Chapter 18
Nanomedicine in Cancer Diagnosis and Therapy: Converging Medical Technologies Impacting Healthcare

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18.1 Introduction

Nowadays cancer diagnosis and therapy is the primary preoccupation of nanomedicine. This focus has given rise to the new field of cancer nanotechnology that involves multidisciplinary, problem driven research cutting across the traditional boundaries of biology, chemistry, engineering and medicine with the aim of creating major advances in cancer detection, diagnosis and treatment [1–4]. The field has received strong support especially in the US where several nanotechnology for cancer centres have been launched and operated since 2004. There is no better definition and overview of this field, than that given in http://nano.cancer.gov/, which outlines the National Cancer Institute's (NCI's) alliance for nanotechnology for cancer. This alliance aims to create a multidisciplinary nanotechnology approach for the creation of solutions for cancer detection, imaging and diagnosis [5]. In Europe a number of academic groups are interested in cancer nanotechnology as well. However only with the advent of Europe FPVII programs have specific calls been announced to support multidisciplinary research in cancer nanotechnology. In the UK, the major cancer research organisation (Cancer Research UK) appears hesitant to support this emerging field, possibly due to the perceived safety risk from nanomaterials currently untested in man. This hesitation is unfortunate. In a recent report "Roadmaps

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in Nanomedicine towards 2020" [6], specialists are now predicting that imaging and therapy in oncology by means of cancer nanotechnology will be a primary opportunity for various "designer" type nanomaterials, nanodevices and nanoparticles currently in discovery and development. Indeed, the global market size for cancer nanotechnology products is predicted to be €30bn by 2015. The particular opportunity presented by cancer nanotechnology is the eventual likelihood of personalised cancer diagnosis and treatment regimes [3].

Personalized therapy of cancer begins with molecular profiling. Golub et al. were first to report how molecular profiling studies, that show variations in gene expression patterns with time and disease status, could be used to inform on the stage, grade, clinical course and response to treatment of tumours [7]. From then on, increasing numbers of such studies have been performed showing that any given metastatic lesion results from a corresponding combination of tumoral, stromal, and inflammatory factors [8, 9]. Following this, causality in cancer has become associated with cancer disease-specific biomarkers validated by histochemical studies of diseased tissue [10]. The identification of such biomarkers by molecular profiling provides the foundation for personalized cancer diagnosis and therapy [11, 12]. In a prime early example of this principle, Erbb2 (HER2) is a tyrosine kinase receptor and cancer disease-specific biomarker found in 25–30 % of breast cancers. Overexpressed HER2 can be targeted for breast cancer therapy using Herceptin that is a potent, anti-HER2 therapeutic monoclonal antibody (biopharmaceutical agent). However, Herceptin has significant drug-use side effects that can be very severe. Accordingly, the Federal Drug Administration (FDA) now requires the proven identification of over-expressed HER2 in breast cancer patients before Herceptin can be prescribed. Typical in vitro diagnostic tests for HER2 that may be used to diagnose the presence of HER2 in breast tumour development include an immunohistochemistry assay and a nucleic acid fluorescence in situ hybridisation (FISH) test. Once these tests can be shown positive, then breast cancer patients may then be prescribed Herceptin with real confidence in probable therapeutic outcomes. In summary, cancer disease-specific biomarker, HER2, is detected as a diagnosis for breast cancer and disease mechanism. Afterwards a biomarker selective biopharmaceutical agent can be administered.

Relevant cancer disease-process biomarkers are many and various. They range from mutant genes, non-coding RNAs (ncRNAs), proteins, lipids, to carbohydrates and may even be small metabolite molecules. The key is that a link(s) should be established clearly from a given biomarker to tumour growth and development. Following on from this, there is a definite requirement for hyper-flexible, platform technologies that can mobilize diagnostic agents for a given biomarker and then deliver biomarker selective therapeutic agents to disease-target cells, also with selectivity. From the various options open to cancer nanotechnology, multifunctional nanoparticles are potentially ideal to meet these twin requirements. Indeed nanoparticles could be envisaged for (a) the detection of biomarkers, (b) the imaging of tumours and their metastases, (c) the functional delivery of therapeutic agents to target cells, and (d) the real time monitoring of treatment in progression. Therefore, if this is the potential, how close are we really?

Where nanoparticles are to be created for the functional delivery of imaging and/or therapeutic agents specific to cancer biomarkers, many factors have to be taken into consideration. This fact can be illustrated with reference to the fields of gene therapy and RNA interference (RNAi) therapeutics where nanoparticles have been devised for functional delivery of therapeutic nucleic acids with some success [13–15]. Where nanoparticles have been successfully designed and used to mediate the functional delivery of therapeutic nucleic acids, an **ABCD** nanoparticle paradigm can be invoked (Fig. 18.1). According to this general paradigm, functional nanoparticles comprise active pharmaceutical ingredients (APIs) (**A**-components) surrounded initially by compaction/association agents (**B**-components – typically lipids, amphiphiles, proteins or even synthetic polymers etc.) designed to help sequester, carry and promote functional delivery of the **A**-components. Such core **AB** nanoparticles may have some utility in vivo but more typically require coating with a stealth/biocompatibility polymer layer (**C**-layer; primary **C**-component – most often polyethylene glycol [PEG]) designed to render resulting **ABC** nanoparticles with colloidal stability in biological fluids and with immunoprotection from the reticuloendothelial system (RES) plus other immune system responses. Finally, an optional biological targeting layer (**D**-layer; primary **D**-components – *bona fide* biological receptor-specific targeting ligands) might be added to confer the resulting **ABCD** nanoparticle with target cell specificity. A key design principle here is that tailor-made nanoparticles can self-assemble reliably from tool-kits of

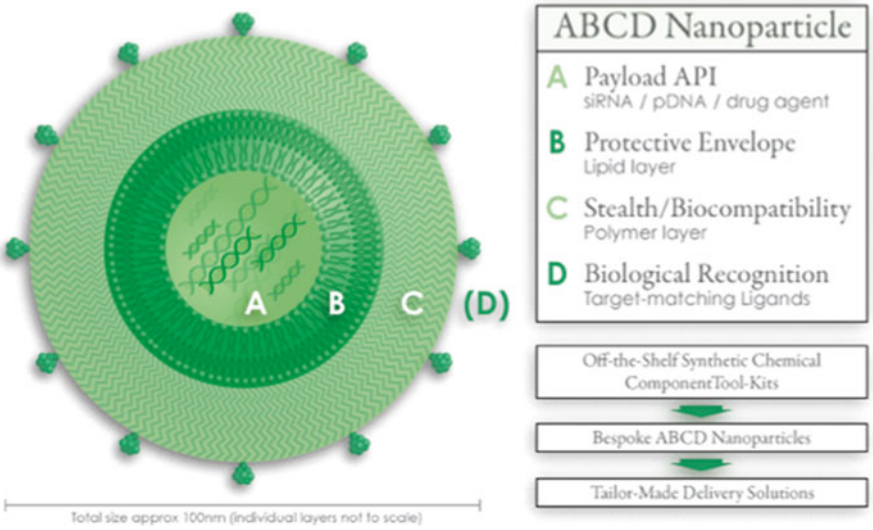

Fig. 18.1 Active pharmaceutical ingredients (APIs) (therapeutic bio-actives or intractactable drugs) are condensed within functional concentric layers of chemical components making up nanoparticle structures designed to enable efficient delivery (trafficking) of active therapeutic agents to disease-target cells. **ABCD** nanoparticle is drawn here assuming that **A**-components are nucleic acids and that **B**-components employed are lipids

purpose designed chemical components [16–26]. Accordingly, the concept of a personalized nanoparticle formulation, assembled in the pharmacy for an individual patient does not seem so far removed from reality.

The **ABCD** nanoparticle paradigm represents a set of well-found principles of design that are being implemented in the real world with the formation of actual nanoparticles leading to actual demonstrated functional properties at least in pre-clinical studies. As such, the design principles laid out in the **ABCD** nanoparticle paradigm are widely corroborated in the literature [1, 27–35]. Clearly functional nanoparticles need to be constructed from a range of chemical components designed to promote functional delivery of different diagnostic and/or therapeutic agents in vivo. In practise this means that nanoparticles need to be equipped to overcome relevant "bio-barriers" in accordance with pharmacological requirements of API use such as site, time and duration of action. Importantly too, with clinical goals in mind, nanoparticles have to be considered different to small and large molecular drugs. For instance, regulations from the FDA state that Absorption, Distribution, Metabolism and Excretion (ADME) studies need to be redesigned in the case of nanoparticles to take into consideration their aggregation and surface chemical characteristics [36].

In terms of cancer diagnosis and therapy, there is one factor that is very much in favour of multifunctional nanoparticle use. Nanoparticles administered in the blood stream (i.v.-administration) frequently accumulate in tumours anyway due to the enhanced permeability and retention (EPR) effect, a behaviour that was identified by Maeda as a means to target anticancer therapeutic agents to tumours [37, 38]. Nanoparticle accumulation in tumours takes place due to the presence of highly permeable blood vessels in tumours with large fenestrations (>100 nm in size), a result of rapid, defective angiogenesis. In addition tumours are characterised by dysfunctional lymphatic drainage that helps the retention of nanoparticles in tumour for long enough to enable local nanoparticle disintegration in the vicinity of tumour cells. The phenomenon has been used widely to explain the efficiency of nanoparticle and macromolecular drug accumulation in tumours [39]. Unfortunately, knowledge of nanoparticle biokinetics, metabolism and clearance is otherwise poor since too few nanoparticle products have been clinically tested. This is a major limitation in the growth of the field of cancer nanotechnology. Nevertheless, cancer nanotechnology is a fast growing field and new data is arriving all the time. In the following sections, the status of nanoparticle use in cancer diagnosis and therapy will be surveyed.

18.2 Nanoparticles for Cancer Imaging and Therapy in Clinical Trials and at Advanced Preclinical Phases of Evaluation

The first nanoparticles used and approved for clinical therapy use were lipid-based nanoparticles (LNPs). Selected structural lipids self-assemble into liposomes that are typically approx. 100 nm in diameter and consist of a lipid bilayer surrounding

an aqueous cavity [40–43]. This cavity can be used to entrap water-soluble drugs in an enclosed volume resulting in a drug-**AB** nanoparticle system [44, 45]. The first reported LNPs of this type were designed to improve the pharmacokinetics and biodistribution of the anthracycline drug doxorubicin. Doxorubicin is a potent anti-cancer agent but is cardiotoxic. In order to minimize cardiotoxicity, doxorubicin was encapsulated in anionic liposomes giving anionic doxorubicin drug-**AB** nanoparticles that enabled improved drug accumulation in tumours and increased antitumour activity while diminishing side effects from cardiotoxicity [46, 47]. This nanoparticle formulation has since been used efficiently in clinic for the treatment of ovarian and breast cancer [48, 49]. Thereafter, Doxil® was devised corresponding to a drug-**ABC** nanoparticle system, comprising PEGylated liposomes with encapsulated doxorubicin. These Doxil® drug-**ABC** nanoparticles (also known as PEGylated drug-nanoparticles) were designed to improve drug pharmacokinetics and reduce toxicity further by maximizing RES avoidance [50–52], making use of the PEG layer to reduce uptake by RES macrophages of the mononuclear phagocyte system (MPS) [53, 54].

The second nanoparticle system used and approved for clinical use were nanoparticles prepared using albumin as a compaction/association agent for sparingly water soluble Taxol®, one of the most potent anticancer drugs known. The resulting protein-based drug-**AB** nanoparticles (130 nm diameter) were christened nab-paclitaxel or Abraxane®. This Abraxane® system was designed to avoid the use of Cremophor EL® solvent (polyethoxylated castor oil) most frequently used to solubilise Taxol® [55–57]. Abraxane® is the first albumin nanoparticle system approved for human use by the FDA. This use of albumin is inspired. Albumin is a natural carrier of endogenous hydrophobic molecules that associate through non-covalent interactions. In addition, albumin assists endothelial trancytosis of protein bound and unbound plasma constituents principally through binding to a 60 kDa glycoprotein cell surface receptor, gp60. The receptor then binds to caveolin-1 with subsequent formation of transcytotic vesicles (caveolae) [58]. In addition, albumin binds to osteonectin, a secreted protein acid rich in cysteine (SPARC), that is present on breast lung and prostate cancer cells, so allowing albumin nanoparticles to accumulate readily in tumours [57, 59]. Currently there are more than 50 clinical trials ongoing using nanoparticles for cancer therapy. Indeed, the majority of these nanoparticles are nab-type (nanoparticle albumin bound) tested for the treatment of various cancer types (http://clinicaltrials.gov).

Otherwise, in terms of leading edge cancer clinical trials, LNPs have also been used in clinical trials for the delivery of biotherapeutic agents in cancer therapy corresponding to leading RNAi effectors known as small interfering RNAs (siRNAs). For instance LNPs corresponding to siRNA-**ABC** nanoparticles, Atu027, ALN-VSP02 and TKM-PLK1 are or have been in various stages of Phase I clinical trials. Moreover, one polymer-based nanoparticle (PNP) system, corresponding to a siRNA-**ABCD** nanoparticle system and christened CALAA-01, has appeared in Phase I clinical trials, with a Phase IIa clinical trial reportedly underway [60]. CALAA-01 employs a cyclodextrin polymer scaffold to entrap RNAi effectors and transferrin as a receptor-specific targeting ligand. Otherwise, advanced LNP (and

even PNP) prototypes, that are either nucleic acid-**AB**, **ABC** or **ABCD** nanoparticle systems, continue to be tested for functional delivery of therapeutic nucleic acids to target cells in animal models of human disease (to liver for treatment of hepatitis B and C virus infection, to ovarian cancer lesions for cancer therapy) and to target cells in murine lungs [61–67]. Rules for enhancing efficient delivery through receptor-mediated uptake of **ABCD** nanoparticles into target cells are also being studied and appreciated [68–71].

From the point of view of using nanoparticle technologies for the imaging of cancer, the ability to combine imaging agents with nanoparticles is central. In terms of the **ABCD** nanoparticle paradigm, the **A**-component now becomes an imaging agent(s) instead of a therapeutic agent. Fortunately, progress with imaging nanoparticles has also been brisk and a number of clinical trials have been expedited. For instance, a heterogeneous LNP system has been described in clinic that consists of a superparamagnetic iron oxide (SPIO) core particle lipid-coated to confer biological function [72]. This LNP system been used as a diagnostic tool for the pre-operative stage(s) of pancreatic cancer [73]. LNPs have also been described for radionuclide delivery to tumour lesions. Typically, these consist of a central liposome, that entraps a radionuclide of interest by analogy to drug-**AB/C** nanoparticles, and whose surface may be modified by targeting antibodies or peptides (**D**-components) in order to derive receptor-targeted nanoparticles [74]. Nanoparticles of this type have been used to entrap the chelate ^{111}In-diethylenetriamine-pentaacetic acid (^{111}In-DTPA). These were administered to 17 patients with locally advanced cancers. Post administration, patients were examined by means of a whole body gamma camera in order to verify pharmacokinetics and biodistribution behaviour. The $t_{1/2}$ of these ^{111}In-labelled nanoparticles was 76.1 h, and levels of tumour LNP uptake were estimated to be approximately 0.5–3.5 % of the injected dose at 72 h. The greatest levels of uptake were seen in the patients with head and neck cancers. However, significant uptake was also seen in the tissues of the RES (namely, liver, spleen, and bone marrow). Nevertheless data support the use of these ^{111}In-labelled nanoparticles for the imaging of solid tumors, particularly those of the head and neck, [75]. Moreover, once delivered to such tumour lesions, the radionuclide may then be used as a therapeutic agent to destroy tumour mass by radiation according to the principles of nuclear medicine.

Potentially important preclinical studies have been carried out recently with imaging LNPs set up for positive contrast magnetic resonance imaging (MRI) [76, 77]. The first described LNPs of this class were formulated by trapping water-soluble, paramagnetic, positive contrast imaging agents [such as $MnCl_2$, gadolinium (III) diethylenetriamine pentaacetic acid (Gd.DTPA), and the manganese (II) equivalent (Mn.DTPA)] in the enclosed volume of a liposome resulting in prototype lipid-based, positive contrast imaging-**AB/C** nanoparticles [78, 79]. Disadvantages were quickly reported such as poor encapsulation efficiency, poor stability, and clear toxicities due to importune contrast agent leakage and poor relaxivity [80]. These problems were obviated when hydrophobic lipidic chains were “grafted” on to contrast agents, thereby enabling these agents to be hosted by a lipid-bilayer [81]. Such lipidic contrast agents formulated in association with the bilayer of a liposome

exhibit improved ionic relaxivity and could therefore be used for dynamic MRI experiments in mice in vivo [82].

A potentially significant variation on this theme involves gadolinium (III) ions complexed with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to which hydrophobic lipidic chains are attached. In particular, gadolinium (III) 2-(4,7-*bis*-carboxymethyl-10-[(*N*,*N*-distearylamidomethyl)-*N*'-amidomethyl]-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid (Gd.DOTA.DSA) was prepared and formulated into passively targeted Gd-**ABC** (no biological targeting layer) and folate-receptor targeted Gd-**ABCD** nanoparticles in conjunction with a number of other naturally available and synthetic lipid components such as (ω-methoxy-polyethylene glycol 2000)-*N*-carboxy-distearoyl-L-α-phosphatidylethanolamine (PEG^{2000}-DSPE) or its folate variant (folate-PEG^{2000}-DSPE), and fluorescent lipid dioleoyl-L-α-phosphatidylethanolamine-*N*-(lissamine rhodamine B sulphonyl) (DOPE-Rhoda) (Fig. 18.2). These bimodal imaging nanoparticle systems demonstrated excellent tumour tissue penetration and tumour MRI contrast imaging in both instances [83–85]. Interestingly, the folate-receptor targeted Gd-**ABCD** exhibited a fourfold decrease in tumor T_1 value in just 2 h post-injection, a level of tissue relaxation change that was observed only 24 h post administration of passively targeted Gd-**ABC** nanoparticles [83, 84]. Preparations for clinical trial are now underway beginning with cGMP manufacturing and preclinical toxicology testing. These Gd-**ABC/D** nanoparticles are potentially excellent nanotechnology tools for the early detection and diagnosis of primary and metastatic cancer lesions. How effective remains to be seen when clinical trials can be performed. On the other hand, these LNPs may well enter into direct comparison with alternative LNPs that have been described by Müller et al. and are known as solid lipid nanoparticles (SLNs). These SLNs could certainly offer an alternative LNP platform for imaging [86–88]. For instance, under appropriate optimised conditions SLNs can carry MRI contrast agents [89], and SLNs containing $[Gd\text{-}DTPA(H_2O)]^{2-}$ and $[Gd\text{-}DOTA(H_2O)]^{-}$have even been prepared for preclinical studies.

In complete contrast, a variety of PNP systems are also beginning to be realized for the delivery of therapeutic agents and/or imaging agents. For instance, dendrimers are a unique class of repeatedly branched polymeric macromolecules with a nearly perfect 3D geometric pattern. They can be prepared with either divergent methods (outward from the core) or convergent methods (inward towards the core). Tomalia was the first to synthesise the 3D polyamidoamine (PAMAM) dendrimers using divergent methods [90]. The methods of Frechet [91] are characterised by generation (G) building using monomers added to a central core. Controlled synthesis results in molecular diameters between 1.9 nm for G1 to 4.4 nm for G4 dendrimers. These G1-G4 dendrimers represent the smallest known nanocarriers yet developed for pharmaceutical and imaging applications associated with cancer [92], including photodynamic therapy (activation therapies) [93], boron neutron capture therapy [94] and hyperthermia therapies in combination with gold nanoparticles [3]. These Gd-**AB** nanoparticles, known as gadolinium (III) dendrimer conjugates, have proven of provisional value in MRI experiments [95]. Unfortunately as delivery systems for therapeutic agents, dendrimers have a tendency post administration to release conjugated drugs before reaching disease target sites.

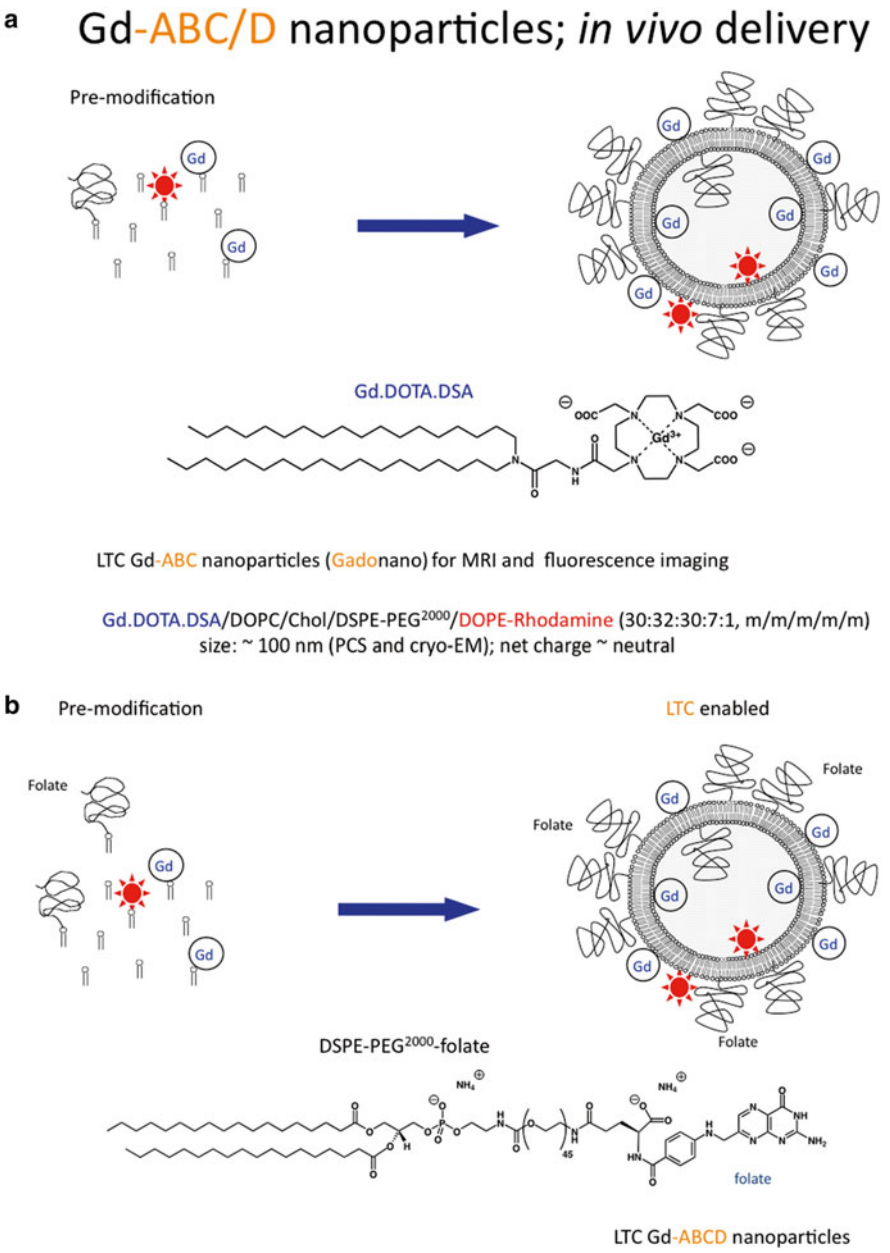

Fig. 18.2 Passively targeted Gd-**ABC** (*top*) and folate-receptor targeted Gd-**ABCD** (*bottom*) nanoparticles for IGROV-1 tumour imaging [83]. These LNPs are long-term circulation (*LTC*) enabled by virtue of the use of bilayer stabilizing lipids and 7 mol% PEG-lipid in the outer leaflet membranes of lipid-based nanoparticle structures

Finally, we turn to inorganic "hard" nanoparticles. Of these the most advanced already in clinical practice are the dextran coated iron oxide nanoparticles that correspond in form to imaging-**AB/C** nanoparticle systems. Ferumoxtran-10 ® is a commercially available ultra-small-superparamagnetic iron oxide particle (USPIO) product [96, 97]. After systemic injection, these nanoparticles collect in lymph nodes, liver, spleen, or brain tissue where are visualized by MRI. In a lymph node with proper architecture and function (healthy), macrophages take up a substantial amount of ferumoxtran-10. This uptake results in a marked reduction in signal intensity and turns the lymph nodes dark when seen by MRI. Infiltration of lymph nodes with malignant cells replaces the macrophages and changes the architecture of the lymph nodes. In malignant lymph nodes there is no ferumoxtran-10 macrophage uptake and they can retain the high signal intensity or display heterogenous signal intensity if micrometastases are involved. This way the grade of tumours and prognosis can be assessed by the presence of micro-metastases [98]. Additionally, iron oxide nanoparticles can be guided in principle to target sites (i.e. tumour) using external magnetic field and they can be also heated to provide hyperthermia for cancer therapy [99].

On another tack, Yu et al. have reported how dextran-coated iron oxide nanoparticles bearing a Cy5.5 near infrared (NIR) probe could also carry doxorubicin thereby allowing both the imaging and drug treatment of cancer lesions. The administration of these bimodal imaging drug-**AB/C** nanoparticles allowed for simultaneous real-time imaging of nanoparticle biodistribution and the measurement of drug pharmacokinetic behaviour alongside the observation of a substantial phenotypic (pharmacodynamic) reduction in tumour size [99]. Similarly bimodal imaging RNAi-**AB/C** nanoparticle systems were realized by coupling RNAi effectors to the dextran coat alongside Cy5.5 near infrared dye. These bimodal imaging nanoparticles were also seen to enable functional delivery of the RNAi effectors to target cells with real-time/diagnostic imaging [100, 101]. Where nanoparticles have a dual function for imaging and therapy, they are increasingly known as theranostic (i.e. *thera*py + diag*nostic*) nanoparticles. Moreover, what was achieved with inorganic "hard" iron oxide nanoparticles was subsequently reported using LNPs. For instance, a multimodal imaging theranostic siRNA-**ABC** nanoparticle system was recently described that had been assembled by the stepwise formulation of PEGylated cationic liposomes (prepared using Gd.DOTA.DSA and DOPE-Rhoda amongst other lipids), followed by the encapsulation of Alexa fluor 488-labelled anti-survivin siRNA. These multimodal imaging theranostic nanoparticles were found able to mediate functional delivery of siRNA to tumours giving rise to a significant phenotypic (pharmacodynamic) reductions in tumour sizes relative to controls, while at the same time nanoparticle biodistribution (DOPE-Rhoda fluorescence plus MRI), and siRNA pharmacokinetic behaviour (Alexa fluor 488 fluorescence) could be observed by means of simultaneous real-time imaging [65]. This concept of multimodal imaging theranostic nanoparticles for cancer imaging and therapy is certain to grow in importance in preclinical cancer nanotechnology studies and maybe in the clinic too.

18.3 Nanoparticle Applications in Triggered and Image-Guided Therapies

Multimodal imaging theranostic nanoparticles may offer substantial benefits for cancer diagnosis and therapy going forward, but only in combination with further advances in nanoparticle platform delivery technologies. What might these advances be and how might they be implemented? As far as imaging nanoparticles are concerned for detection of cancer, provided that all that is required for diagnosis is nanoparticle accumulation within cancer lesions, then current imaging nanoparticle technologies may well be sufficient. However, for personalized medicine to really take off, the detection of cancer disease specific biomarkers in vivo is really required. In order to achieve this, considerable attention may well have to be paid to the appropriate design and selection of ligands (**D**-components) for the biological targeting layer (or **D**-layer).

As far as nanoparticles for cancer therapy are concerned, the opportunities for delivery are relatively limited at this point in time, primarily due to the facile partition of current nanoparticles post-administration to liver and to solid tumours in vivo and in clinic. In order to enable partition to other organs of interest and even to diseased target cell populations within, there is now an imperative to introduce new design features involving new tool-kits of chemical components. Moreover the **ABCD** nanoparticle paradigm itself has one primary design weakness in that the stealth/biocompatibility polymer layer (or **C**-layer) (typically PEG, main **C**-component) does not prevent nanoparticle entry into cells but may substantially inhibit functional intracellular delivery of the therapeutic agent, unless sufficiently removed by the time of target cell-entry or else during the process of cell-entry. Learning the rules for the control of nanoparticle biodistribution and of therapeutic agent cargo pharmacokinetics may take several years yet even though rule sets are emerging. Therefore, overcoming the **C**-layer paradox should be the primary focus for therapeutic nanoparticle development over the next few years. Accordingly, there has been a growing interest in the concept of nanoparticles that possess the property of triggerability. Such nanoparticles are designed for high levels of stability in biological fluids from points of administration to target cells whereupon they become triggered for the controlled release of entrapped therapeutic agent payload(s) by changes in local endogenous conditions (such as pH, $t_{1/2}$, enzyme, redox state, and temperature status) [61–66, 102], or through application of an external/exogenous stimulus (Rosca et al. 2014, manuscript in submission). While much of previous work on this topic has revolved around change(s) in local endogeneous conditions [61–66, 102], the development of appropriate exogeneous stimuli looks to be a real growth area for the future. In principle, all **ABC** and **ABCD** nanoparticle systems could be triggered to exhibit physical property change(s) appropriate for controlled release through interaction with light, ultrasound, radiofrequency and thermal radiation from defined sources. So how might this be harnessed using “soft” organic and “hard” inorganic nanoparticles?

Today the journey towards "soft" organic LNPs for cancer therapy that can be described as truely triggered multimodal imaging theranostic drug-nanoparticles appears well underway. A few years ago thermally triggered drug-**ABC** nanoparticles (now known as Thermodox®, Celsion) were described based upon Doxil®. Thermodox® nanoparticles are formulated with lipids that included lysophospholipids in order to encapsulate doxorubicin within thermosensitive, nanoparticle lipid bilayer membranes [103, 104]. At induced temperatures above 37 °C, these membranes appear to become unexpectedly porous allowing for substantial local controlled release of drug. Needham et al. were first to demonstrate the use of such thermally triggered drug-**ABC** nanoparticles for the controlled local release of drug into target tissues in vivo [105], thus allowing for the potential treatment of tumours more efficiently than was achieved following administration of the thermally insensitive, Doxil® parent system [106]. Thermodox® is currently the subject of phase III HEAT studies and phase II ABLATE studies. In the latter studies, Thermodox® is administered intravenously in combination with Radio Frequency Ablation (RFA) of tumour tissue. In this case, the RFA acts as an exogenous source of local tissue hyperthermia (39.5–42 °C) that simultaneously acts as a thermal trigger for controlled release of encapsulated doxorubicin from the central aqueous cavity of Thermodox® nanoparticles. The company's pipeline going forward is focused on the use of Thermodox® nanoparticles under thermal triggered release conditions for the treatment of breast, colorectal and primary liver cancer lesions [107, 108]. This is the first time that thermally triggered drug-**ABC** nanoparticles have been devised and used in clinical trials.

A further evolution of this concept has now been more recently reported with the simultaneous entrapment of both doxorubicin and a MRI positive contrast agent, $Gd(HPDO_3A)(H_2O)$, into thermally triggered drug-**ABC** nanoparticles [109]. High Frequency Ultrasound (HIFU) was used as an alternative thermal trigger for the controlled release of encapsulated drug at 42 °C. The simultaneous release of MRI contrast agent enabled the observation of release in real time and led to an estimation of doxorubicin nanoparticle release kinetics. Researchers involved in Thermodox® have similarly reported on the development of thermally triggered drug-**ABC** nanoparticles with co-encapsulated doxorubicin and the MRI contrast agent Prohance® [110]. Using HIFU as a thermal trigger once more, they were able to promote controlled release of drug in rabbits with Vx2 tumours, and monitor drug release in real time by MRI [111]. The same researchers also developed an algorithm to simulate the thermal trigger effects of HIFU [112]. Simulation data were in agreement with mean tissue temperature increases from 37 °C to between 40.4 °C and 41.3 °C, resulting in quite heterogeneous drug release kinetic behaviour [112]. By contrast, we have striven to draw inspiration from the Gd-**ABC** and Gd-**ABCD** imaging nanoparticle systems described above [83–85, 113, 114], and Thermodox® data, in order to derive thermally triggered theranostic drug-**ABC** nanoparticles. These might also be described as thermal trig-anostic drug-**ABC** nanoparticles (shortened to the acronym thermal TNPs) (Fig. 18.3). By description, these nanoparticles are enabled for thermally triggered release of encapsulated drug in tumours by means of ultrasound together with real time, diagnostic imaging of nanoparticle

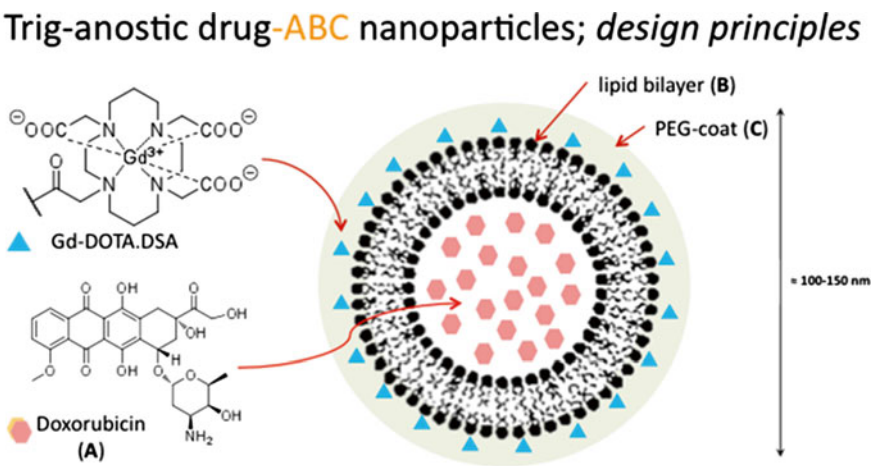

Fig. 18.3 Schematic of thermal trig-anostic drug-**ABC** nanoparticles (thermal TNPs) enabled for thermally triggered release of encapsulated drug in tumours by means of ultrasound together with real time, diagnostic imaging of nanoparticle biodistribution by MRI with drug pharmacokinetics

biodistribution with drug pharmacokinetics. Critical to this proposition is the use of Gd.DOTA.DSA once again. Going forward, lipidic MRI agent use should be supplemented with other imaging agents leading to new families of triggered multimodal imaging theranostic drug-**ABC** nanoparticles. These could also be described as trig-anostic^{n} drug-**ABC** nanoparticles where n is number of imaging modes employed, a description that could also be shortened to the acronym ^{n}TNPs.

In the case of "hard" inorganic nanoparticle systems, gold nanoparticles provide for a useful illustration. These belong to a class of nanoparticles known as nanoshells with tunable optical resonances. These nanoshells consist of a core, in this case silica that is surrounded by a thin metal shell, in this case gold [115]. These particles exhibit highly tunable surface plasmon resonances that absorb NIR radiation from a bespoke laser source and then transmit locally causing local tissue damage while leaving surrounding tissue intact [116]. Nanoshells are currently under evaluation in a number of clinical settings after a 5 years period of intensive preclinical development [117]. Obviously, in this instance, nanoshells are triggered to act in effect as their own "therapeutic agent", but nanoshells can also be administered in combination with anti-cancer therapeutic antibodies opening up options of combining

anti-cancer antibody therapy with hyperthermia therapy [118]. In hyperthermia treatment, nanoshells may be replaced shortly by nanorods in the next steps of development in these "hard" nanoparticle systems [119].

A peak of design must then be represented by the development of targeted trig-anostic^{n} therapeutically multifunctional drug-**ABCD** nanoparticles. These might also be described as targeted trig-anostic^{n} drug^{m}-**ABCD** nanoparticles, where *n* is number of imaging modes employed in nanoparticle and *m* is the number of active therapeutic agents (APIs) encapsulated/entrapped, a description that reduces to the corresponding acronym of targeted $^{n}T_{m}$NPs. Amazingly, while LNP and PNP systems of this type have yet to be devised, nanoshell structures have now been reported that have been pre-doped with MRI probes (by introduction of a 10 nm iron oxide layer over the silica core) and/or NIR probes (indocyanine green dye), then set up (with streptavidin) for surface conjugation of anti-HER2 antibodies (biotin labelled) with an additional surface PEG biocompatibility layer (introduced by disulphide post coupling bond formation). Such ensembles can be described readily as targeted trig-anostic^{2} drug^{2}-**ABCD** nanoparticle systems (or targeted $^{2}T_{2}$NPs) enabled for real time/diagnostic bimodal MRI and NIR contrast imaging accessed in combination with the capability for dual targeted and triggered chemotherapy (by anti-HER-2 antibodies) and photo-thermal ablation therapy (post illumination with a 808 nm wavelength NIR laser) either in vitro or in vivo [120, 121].

18.4 Conclusions and Future Perspective

Nanotechnology is revolutionising research and development in healthcare. Currently, the most advanced clinical-grade nanotechnologies in cancer are lipid-based and some "hard inorganic" nanoparticles. Recent studies show more evidence that biocompatibility and safety of nanoparticles depends on the material, and surface chemistry properties. Even quantum dots that have been previously characterised as toxic can be adapted for apparently safe use in non-human primates [122]. Unfortunately, there is still some scepticism from the big pharma industry and from clinicians themselves regarding the efficacy and safety of nanoparticle technologies. Such scepticism will only be solved with the advent of reliable cGMP-grade manufacturing processes and reliable preclinical ADME/toxicology data, followed by a range of successful first in man-studies. While these data are being acquired, nanoparticle technologies continue to be innovated in the laboratory. In this case, there appears to be an increasing push towards targeted trig-anostic^{n} drug^{m}-**ABCD** nanoparticles ($^{n}T_{m}$NPs) enabled for both targeted and triggered release of *m* active therapeutic agents (APIs) (including small molecule drug entities), all monitored simultaneously by real time/diagnostic imaging using *n* different imaging agent probes integrated into individual nanoparticles. Of the latter, both NIR and ^{19}F-NMR spectroscopy probes [123], could have real clinical potential alongside MRI. Such functional multiplicity offers the very real opportunity for highly personalized, hyper-functionalized drug-nanoparticles tailor-made (designed and assembled) from

tool-kits of chemical components that have themselves been highly refined for specific, personalized delivery applications. As this vision takes shape, so we will be looking on a very different world of innovative, interactive healthcare products with vastly more potential to treat and even to cure cancer than has ever been seen before.

And what of routine personalized cancer diagnosis and therapy? Do current advances in nanoparticle development allow us to close the virtuous circle of molecular profiling to personalized cancer nanomedicine? At this stage the answer must be, "not yet" or "status unproven". Clearly cancer imaging and therapy using nanoparticle technologies looks and is entirely becoming clinically realistic. But we are not yet at the point where patient specific, cancer disease-specific biomarkers can be detected in vivo using nanotechnology followed in the clinic by nanoparticle-mediated functional delivery of biomarker specific therapeutic agents. However, at least where ncRNAs are concerned, the prospect of such a cycle does appear imminent. As ncRNA profiling of cancers take place, so one can envisage a time when the follow on design of nanoparticles for the functional delivery of RNAi effectors targeted against specific cancer biomarker ncRNAs could become routine. Once this can be achieved, then the virtuous circle of personalized cancer nanomedicine will be properly closed.

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