to limit the photosensitivity that had plagued first-generation porphyrin photosensitizers. As with many porphyrin-like compounds, these compounds are limited in their application by their poor aqueous solubility. Similar to the porphyrins discussed above, HPPH can be functionalized so that Gd(III) complexes can be attached covalently. It was this thinking that led Li et al.\(^{25}\) to synthesize a class of chlorophyll-A-based compounds that were
conjugated to the aminobenzyl-DTPA MRI contrast agents 9 (Fig. 5). Studies with one of these compounds showed not only that it retained both the MR and PDT properties of Magnevist and HPPH, respectively, but due to the possibility of attaching two separate Gd(III) centers, it had a higher relaxivity and showed a higher cellular uptake than either of the individual components. This compound also showed relatively low skin phototoxicity, as was expected for a HPPH derivative. Its usage as a theranostic, however, was limited by its limited aqueous solubility and therefore high doses could not be administered in vivo. In order to address this issue, Spernyak et al. synthesized a second group of compounds where the number of DTPA moieties was increased from two to three and six in order to increase the aqueous solubility of the conjugate. Unfortunately, those compounds containing six Gd(III) centers showed a decreased PDT efficacy. Those compounds containing three Gd(III) centers, however, showed an increase in MR contrast whilst retaining their PDT effectiveness. Future work will focus on increasing the tumour selectivity by the incorporation of a variety of tumour-targeting groups.

2.3.2 Neutron capture therapy agents. For several decades, neutron capture therapy (NCT) has been performed almost exclusively using boron-based compounds due to the high propensity for the naturally-occurring $^{10}$B isotope to capture thermal neutrons, resulting in a nuclear fission process that liberates tremendous amounts of kinetic energy (~2.4 MeV) that can destroy tumour cells. BNCT has been used for the treatment of intractable and aggressive tumours such as glioblastoma multiforme (GBM), the most common malignant brain tumour. The only clinically-utilised boron-based agents $\gamma$-boronophenylalanine (BPA, 10) and borocaptate ion (BSH, 11) (Fig. 6) have some significant drawbacks. In general, these two agents have limited tumour-targeting capabilities and they are not able to be imaged in vivo which does not allow for the real-time monitoring of the drug uptake, biodistribution and tumour localization which limits their theranostic potential.

Gd possesses some key advantages when compared to B for NCT. Firstly, Gd(III) can be used as an MRI contrast agent. Furthermore, as mentioned earlier, $^{157}$Gd has the highest neutron capture cross-section of any of the naturally-occurring elements and as such it should be approximately 66-times more effective as a NCT agent than $^{10}$B, all things being equal. Initial explorations into the use of GdNCT were first published in the 1980s but these agents have failed to gain any traction within the medical community. One major reason is that the high-energy ACK electrons formed upon neutron capture by the $^{157}$Gd nucleus cannot travel more than a few nm, and therefore it is critically important that Gd is localized in close proximity to key sub-cellular components in order to trigger irreversible cellular damage.

The design of many GdNCT agents to date has focused on combined BGd-NCT agents in order to maximise cytotoxicity from the combined effects or to use the B component for NCT and the Gd component as a MRI imaging agent. Some of the preliminary work in this area was performed by Nemoto et al. whereby a boron cluster was attached to the commonly-used MRI contrast agent Gd–DTPA via a palladium-catalyzed allylation reaction (12, Fig. 7) The in vivo properties of this compound were then studied by Nakumara et al. where rats bearing an AH109A (rat hepatocellular carcinoma) tumour were administered with the compound and MRI images taken at short intervals over the period of one hour. This study revealed that while this B–Gd compound did show an increased tumour-retention time when compared to Gd–DTPA alone, it did not show any significant selectivity for tumour cells but it did show some accumulation in the necrotic tissue associated with the tumour. Takahashi et al. then explored the linking of a well-known BNCT agent, BPA (15), in order to improve the tumour targeting of this agent (Fig. 7). This BPA-coupled Gd–DTPA agent was then assessed in vivo against the BPA agent and it was found to have a higher tumour selectivity and, furthermore, was excreted from the animal quickly. The Geninatti-Crich group have published a large amount of work regarding Gd–carborane compounds (14) that are shuttled into cells using low-density lipoproteins (LDLs) (Fig. 7). These LDLs can carry up to 2000 boron atoms and are transported into the cells via receptor-mediated endocytosis. Initial studies were conducted in order to determine their uptake into human hepatoma (HepG2), murine melanoma (B16) and human glioblastoma (U87) cell lines, all of which are known to overexpress LDL receptors. Of the three cell lines tested, the minimum concentration of boron required for effective NCT was achieved in the B16 and HepG2 cells. The researchers then proceeded to in vivo studies involving B16 tumour-bearing mice. It was found that there was an impressive decrease in tumour growth in these mice when dosed with the compound upon irradiation with thermal neutrons. Alberti et al. then extended the study of this compound by exploring its in vivo effect on mouse
mammary tumour (TUBO) and human mesothelioma (ZL34), respectively. In all tumour lines investigated, there was an impressive decrease in tumour size. However, in both the melanoma and mammary tumour lines, there was an increase in tumour growth at day 30. This result was attributed to the presence of radiation-resistant cells in these tumour lines. This effect was not seen in the mesothelioma cell line, indicating that this line may be more susceptible to NCT. Alberti et al. also explored a different approach to targeting cancer. The above compound was further functionalized with cholesterol (13). This modification provided greater stability when the cholesterol derivative was incorporated into liposomes.

While linking B agents to Gd MRI agents has proved to be a promising strategy in the design of new Gd theranostic agents for NCT, it is also possible to include the Gd center only and exclude the B atom entirely. Initial studies exploring the use of clinical MRI contrast agents in NCT were promising with a number of in vivo studies performed against VX-2 subcutaneous tumours in New Zealand White rabbits and Ehrlich tumours inoculated subcutaneously into nude mice. Despite the initial successes in these animal models, human trials were far less successful with studies showing that the tumour uptake of these agents was insufficient for successful GdNCT.

Morrison et al. have reported a series of arylphosphonium cation-linked Gd(III) agents (16) with high tumour cell selectivity and low inherent cytotoxicity in the absence of thermal neutrons (Fig. 8). Arylphosphonium cations are delocalized lipophilic cations (DLC) which allow otherwise hydrophilic compounds to pass through the phospholipid bilayer of cells and, moreover, the mitochondrial membrane. The delocalized positive charge of these salts also allows for the active targeting of the mitochondria in most cancers which possess an elevated mitochondrial membrane potential. These compounds showed limited cytotoxicity in both human carotid artery endothelial (HCTAEC) and human glioblastoma (T98G) cell lines and an impressive selectivity for tumour cell lines with the highest being ca. 23 times more selective for tumour cells over normal, healthy cells. This study also showed that an increase in lipophilicity showed a decrease in tumour cell selectivity, thus highlighting the need to tune the lipophilicity of the Gd agent in order to maximize both tumour cell uptake and selectivity. Kim et al. reported a Gd complex that was linked to a benzothiazole–aniline (BTA) group (17, Fig. 8). These BTA groups have been shown to have interesting anti-inflammatory, antimicrobial and anticancer effects. The Gd(III) complex showed excellent antiproliferative properties and selectivity for cancer cells.
With the added potential of NCT, this compound could also show potent anti-cancer properties.

3. Nanoparticles as theranostics

One theranostic strategy that is gaining a great deal of popularity in recent years is the use of nanoparticles. Nanoparticles are much larger than conventional small molecule therapeutics and can be loaded with many diagnostic and therapeutic components. Nanoparticles can also be used to deliver higher levels of drug in a more localized area than small molecules, leading to a more effective cancer treatment and imaging option. Nanoparticles are generally taken up into tumours by the enhanced permeability and retention (EPR) effect which allows nanoparticles to enter the tumour microenvironment. It also allows nanoparticles to circumvent some of the multi-drug resistance factors that plague current small-molecule chemotherapeutics. Nanoparticles can facilitate the transport to tumour sites of small molecules or other components that are otherwise unsuitable for biological therapeutics due to their poor localization or aqueous solubility. The application of such nanoparticles in a clinical context is limited, however, as it is a great challenge to prepare nanoparticles that allow small molecules to be released selectively within the tumour site only.37

3.1 Micellar nanoparticles

The idea of using polymeric micelles to encapsulate chemotherapeutic agents in order to increase their effectiveness (either by increasing their water solubility and/or improving their pharmacokinetics) has been considered since the late 1990s, with a range of block-polymer micelles proceeding into clinical trials.38 However, these micelle-based carriers can be associated with serious practical issues. For example, micellar structures tend to disintegrate when the concentration is below that of the critical micelle concentration (CMC). This issue can become a problem in vivo where the micelles are diluted within the blood and/or tissues and are exposed to multiple stress factors inside the body. This micellar disintegration process can lead to an undesirable release of drug and/or imaging agent well before the desired target is reached. In order to counter this problem, it has been shown that by cross-linking various parts of the micelle (core, shell and core/shell interface) using either disulfide, pH sensitive, or light sensitive bonds, it is possible to increase the stability of the nanoparticle and only release the chemotherapeutic and/or imaging agent when it reaches the specific tumour region whereby bond cleavage can occur. Li et al.39 used this technique to impart good physiological stability to their theranostic micelles. In this species, the central core was composed of a hydrophobic hyperbranched polyester. This central core acted as the storage component for the hydrophobic chemotherapeutic agent paclitaxel. It also acted as an anchor point for the hydrophilic part of the micelle as this micellar component can be cross-linked to its central core. This was achieved by the use of poly(c-caprolactone) which formed the hydrophobic inner-layer, and was subsequently linked to a multi-arm star-block copolymer. The branching arms of this polymer were composed of monomethyl ether methacrylate and 3-azidopropyl methacrylate, which were then linked to the tumour targeting moiety, alkynyl folate, and the MRI contrast agent, alkynyl-DOTA-Gd. This block polymer formed the hydrophilic outer corona (Fig. 9). This micelle was found to exhibit excellent cytotoxicity with good targeting of cancer cell lines while also exhibiting reasonable MRI contrast ability.

In some cases, however, the cross-linking step is unnecessary, and the polymer micelles still remain stable in vivo. This was found to be the case by Gong et al.40 who engineered a polyethylene glycol (PEG) nanomicelle which encapsulated the NIR dye IR825, a PDT agent. The nanomicelle was further functionalised to incorporate chlorin 6 Ce6, a porphyrin that can encapsulate Gd3+ ions. This led to the creation of a nanoparticle that was capable of PTT/PDT as well as multimodal imaging (fluorescence, MR and photoacoustic imaging) and which also showed good tumour growth inhibition.

3.2 Fullerenes

Fullerenes are carbon-based molecules which have many interesting properties largely due to their electronic properties. They possess delocalized π-molecular orbitals, however the configuration of the planar 6-membered rings causes strain on the conjugated system. The fullerenes themselves are poorly water-soluble but are relatively easy to functionalize in order to increase their aqueous solubility. This functionalization further changes the electronic properties allowing for some interesting chemistry to be observed. One of the common approaches to using fullerenes in theranostic medicine is to encapsulate a Gd center within a fullerene.31–44 Li et al.42 have synthesized an encapsulated ‘gadofullerene’ and then functionalized the fullerene with cytokine interleukin-13, which is overexpressed
in human glioblastoma cell lines (Fig. 10). The compound was further functionalised with amine groups, which are capable of maintaining a positive charge at physiological pH, in order to further increase water solubility and tumour uptake. While this group of researchers has yet to report cytotoxicity studies, the preliminary tumour uptake and MRI studies are promising. Another area that shows potential in the field are carbon dot nanoparticles. Guan et al.\textsuperscript{43} have synthesized a multifunctional nanoparticle containing both carbon dots and gadofullerenes. The strategy of utilizing Gd metallofullerene nanocrystals was thought to enhance the tumour accumulation of the carbon dots and also enhance the relaxivity of the nanocrystals. This gadofullerene contains the central fullerene core encapsulating Gd, however, in contrast to the previous example, the outer shell supports a negative rather than positive charge. This negative charge allowed the positively-charged carbon dot to electrostatically interact with the gadofullerene. The stability of this arrangement was then improved by further surface functionalization using carboxylic acid PEG derivatives. This agent showed an increased relaxivity and ultra-efficient tumour abatement when irradiated with a 635 nm laser with almost complete eradication after 15 days. Wang et al.\textsuperscript{45} have synthesized a core–satellite gadofullerene therapeutic that showed almost complete tumour eradication in \textit{in vivo} models and 100% survival rate over the course of the trial. This compound consisted of a doxorubicin-loaded polydopamine core nanoparticle. This nanoparticle was then decorated with PEG and Gd-encapsulated fullerenes. This agent proved to be largely non-toxic until irradiated with NIR radiation, which caused the release of the doxorubicin payload. Due to the large number of Gd-fullerenes decorating this nanoparticle, this species also showed very good relaxivity for MRI contrast.

3.3 Liposomal nanoparticles

A popular technique for the delivery of chemotherapeutic drugs to tumours using nanoparticles involves the use of mesoporous silica nanoparticles (MSN). Like fullerenes, mesoporous silica nanoparticles create a cage-like structure that can be used to encapsulate drugs. They can also be functionalized internally or externally to increase bioavailability or to covalently link the payload drug and/or imaging agent. Silica nanoparticles are not particularly biocompatible, but it has been found that coating these silica nanoparticles in a lipid bilayer vastly improves drug delivery. One of the first examples in using MSNs as therapeutic agents was performed by Kalluru et al.\textsuperscript{45} In these entities, Gd and Eu oxide nanoparticles were encapsulated within the mesoporous silica framework. These structures were then decorated with amine functional groups to allow for the attachment of PEG and folate functionalities in order to improve their aqueous solubility and also allow for the active targeting of cancer cells. This combination of features led to a MSN which was capable of bimodal imaging (MR and fluorescence) as well as therapeutic NIR-mediated release of a doxorubicin (DOX) payload for PDT, and it showed almost complete \textit{in vivo} tumour eradication after 14 days post-treatment. Sun et al.\textsuperscript{46} expanded upon this work to produce MSN containing Gd$_2$O$_3$ nanoparticles that were also loaded with DOX, as in the previous example. These researchers, however, coated this particular MSN with thermosensitive indocyanine green (ICG) loaded liposomes that were functionalized with folate for tumour-targeting purposes. ICG is a FDA-approved drug that is used in both PTT and PDT. Therefore, ICG can be activated and the local production of heat will cause the thermosensitive liposomes to break down, allowing for the release of DOX in a targeted manner. This agent showed similar anti-tumour activity to the previous generation, but it possessed some distinct advantages as it is capable of photoacoustic imaging in combination with MR and fluorescence.

3.4 Activation and guiding irradiation by X-ray (AGuIX) nanoparticles

First developed by Tillement et al.,\textsuperscript{47} the AGuIX nanoparticles are a unique class of nanoparticle that are currently undergoing clinical trials where phase 1 trials showed no acute severe or life threatening adverse affects up to a dose of 100 mg kg$^{-1}$. The central structure consists of a Gd$_2$O$_3$ core with a polysiloxane shell. This core is then decorated with DOTA ligands. Upon immersion in aqueous solution, the core collapses as the DOTA ligands detach and the Gd$_3^{3+}$ ions from the Gd$_2$O$_3$ core. These collapsed shells then fragment to form small rigid platforms (SRPs). The SRPs retain the properties of the initial nanoparticle prior to collapse. While the chelation of Gd$^{3+}$ was found to be quite efficient, there were still free DOTA moieties available which allowed for the complexation of radioactive $^{111}$In. This compound has shown excellent \textit{in vivo} imaging using four techniques: MRI; fluorescence imaging; SPECT,
and CT. MRI was particularly effective, as the rigid structure worked to further enhance tissue contrast. This nanoparticle was also found to be a good radiosensitizer due to the presence of high-Z elements, with a median animal survival time of 108 days when compared to that in the presence or absence of clinical Gd contrast agents (38 days and 44 days, respectively) in the treatment of gliomas.

3.5 Upconversion nanoparticles

Upconversion nanoparticles (UCNPs) are a class of nanoparticle in which certain rare-earth ions are doped into nanocrystals. These nanocrystals are capable of absorbing infrared light, which is then upconverted and re-emitted in the visible spectrum (Fig. 11). Another important feature of these nanomaterials is that high-Z elements, such as those used in UCNPs, have been shown to amplify a local radiation dose in radiotherapy (RT). This feature alone allows UCNPs to be effective therapeutic agents, though it can be improved upon in a number of ways. By further doping the nanoparticles with other rare-earth elements, other diagnostic methods can be employed. These nanomaterials can also be coated in silica and further functionalized with other diagnostic or therapeutic entities such as copper nanoparticles (CuNP), which are known to be effective photothermal ablation (PTA) agents, or any of the previously discussed theranostics. Many of these upconversion nanoparticles have very similar central nanocrystals, and all contain Gd$^{3+}$ which displays excellent MR contrast properties. The focus of current research endeavours is on utilizing these agents to increase the effectiveness of other well-known binary therapies, as well as creating methods of excitation that do not involve a 980 nm laser which is associated with tissue overheating issues due to the high absorption of this wavelength by water molecules.

An UCNPs can also be encapsulated in such a way that they act as “rattle-like” carriers for small-molecule cancer therapeutics such as cisplatin. In order to prepare these compounds, Fan et al.$^{48}$ coated the UCNP with two layers of biocompatible dense silica. Subsequent hot-water etching was used to break the Si–O–Si bonds in a controlled manner to form the rattle cage. The cisplatin was then absorbed by the nanoparticle. This compound showed good stability in aqueous solution and exhibited a higher in vivo cytotoxicity than that of free cisplatin. These researchers further developed this method to create a trimodal theranostic nanoparticle. In this nanoparticle, the rattle cage was generated in a similar manner but, in addition to the encapsulation of a chemotherapeutic in the inner cavity (doxetaxel), a radiosensitizer (hematoporphyrin) was covalently grafted onto the silica shell.

Xiao et al.$^{49}$ synthesized a UCNP where the internal nanocrystal core contained Yb$^{3+}$, Er$^{3+}$ and Gd$^{3+}$ ions which enabled it to be used for upconversion luminescence (UCL), MRI, and computed tomography (CT). This UCNP was then coated with silica and decorated with CuNPs, for application as a photothermal agent. Finally, PEG was grafted onto the nanoparticle to prevent self-aggregation and to assist with the solubilization of the nanoparticle in aqueous media. The synergistic nature of the therapeutic aspects of these nanoparticles proved to be extremely effective with no tumour recurrence found after 120 days when animals were treated with both RT and PTA. The nanoparticles also showed excellent imaging capabilities in UCL, MRI and CT, with all techniques able to be performed simultaneously, allowing these agents to be used for full body imaging and targeted treatments.

One of the key issues with upconversion nanoparticles is that functionalization of the outer shell can be quite challenging. Chen et al.$^{50}$ approached this problem by first coating the UCNP with bovine serum albumin (BSA), which has previously been used as a nanodrug carrier as it is able to bind hydrophobic drugs within the hydrophobic domain of the protein. The hydrophobic photosensitizer molecules rose bengal (for PDT) and IR825 (for PTT) were bound to the BSA-functionalized UCNP. This functionalized UCNP showed good solution stability and excellent cytotoxicity when animals were treated with PTT and PDT, with only minimal tumour growth observed after 14 days.

Another limitation of UCNPs is the lack of high upconversion emission with low excitation power density. NIR dyes have a larger absorption coefficient than rare-earth nanoparticles.$^{51}$ There are also certain dyes that have an emission wavelength of 808 nm which overlaps with the absorption wavelength of Yb$^{3+}$ and Nd$^{3+}$. This means the dyes can act as an antenna, improving the effectiveness of the UCNP. Xu et al.$^{52}$ used this technique to synthesize a trimodal UCNP where they used IR-808 as the antenna dye for the UCNP. The UCNP was then functionalized with the photosensitizers Ce6 and MC540 which can absorb light in the wavelength region that is emitted by the UCNP. Approaching the problem in this way meant that the high cytotoxicity seen with these drugs was maintained but the significant overheating issues that were sometimes observed with the conventional 980 nm laser could be avoided.

These UCNPs can also be encapsulated within micelle-like structures. This particular strategy means that the problem of functionalization of the shell is largely removed as the micellar component has many more options for further functionalization. This approach also works to mitigate the risk of the encapsulated drug being prematurely released from the UCNP. This strategy was employed by Han et al.$^{53}$ in order to create an antibody-targeted micellar nanoparticle. These researchers synthesized a
UCNP-based nanoparticle which was then grafted with anti-EpCAM antibodies able to selectively target the epithelial cell adhesion molecule (EpCAM), a biomarker for cancer stem cells. The chemotherapeutic mitoxantrone (MX) was then loaded into the nanoparticle. In vivo data involving this nanoparticle showed an increase in tumour uptake when compared with non-targeted molecules, and also exceptional anti-tumour activity.

4. Conclusion

The design and development of Gd theranostics is a rapidly emerging field. This tutorial review has focussed on the design, synthesis, biological and, in some cases, clinical evaluation of Gd(III) complexes or nanoparticles for theranostic purposes. There are many other examples of Gd-based MRI contrast agents that could also be adapted as theranostics as their MRI properties alone are impressive, and their incorporation into tumour-selective (nano)molecules could lead to the development of more effective binary therapy agents. It is the authors’ opinion that with greater awareness of the unique possibilities that Gd offers in the theranostic realm, its incorporation into a single platform for both imaging and therapy will mean that more effective theranostic agents will likely be developed in the near future. Amongst the many different theranostic strategies being investigated in medicine today, Gd theranostics could indeed result in much improved clinical outcomes for those cancer patients diagnosed with intractable and aggressive tumours in the future.

Conflicts of interest

There are no conflicts to declare.

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