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Rethinking cancer nanotheranostics

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Abstract

Advances in nanoparticle synthesis and engineering have produced nanoscale agents affording both therapeutic and diagnostic functions that are often referred to by the portmanteau ‘nanotheranostics’. The field is associated with many applications in the clinic, especially in cancer management. These include patient stratification, drug-release monitoring, imaging-guided focal therapy and post-treatment response monitoring. Recent advances in nanotheranostics have expanded this notion and enabled the characterization of individual tumours, the prediction of nanoparticle–tumour interactions, and the creation of tailor-designed nanomedicines for individualized treatment. Some of these applications require breaking the dogma that a nanotheranostic must combine both therapeutic and diagnostic agents within a single, physical entity; instead, it can be a general approach in which diagnosis and therapy are interwoven to solve clinical issues and improve treatment outcomes. In this Review, we describe the evolution and state of the art of cancer nanotheranostics, with an emphasis on clinical impact and translation.

An optimized cancer therapy would deliver the right type of therapy to the right target, to achieve localized control of the disease efficiently with minimal systemic toxicity. This task is daunting, because there is considerable variation among tumours and individual patients. Increasingly, it is clear that the battle against cancer cannot be won with a single formulation. Rather, it will require the careful coordination of diagnosis and therapy, stratification of patient and tumour subpopulations, and treatments tailored to individual needs. Emerging nanotechnologies offer a promising opportunity for this new campaign: extensive efforts from the past decade have produced a large arsenal of nanoplatforms with diversified capabilities for drug loading and release, and for tumour targeting. It is possible to impart imaging functions to these nanoplatforms, such that cancer can be diagnosed for individualized therapy, and therapy can be monitored non-invasively and in real time. Such a

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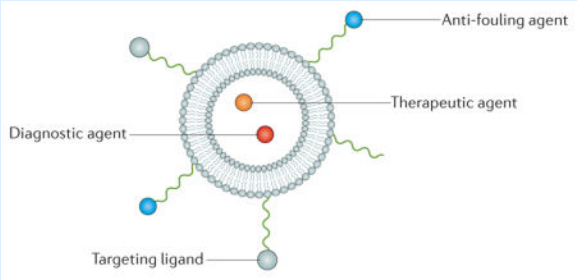
The authors declare no competing interests.

nano-enabled amalgamation of therapy and diagnosis is often known as ‘nanotheranostics’ (BOX 1) and consolidates advances in nanomaterials with those on other fronts, including imaging, biomarkers and therapy. A timeline of advances in nanotheranostics is provided in FIG. 1.

Box 1

Conventional nanotheranostics

A traditional nanotheranostic agent is integrated with both diagnostic and therapeutic moieties. Recently, nanotheranostics with diagnostic moieties and therapeutic moieties on separate nanoentities have demonstrated unique utility for cancer theranostics. To improve nanoparticle pharmacokinetics, nanotheranostics are often coated with a layer of anti-fouling agent and, sometimes, coupled with a ligand for active targeting.



Diagnostic agents

- Positron emission tomography: ^{64}Cu and ^{68}Ga
- Magnetic resonance imaging: Gd^{3+}, Mn^{2+} and iron oxide nanoparticles
- Ultrasound imaging: microbubbles
- Computed tomography: I and Au
- Optical imaging: quantum dots and fluorophores
- Single-photon emission computed tomography: ^{99m}Tc and ^{123}I
- Photoacoustic imaging: Au nanostructures and porphyrin

Therapeutic agents

- Chemotherapy: doxorubicin and paclitaxel
- Radiation therapy: Au, Hf and Gd
- Immunotherapy: cancer vaccines and immune checkpoint inhibitors
- Photodynamic therapy: indocyanine green
- Photothermal therapy: Au nanostructures
- Gene therapy: small interfering RNA, plasmids and CRISPR

At present, such advances have been largely confined to academic settings. Moreover, most nanotheranostic publications imply that imaging and therapy are performed essentially independently, rather than in an integrated protocol. We need to ask how to exploit these advances to solve previously unanswerable clinical questions. Also, we need to determine to what extent and in what ways integrated nanotheranostics are advantageous over discrete steps of imaging and therapy. How can nanotheranostics promote the development of nanomedicine? How can nanotheranostics transform from 'bench to bedside'?

One debate throughout the development of nanomedicine has been about the efficiency of the enhanced permeability and retention (EPR) effect in solid tumours. Discussions have included the advantages and disadvantages of passive and active targeting, and recent debates have been fuelled by a controversial paper in which it was concluded that the average tumour uptake of nanoparticles is only 0.7% of the injected dose[1]. When the EPR effect was first discovered, it was extolled by many as the Achilles' heel of cancer[2]; it is clear now that this is not the case. Delivering nanoparticle drugs to cancer cells is an extremely complicated process[3–5]. It requires that nanoparticles evade immune surveillance and avoid adsorption of serum opsonin proteins, and selectively extravasate at a tumour site. It also requires nanoparticles to overcome cancer cell intravasation, thick tumour stroma (the supportive tissue), uptake by macrophages, high interstitial fluid pressure (IFP) and slow diffusion, and to achieve homogeneous distribution throughout the tumour. From this perspective, the conventional EPR model, at least in its classical definition, is oversimplified and inadequate to predict nanoparticle accumulation in the tumour. Moreover, nanoparticle deposition in tumours is not governed by only one effect, such as EPR, but by a group of factors. Abstraction of delivery rules without taking these variables into consideration underlies many of the contradictions and controversies (for example, passive targeting versus active targeting) in this field[6]. Even worse, the initial assumption was that the magnitude of the EPR effect would be of the same order among different solid tumours, or at least among those of the same origin. This led to early, unrealistic hopes for nanomedicine[7]. The right attitude, we now believe, is to embrace the benefits of nanotechnologies but acknowledge their limitations. We need to give up the one-for-all or all-in-one obsession; instead, nanotherapies, perhaps even more than other modalities, need to work closely with diagnosis and be given only to the right patients[8]. Nanotheranostics, we believe, can and should have an important role in this new campaign[9–13].

In this Review, we discuss fundamental notions of nanotheranostics and review the history and state of the art of cancer nanotheranostics, including immune nanotheranostics to treat the suppressed immune system. We focus on methodologies with potential to make an impact in the clinic, such as patient stratification to identify subpopulations that are most likely to benefit from nanotherapy; tracking drug release and penetration within tumours; imaging-guided focal therapy; and the monitoring of therapeutic responses. Additionally, we lay out challenges and opportunities of cancer nanotheranostics, such as comprehensive individual tumour characterization, understanding and predicting nanoparticle–tumour interactions, and tailoring nanomedicines for optimized treatment. Many of these developments are at an early stage — we are still at the dawn of personalized medicine — but hold potential to revolutionize drug research and development, and clinical oncology.

Key concepts and the status quo

With both diagnostic and therapeutic functionalities, cancer nanotheranostics have been explored for applications beyond diagnosis or therapy alone. Specifically, some unique applications range from patient stratification to patient subpopulation screening, from monitoring intratumoural drug release to optimizing it for therapeutic efficacy, and from image-guided local therapy to therapy response monitoring. These unique applications are especially important in the context of tumour heterogeneity among patients, which demands personalized theranostic strategies.

Patient stratification

It is common to take labelled nanotherapeutic particles (FIG. 2) of similar compositions, but different sizes or shapes, and determine their optimal morphology by comparing their tumour uptake in the same animal model. However, it is rare to inject the same nanotherapeutics into tumour-bearing animals and stratify these ‘patients’ on the basis of differences in tumour uptake. This probably stems from the assumption that tumours in preclinical models often share similar background and characteristics. In particular, tumour variations among xenograft models are often considered small and their responses to nanotherapy comparable, which is not necessarily the case. For example, iodine-labelled liposome nanoprobes were injected into rats bearing breast tumour xenografts and tumour uptake was assessed by mammography[14]. Based on tumour uptake, the animals were divided into good- and bad-prognosis groups and treated with liposomal doxorubicin. The therapeutic efficacy correlated well with the classification, with the good-prognosis group showing slower tumour progression. This suggests that even in artificial xenograft models, tumour variation may have a considerable, underappreciated impact on treatment efficacy. For large animals bearing spontaneous tumours, the discrepancy is even greater. For example, a positron emission tomography–computed tomography (PET–CT) study of ^{64}Cu-labelled liposomes in 11 canine patients with cancer[15] found EPR-mediated tumour uptake in 6 out of 7 carcinomas but only 1 out of 4 sarcomas. Further, because tumour heterogeneity is increasingly recognized in both human patients and xenograft tumour models, it is certainly biased to choose to explore nanotheranostic applications solely in tumour models for which a nanomedicine works well. Therefore, we encourage researchers to consider tumour heterogeneity when exploring nanotheranostics, by using multiple relevant models and humanized tumour models, despite the inevitably elevated cost and effort involved.

From a technological perspective, patient stratification with nanotheranostics may not be far away (FIG. 3). In 2001, ^{111}In-labelled PEGylated liposomes (with no drug loaded) were injected into human patients who had different types of locally advanced tumours[16]. Effective tumour accumulation was found in 15 out of 17 cases, but there were large variations in tumour uptake among tumour types. Then, in a 2009 phase II clinical trial, a nanoparticle formulation consisting of *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer combined with doxorubicin was mixed with a ^{123}I-labelled analogue and injected into patients with different cancer types[17]. Accumulation of nanoparticles in primary tumours and, in some cases, in metastases, was observed. Moreover, liposomal doxorubicin

plus cisplatin was investigated for treatment against unresectable malignant pleural mesothelioma[18], and tumour uptake, quantified by ^{99m}Tc-liposome imaging, was positively correlated with patient response and survival[19]. However, in the above studies, the nanotheranostic agents were not assessed for patient stratification, but to evaluate the therapeutic efficacy of the nanomedicines. As far as we know, there is currently no nanotheranostic formulation in the pipeline for translation to clinical practice. Despite the technical soundness of the methodology, much work needs to be done to bring it forward for regulatory approval[20].

A specialized probe for each nanotherapeutic formulation would be ideal but is practically challenging in the short term. For the present, it is probably more realistic to develop generic imaging probes that can adequately predict the performance of a wide range of nano-therapeutics[21]. Recently, ferumoxytol, a clinically used iron oxide nanoparticle formulation, was assessed as such an imaging agent[22]. Dye-labelled ferumoxytol (~30 nm) and nanoparticles (~90 nm) consisting of poly(ethylene glycol) methyl ether-block-poly(lactide-co-glycolide) (PEG–PLGA) block copolymer were injected into mice bearing subcutaneous human fibrosarcoma, and high-resolution intravital microscopy was used to study the tumour accumulation of both particles. Despite the relatively large difference in size and composition, ferumoxytol showed a similar tumour accumulation pattern to the PLGA–PEG nanoparticles and can well predict the distribution of PLGA–PEG nanoparticles within tumours and microvasculature. Ferumoxytol-based MRI can adequately predict accumulation and treatment response of paclitaxel-loaded therapeutic nanoparticles. This approach is now being assessed in a clinical trial, where ferumoxytol-based MRI is used to predict patient response to MM-398, an irinotecan liposomal formulation[23]. Note that ferumoxytol is not an ideal imaging agent, but was developed to treat iron-deficiency anaemia in adult patients with chronic kidney disease. In the next stage, it will be worthwhile to develop dedicated tumour imaging probes[22,24], for example radioisotope-labelled liposomes (with no drug loaded), to screen patients for specific nanotherapeutics. In one relevant study, PET-radiolabelled nanoparticles were demonstrated to predict the efficacy of nanotherapies in animal models[24]. In particular, a zirconium-89 nanoreporter (^{89}Zr-NRep) co-administered with nanomedicines including Doxil precisely quantified the biodistribution of nanomedicines. Although substantial intertumour heterogeneity in accumulation was revealed by ^{89}Zr-NRep PET imaging, the therapeutic efficacy also positively correlated with the tumour accumulation of nanomedicine.

Monitoring intratumoural drug distribution

In many nanoparticle studies, the overall tumour uptake is considered the most important criterion in nanocarrier screening. However, this notion is potentially problematic. The intratumoural distribution of nanoparticles is never homogeneous but is affected by factors such as interstitial fluid pressure (IFP), blood flow, diffusion and stroma thickness[25] (FIG. 4). In particular, it is difficult for nanoparticles to migrate into the central, often hypoxic and sometimes necrotic, regions of a tumour[26–29], where the cancer cells have a high propensity to acquire stem-like phenotypes and enhanced tumorigenicity[30]. Hence, nanomedicines with comparable tumour uptakes may show different treatment outcomes because of discrepancies in intratumoural distribution. In addition to evaluating overall tumour uptake,

it is equally important to develop tools to map intratumoural nanoparticle deposition and drug release, and then include the information in prognosis[11].

In preclinical studies, this can be achieved by intravital microscopy. The high spatial and temporal resolution of this technology permits in-depth analysis of interactions between nanoparticles and tumours, and can guide therapeutic optimization. For example, this method was used to investigate tumour accumulation and drug release of doxorubicin-loaded, temperature-sensitive liposomes, whereby drug release induced by hyperthermia intravascularly was found to lead to increased free drug in the tumour interstitial space[31]. A phase III clinical trial of a temperature-sensitive liposomal doxorubicin, ThermoDox, with radiofrequency ablation is under way for the treatment of hepatocellular carcinoma[32]. Intravital microscopy was also used to study the dynamic interaction between tumours and single-walled carbon nanotubes[33]. Unlike in previous thoughts, the nanotubes were first taken up by Ly-6C^{hi} monocytes and delivered to tumours by cell-mediated transportation. In another example, intravital microscopy was used to study micelle nanoparticle delivery to BxPC3 tumours. In addition to static pores in the endothelium, dynamic vascular bursts were identified that mediate nanoparticle extravasation at the tumour sites[34].

In the clinic, intravital microscopy has limited use owing to its invasiveness. Instead, MRI-based imaging methods have been developed[35]. MRI affords high spatial and temporal resolution, which is essential for in-depth analysis of the intratumoural behaviour of a nanomedicine. MRI-based imaging can be achieved by imparting a T_1 or T_2 imaging probe into nanoparticles, preferably ones that are currently used in the clinic. Different nanoparticle-based systems for drug-release monitoring are depicted in FIG. 5 and detailed as follows. For example, both Gd-DTPA and (1,2-diaminocyclohexane)platinum(II) (DACHPt) were incorporated into polymeric micelles through reversible chelation. The R_1 (relaxivity of T_1) of the resulting micelles was 24 times higher than that of Gd chelates, making it an effective nanotheranostic agent for MRI-based tumour accumulation tracking[36]. However, probably a more advantageous approach to monitoring drug release is to convert nanoparticles into a switchable MRI probe that experiences a signal change following drug liberation. This approach has been used successfully in liposome-based nanotherapeutics, where Mn^{2+}/Gd^{3+}-based salts or chelates are loaded into the interior of nanoparticles along with drug molecules. Owing to limited access to the bulk water, Mn^{2+}/Gd^{3+} induces only slight T_1 shortening in an intact liposome particle; when the lipid layer is breached, for example, in the tumour extracellular environment, the payloads are released to the aqueous surroundings, causing hyperintensities on a T_1-weighted image that can serve as indicators of drug release. For example, in one study, MRI was used to monitor drug release with $MnSO_4$–doxorubicin liposome nanoparticles[37,38]. In another study, Gd(HPDO3A) was incorporated into doxorubicin liposomes to track drug release[39]. In both cases, good spatial correlation was found between T_1-shortening effects and doxorubicin liberation, thereby demonstrating that MRI can be used to derive intratumoural drug-dose painting[38]. Such an activatable MRI imaging approach has also been seen with other types of nanotherapeutics. For example, it was shown that drug molecules can be adsorbed to the coating of ferumoxytol and released in the acidic tumour extracellular environment[40]. This is accompanied by a significant change of the T_2 relaxation time, making ferumoxytol an interesting theranostic nanoplatform. In another study, arsenic trioxide (ATO) and Mn^{2+}

were combined to prepare water-insoluble manganese arsenite complexes[41]. In the tumour microenvironment, acidic stimuli triggered the simultaneous release of manganese ions and ATO, leading to a shortened T_1, which could then be monitored by MRI in real-time.

Some nanomedicines are designed in such a manner that the drug release is governed by an exogenous rather than endogenous stimulus, such as ultrasound, heat, photo-irradiation or X-rays[42,43]. Imaging guidance is important for these nanomedicines, because the timing to apply the stimulus may greatly affect intratumoural drug distribution. For example, Mn^{2+}-doxorubicin temperature-sensitive liposomes were injected into fibro-sarcoma-bearing rats, and heat was delivered to the tumour centres by means of a catheter[38]. The total amount of drug released was found to be higher when the liposomes were administered during, but not after, hyperthermia. However, in this case, drugs accumulated mostly at the tumour periphery, where large arteries are located. By contrast, when nanoparticles were injected before hyperthermia, there was relatively low overall drug uptake in tumours, but the perfusion was substantially greater. This is good evidence that intratumoural drug distribution can be independent of overall tumour uptake and should be monitored separately. It is worth mentioning that recent advances in high-intensity focused ultrasound (HIFU) allow hyperthermia to be delivered to tumours in a non-invasive manner. This makes heat-regulated drug release with temperature-sensitive liposomes highly promising in the clinic[44]. MRI-based drug-release monitoring may play an important role in the clinical translation of this technology[45]. Ultrasound imaging will be another important tool to guild HIFU ablation and to monitor drug release[46].

It may be possible to use one nanotheranostic agent to monitor both tumour uptake and drug release. This can be achieved by exploiting multiple imaging modalities (for example, PET for tumour uptake analysis and MRI for assessment of intratumoural drug release). Alternatively, multiplexed MRI can be used to track the two processes. For example, both iron oxide nanoparticles and Gd-DTPA were incorporated into 5-fluorouracil (5-FU)-loaded PLGA nanospheres and microspheres[47]. Tumour targeting was tracked by T_2-weighted MRI, while the drug release was monitored by T_1-weighted MRI. In another example, Tm(HPDO3A)(H_2O) and NH_4PF_6 (used as ^1H chemical exchange saturation transfer (CEST) and ^{19}F contrast agents, respectively) were loaded into liposomes[48]. The CEST signal was strong for intact nanoparticles, but dropped significantly when the liposome membrane was breached during drug release. By contrast, the ^{19}F signal was quenched in intact nanoparticles, but enhanced when Tm(HPDO3A)(H_2O) was released to the surroundings. In the next stage of development, more effort should be invested in developing nanotheranostics that permit simultaneous assessment of overall tumour uptake and intratumoural drug release, and tools that can integrate the information for accurate prognosis.

Clinical imaging has its intrinsic limitations. Even with advanced nanotheranostics, it is not possible to use current clinical imaging technologies to examine nanodrug dynamics at the cellular or molecular levels, which is critical to the delivery of certain therapeutics, especially small interfering RNA (siRNA) and microRNA[49–51]. Companion biopsy and histological studies analysis may complement current imaging technologies to reveal

information in a relatively systemic manner. However, protocols remain to be established at the clinical level.

Imaging-guided focal therapy

Nanoparticle-enabled focal therapy, such as photodynamic therapy (PDT) and photothermal therapy (PTT)[52–56], has advantages including low systemic toxicity, no induced resistance and high tumour selectivity. Unlike the delivery of chemotherapeutics, the tumour selectivity in PDT and PTT is mainly governed by photo-irradiation rather than nanoparticle distribution. Still, it is important to monitor nanoparticle tumour accumulation so that the irradiation can be given at the best time interval (for example, to achieve the highest tumour-to-normal-tissue ratio of nanoparticle accumulation) for optimal treatment outcomes. However, in most nanoparticle-based PDT and PTT studies, imaging and therapy are conducted separately, rather than as an integrated protocol. Another problem is that, owing to limited tissue penetration of light, PDT and PTT are more promising in the treatment of cancers that are close to the skin, internal linings accessible by endoscopy, or organ surfaces that can be exposed during surgery. Therefore, it is important to assess nanotheranostic PDT and PTT in relevant animal models, with clinically compatible methods of light delivery and an emphasis on minimizing collateral damage to surrounding normal tissues. These assessments are rarely found in current studies.

Despite the problems outlined above, encouraging progress is being made. In one recent study, a porphyrin lipoprotein (PLP)-mimicking nanoparticle was investigated for imaging-guided surgery and PDT[57]. Each PLP contains multiple porphyrin molecules and can efficiently chelate with ^{64}Cu and produce singlet oxygen ($^1O_2$) under photo-irradiation. PLPs also afford switchable near-infrared fluorescence, which is quenched in intact nanoparticles, but activated when the particles are disassembled. When tested in VX-2 buccal carcinoma rabbit models, PLPs showed selective accumulation in primary tumours and metastatic nodes. This was attributable to the EPR effect and possibly the high affinity of porphyrin against cancer cells. The tumours were visualized by both PET imaging and intraoperative fluorescence imaging, enabling precise photo-irradiation and tumour eradication. In another example, a PTT agent called porphysome was used, which features high porphyrin packing density and high light-to-heat conversion efficiency[55]. When tested in a rat orthotopic prostate cancer model, ^{64}Cu-porphysome showed good tumour homing effect, manifesting a tumour-to-normal-prostate ratio of 6:1. This was followed by MRI-guided insertion of an optical fibre to the tumour region to initiate PTT, and magnetic resonance thermometry to monitor regional temperature changes. The zone of temperature increase matched the tumour boundary well, leading to selective PTT damage while minimally affecting normal prostate tissues. These systems underscore the unique capability of nanotheranostic agents for imaging-guided focal therapy.

The good light-to-heat conversion efficiency of most PTT agents also makes them capable photoacoustic imaging (PAI) agents. Therefore, it is possible to track PTT nanoparticles by PAI, saving the need for an extra imaging probe. For example, several nanoparticle-based PTT agents with multiple imaging capabilities, including gold nanovesicles[58], gold nanorods and melanin-coated Fe_3O_4 nanoparticles[59], have been reported. In an orthotopic U87MG

mouse model, it was shown that PAI can be used to guide selective photothermal ablation induced by hollow gold nanospheres[60]. It was demonstrated that the migration of gold-plated carbon nanotubes, or golden carbon nanotubes (GCNs), can be tracked by PAI. In particular, antibody–GCN conjugates home to lymphatic endothelial walls, and the PAI results can then guide a laser to induce localized damage to the lymphatic vessels with great accuracy[61].

Nanoparticle-enhanced radiation therapy is less studied than PDT and PTT, which is unfortunate because it is a mainstay in clinical oncology and nanotechnology holds great potential to enhance its efficacy. Unlike PDT and PTT, clinical radiation therapy has almost no difficulties with tissue penetration. With advanced radiation planning and delivery (for example, intensity-modulated radiation therapy and stereotactic body radiotherapy), X-rays can be delivered in a 3D conformal manner to cover tumours lying under deep tissues[62]. However, one main problem of radiation therapy is that some cancer cells are refractory to the treatment. To improve efficacy, chemotherapy is often administered during radiation therapy to sensitize the cancer cells (known as chemoradiation therapy)[63–66]. However, the trade-off of the accompanying chemotherapy is increased systemic toxicity and even increased mortality[67]. Nanomedicines may find wide application in this context by reducing side effects and increasing drug bioavailability. For example, promising results were obtained from a series of clinical studies conducted to investigate liposomal doxorubicin as a radiosensitizer for non-small-cell lung cancer (NSCLC), breast cancer and bladder cancer management[68,69]. However, nanoparticle-based chemo-radiotherapy has not yet been adopted in the clinic. In addition to the relatively slow clinical translation of nanomedicine (and thus limited options for these therapeutics), another important reason is that current clinical nanomedicines were not designed as adjuncts to radiotherapy, and hence their radiosensitizing effects are suboptimal. Fortunately, efforts are emerging to develop dedicated nanotherapeutics for chemoradiation therapy. For example, Genexol-PM, a clinically used micelle formulation, was shown to improve paclitaxel accumulation in rodent NSCLC tumours[70]. This led to enhanced radiation therapy efficacy, while reducing toxicity to healthy lung tissues and other major organs. Nanoparticle formulations of histone deacetylase inhibitors[71] and wortmanin[72,73] were also investigated as radiosensitizers with encouraging results.

In addition to chemotherapeutics, nanoparticles containing high-atomic-number elements (for example, gold, iodine and gadolinium) have also been used for radiosensitization. With high absorption cross sections, these heavy-element nanoparticles can improve energy deposition in tumour areas, leading to improved radiation therapy efficacy and reduced radiation doses[74]. For example, it was shown in Panc-1 xenograft models that systemic administration of Arg–Gly–Asp (RGD) peptide-conjugated gold nanoparticles and conformal-image-guided radiation therapy can lead to site-specific damage of tumour neoendothelium[75]. In another study, it was shown that NBTXR3, a 50 nm hafnium oxide nanosphere, can substantially enhance radiation therapy efficacy when intratumourally injected into different tumour xenograft models[76]. This nanoparticle formula is in phase II/III clinical trials for improving radiation therapy against advanced soft tissue sarcoma of the extremities[77], and in phase I/II for head and neck cancer, liver cancers (both hepatocellular carcinoma and liver metastases), prostate cancer and rectal cancer radiation

therapy. Recently, AGuIX, a ~3 nm polysiloxane and a Gd-DTPA conjugate, was investigated as a nanoparticle radiosensitizer[14]. With a high Gd content, these nanoparticles showed good T_1 contrast and impressive radiosensitizing effects. For example, in a clonogenic assay against B16F10 cells, high sensitivity enhancement ratio and dose enhancement fractions were observed[78]. Owing to efficient renal clearance, these nanoparticles induced little systemic toxicity, which was confirmed in mouse and cynomolgus monkey models[79]. When injected into murine brain metastasis models, AGuIX efficiently accumulated in tumours, as verified by T_1-weighted MRI. Whole-brain radiotherapy was applied at a time post-injection determined from the imaging results for optimal tumour selectivity. The radiosensitizing effect of AGuIX was also demonstrated in mouse pancreatic xenograft models and a good safety profile and imaging-guidance potential was shown in cynomolgus monkeys[79]. This nanotheranostics is now under clinical trial for treating brain metastasis[80].

It is possible to combine radiation therapy with another focal treatment. A good example is the combination of radiation therapy and PDT, which can induce a synergistic effect in cancer cell killing[81]. However, synergy has been shown only to occur when the two modalities are applied simultaneously[82], which is not practical in the clinic. New scintillator nanoparticles, such as those made of Tb_2O_3 (REF. 83), LaF_3:Tb (REF. 84) and $SrAl_2O_4$:Eu (REFS 85,86), can be used to down-convert X-ray photons to visible photons, and in turn, activate a PDT process. Alternatively, Cherenkov radiation from radionuclides can be harnessed to activate titanium dioxide nanoparticles and produce reactive oxygen species[87]. Although different, the two approaches both constitute an effective PDT and radiation therapy combination, which explains the efficient killing of cancer cells, including those that are refractory to radiation therapy alone. The combination of such a radiation therapy and PDT also represents a significant advance for overcoming the shallow penetration of conventional PDT.

Nanoparticle probes can also function as intraoperative imaging agents to guide surgical procedures. This is important because tumours are often irregular in shape, and the margins are difficult to identify during surgery. Incomplete resection may cause lethal recurrence, whereas removal of normal tissues induces excessive morbidity. Previously, intraoperative MRI, often facilitated by Gd agents, has been investigated to guide surgery in the clinic. However, this method has certain restrictions, including narrow imaging time windows, high injection doses and suboptimal specificity. To address these issues, there has been an interest in developing intraoperative optical imaging probes, which potentially offer better sensitivity and specificity with regard to margin delineation. For example, plasmonic nanobubbles were investigated as intraoperative acoustic probes[88]. These nanobubbles are not introduced exogenously but generated *in vivo* from around gold nanoclusters under short laser pulses. In another study, a MRI–PAI–Raman trimodality nanoprobe was evaluated for assisting surgical removal of brain tumours. In this probe, the MRI component allows for preoperative detection and surgical planning, while the PAI component can be used to guide bulk tumour resection during surgery, and the Raman imaging enables accurate removal of residual microscopic tumour burden[89]. Another type of material that has been used for tumour imaging is silica nanoparticles, called Cornell dots or C dots[90]. Specifically, the C dots were encapsulated with the cyanine dye Cy5 and ^{124}I, and conjugated with cyclic RGD peptide on

the surface, making them compact optical-PET probes with great tumour affinity as well as good renal clearance. A recent study revealed that the ultrasmall C dots can also be used for cancer treatment by nanoparticle-induced modulation of iron and reactive oxygen species levels within cancer cells and the tumour microenvironment, to induce ferroptosis in nutrient-deprived cancer cells, and to suppress tumour growth[91].

Therapy response monitoring

Post-therapy response monitoring is an appealing concept that has frequently appeared in nanomedicine papers[92]. The rationale is rapid prognosis after each cycle of treatment, so that timely adjustments can be made long before the conventional endpoints. In preclinical studies, tumours are often implanted subcutaneously, and their dimensions can be easily measured by calipers. Tumours that are inoculated into internal organs have often been genetically engineered to express fluorescent, bioluminescent, MRI or PET reporters, so that their tumour load can be monitored non-invasively[93,94]. However, these techniques are obviously not viable in the clinic. Hence, many researchers suggest introducing imaging functions into nanoparticles to permit therapy response monitoring. Although this sounds logical, it is not clear exactly how this could work in a clinical setting and what the benefits might be. The concept of early response monitoring originates from nuclear diagnosis (PET and single-photon emission computed tomography), with an emphasis on sensitive detection of alterations at the molecular or genetic level[95]. However, owing to the relatively large sizes and limited access to intracellular targets, nanoparticles are suboptimal or even inadequate for the task.

Perhaps nanotheranostics can find applications in sensing a change in the tumour microenvironment (TME) that is relevant to progression in therapy. Compared with a healthy microenvironment, the TME has some distinct physiological features such as lower pH and more hypoxia in some tumours, and may have altered signature of molecular biomarkers, such as matrix metalloproteinase (MMP). Therefore, it is logical that effective therapeutics would normalized these TME parameters, and thus monitoring these TME parameters would help to monitor therapy response and provide feedback to evaluate previous therapeutic efficacy and guide future therapy. For example, optical nanoprobes with a sharp pH response ($\Delta pH_{10–90\%} < 0.25$) were used in the acidic tumour extracellular environment[96]. However, because of the limited tissue penetration of light, this technique is yet to find its way to the clinic. A Mn^{2+}-doped calcium phosphate nanoprobe was reported, which disintegrates at low pH, liberating Mn^{2+} and mediating signal enhancement on T_1 images[97]. However, MRI has low sensitivity, takes a relatively long acquisition time (up to 1 h) and is difficult for quantification. We and others have developed a series of optical probes that can detect changes in MMP concentration in the tumour extracellular matrix[98]. This means, however, that the therapeutic and imaging functionalities of the same nanoparticle target different components of a tumour, which is considered unfavourable. Moreover, there are different sets of requirements on pharmacokinetics, clearance and dosage for diagnostic and therapeutic agents, which can hardly be satisfied within a single nanoscale particle.

For the present, it is probably more realistic to use separate diagnostic methods to follow nanotherapy, rather than imposing the function of imaging on a nanomedicine. This by no

means defies the concept of treatment response monitoring; by contrast, owing to the importance of the notion, it is worth breaking the stereotype that a nanotheranostic must be a physical object concurrently carrying both prognosis (or diagnosis) and therapy functionalities. In several successful studies, conventional diagnostic tools, such as ^{18}F-fluorodeoxyglucose (^{18}F-FDG), 2-fluoropropionyl labelled PEGylated dimeric RGD peptide (^{18}F-FPPRGD$_2$), diffusion-weighted MRI and blood-oxygenation-level-dependent MRI[99–103], have been successfully used for early prognosis for nanotherapy with adequate prediction accuracy. Because the concept is no different from traditional prognosis, we will not expand the discussion here. Therapeutic response monitoring should provide feedback that can guide follow-up regimens. This has rarely been demonstrated in nanotherapy, probably owing to a lack of established treatment options, and needs to be explored in more depth.

Challenges and new opportunities

One of the main issues in nanomedicine development has been underestimation of tumour heterogeneity[104]. This is largely attributable to an oversimplified pharmacokinetic model, in which the tumour is seen as a leaky sponge into which nanoparticles can be efficiently deposited, given a sufficiently long circulation half-life. Based on this model, nanomedicine research has focused on inhibiting or minimizing opsonin adsorption, reticuloendothelial system uptake and renal clearance. It is increasingly clear that this model downplays the impacts of microvessel density, vessel leakiness, interstitial fluid pressure, blood flow, stromal thickness and macrophage abundance, as well as variations of these factors among tumours[105,106]. Each of these properties may strongly affect nanoparticle accumulation and penetration in tumours. For optimal nanoparticle delivery, it is important to treat each tumour as a unique and complex organ. Instead of attributing all factors to the EPR effect, which is almost impossible to quantify, it is useful to look into each individual factor and combine the information for comprehensive prognosis. Nanotheranostics can play an important role in this arena, but because of the large number of variables, it is not always feasible to derive full information from one type of nanoparticle. Similar to the case of therapeutic response monitoring, we suggest for many of the new challenges discussed below not to treat nanotheranostics as a physical entity, but as a generic approach in which diagnosis is exploited to guide or assist a nanotherapeutic procedure. Only then can we maximally harness advances on multiple fronts to identify variations among tumours and devise optimal therapeutic regimens, and thus develop innovative and effective cancer nanotheranostics. In this section, we will specifically discuss tumour heterogeneity (especially tumour vasculature variation), approaches to tailor nanoparticles and enhance the EPR effect, and emerging cancer immunotherapeutic nanomedicine.

Variation in vascular density among tumours

Although high vascular density is considered a common tumour characteristics and a key factor contributing to the EPR effect, there is great variation in vascular density among tumours. For example, tumour microvessel density may be only 14.8 vessels per microscopic field (mm^2) for squamous cell carcinoma, but up to 145 vessels per field for colorectal carcinoma[107]. Analysing individual tumour microvessel density would provide

valuable information with which to predict nanoparticle accumulation in tumours. Tumour vascular density can be assessed non-invasively by CT-based[108], MRI-based[109] or ultrasound-based[110] angiography.

There is also considerable variation in the leakiness of tumour vasculature. In preclinical studies, vasculature leakiness is often assessed by an extravasation assay that uses Evans blue dye[111]. However, because this is a histological method, it is invasive and has clear limitations in the clinic. Alternatively, vasculature leakiness can be evaluated by dynamic contrast-enhanced MRI, which provides functional information on capillary blood flow and volume in tumours[112,113]. Vascular leakiness may also be assessed by MRI angiogram with gadofosveset, which is an albumin-binding blood tool agent that has recently obtained regulatory approval[114]. Recently, we developed a series of Evans-blue-derived radiotracers, which includes a 1,4,7-triazacyclononane-N,N′,N″-triacetic acid (NOTA)-conjugated truncated form of Evans blue (NEB)[115]. In particular, ^{68}Ga-NEB is now under clinical trials for diagnosis of haemangiomas[116] and evaluation of lymphatic disorders[117].

It is difficult to differentiate the impact of microvessel density from that of the vessel leakiness; the compound effect of both is measured in all methods mentioned above. For better assessment of vessel leakiness, it is beneficial to evaluate a group of nanoprobes with similar compositions but varied sizes. This is commonly seen in nanoparticle optimization, but has rarely been pursued as a tool in tumour imaging.

In addition to vasculature leakiness and density, increasing attention is being paid to other tumour properties that may regulate nanoparticle deposition. A methodical model that takes into account spatially distributed diffusion-convection properties was developed to depict nanoparticle uptake by tumours[118]. The simulation suggested that the complex transport micro-environment was responsible for the large inter-subject variations in nanoparticle tumour accumulation. In particular, the IFP was identified as an important contributing factor in restricting transvascular transport of nanoparticles and their migration within tumours. In another study, multiple imaging methods were used to investigate liposome accumulation in tumours, including CT angiography to examine tumour vascularity, perfusion CT to assess blood flow and fluorescence molecular tomography to assess nanoparticle spatial-temporal distribution[108]. Their study suggested that liposome deposition is highly dependent on regional blood flow, which shows large intertumour and intratumour variation. Such variations in tumour blood flow and IFP, along with other heterogeneities, such as collagen density and perivascular cancer cell density, all contribute to the complexity of nanoparticle delivery to tumours.

In summary, accurate prognosis should be based on thorough characterizations of each contributing or restricting factor within a tumour, rather than on the broad, sometimes vague, EPR effect. Piecing together the information to enable precise predictions will demand extensive efforts as individual diagnostic tools become increasingly available.

Tailored nanoparticles for optimal tumour uptake

In many studies, nanoparticle extravasation at a tumour site is considered the endpoint of the delivery, and to improve tumour uptake, an extended circulation half-life is favoured. Hence,

tremendous efforts have been spent on elucidating the relationship between the physicochemical properties of nanoparticles and their circulation times. However, extravasation at a tumour site is only half of the journey for nanomedicine transportation. For effective treatment, nanoparticles (or their payloads) need to distribute evenly throughout tumours, and this is not easy for many nanoparticles, whose migration within tumours is governed by the slow process of diffusion, rather than convection. Potential differences in tumour distribution constitute a source of heterogeneity for nanoparticle-based therapy. We also need to understand the impact of nanoparticle physicochemical properties on intratumoural migration and distribution, based on tumour characterizations, to be able to predict whether a patient is suitable for particular nanomedicines. Unfortunately, the required investigations are still in their infancy.

In TABLE 1, we summarize nanoparticle design rationale with an emphasis on enhancing perfusion and retention of nanomedicines within tumours. Taking size, the most widely studied nanoparticle physical property, as an example, it is now generally accepted that nanoparticles larger than 200 nm or smaller than 5 nm are inappropriate for tumour targeting, because they are rapidly cleared from the body. There is no consensus, however, on what sizes within the 5–200 nm range are optimal. For example, 90 nm Doxil was chosen on the basis of early studies suggesting that ~100 nm was a good balance between high drug loading and favourable pharmacokinetics. However, later studies found that in many solid tumours, Doxil accumulated mainly at the periphery and perfused only minimally into the centre. By contrast, smaller nanoparticles, despite having advantages in tumour penetration, are not necessarily associated with high tumour uptake. This has been explained by means of a pharmacokinetic model for quantitative analysis of the EPR effect[119]. According to this model, the EPR effect, although aiding nanoparticle extravasation, also permits nanoparticles to re-enter the blood circulation. This is important because the tumour interstitial fluid has a much slower flow rate than the pulsatile flow; hence, compared with nanoparticles in the vessel lumen, those in the interstitial space stay much longer at an endothelial defect, meaning a high probability of intravasation, especially for small nanoparticles. This effect was observed in a study in which 20 nm, 50 nm and 200 nm drug–silica nanoconjugates were injected into MCF-7 breast-tumour-bearing mice[120]. Nanoconjugates with a diameter of 200 nm were poor in tumour extravasation, whereas 20 nm nanoconjugates were quickly cleared from the tumour region. The 50 nm particles, by contrast, seemed to offer a good balance, leading to the highest tumour tissue retention.

Such rules of thumb are not strict but are largely dependent on individual tumours. For example, the tumour accumulation and treatment efficacy was compared for polymeric micelle nanoparticles of different diameters (30, 50, 70 and 100 nm) in animals bearing either highly permeable C26 colon tumours or poorly permeable BxPC3 pancreatic tumours[121]. With C26 tumour models, there were no significant impacts of size on either delivery or treatment efficacy. By contrast, with BxPC3 tumour models, 50, 70 and 100 nm nanoparticles showed poor tumour penetration owing to their relatively bulky sizes. However, 30 nm nanoparticles could diffuse into the central areas of tumours, leading to the best treatment outcome. In another important study, disposition of liposomes in the tumours was found to be dependent on both nanoparticle size and local blood flow, with relatively

large nanoparticles (for example, 100 nm) accumulating more in the fast-flow regions and small nanoparticles (for example, 30 nm) in the slow-flow regions[108].

Such complexity in nanoparticle–tumour interactions again underscores the flaws of a one-size-fits-all approach. For optimal treatment, nanoparticle size may need to be tailored to suit tumour haemodynamics and pathology. Nanoparticles with varied sizes have not been pursued as a tool in tumour imaging, and this approach may face challenges in clinical translation owing to toxicity and cost concerns. This notion can also be expanded to other nanoparticle properties, such as shape, rigidity and surface charge. Whereas there have been tremendous efforts to elucidate the impact of these physicochemical properties on nanoparticle blood circulation half-lives and uptake in the reticuloendothelial system, their influences on intratumoural distribution and penetration have not been adequately studied. In addition, recent investigation of the protein corona effect of nanoparticles has identified key factors that influence nanoparticle–tumour interactions, such as the physicochemical properties of the nanoparticles, exposure time, protein type, and nanoparticle concentration[122,123].

Tumour-tailored targeting approaches may also include active targeting. The results of a recent simulation suggest that tumour-targeting ligands may improve nanoparticle retention after extravasation and minimize intravasation[119]. The approach would probably work better for relatively small nanoparticles, which are more susceptible to washout from the interstitial space. For example, conjugating a ligand that targeted epidermal growth factor receptor (EGFR) to a 30 nm liposome nanoparticle was found to increase deposition in tumours[108]. By contrast, adding the same EGFR-targeting ligand to 100 nm liposomes had minimal impact. One should also keep in mind that other factors are relevant in tumour deposition. For example, in the study mentioned above, increased tumour uptake for 30 nm active targeting particles was only seen in regions of slow flow rate. Overlooking these impacts of tumour specifics is probably behind many of the controversies regarding active versus passive targeting.

Another approach explored to improve tumour delivery is ‘size expansion’. Briefly, molecules or small nanoparticles are injected systemically and undergo self-assembly within tumours. This approach enables enhanced tumour uptake through favourable extravasation and minimized intravasation. An example was the use of pH-sensitive PDPA-b-PAMA/SA (succinic anhydride (SA)-modified poly(2-diisopropylaminoethyl methacrylate)-block-poly(2-aminoethyl methacrylate hydrochloride)) micelles, which agglomerated in the acidic tumour microenvironment, forming aggregates that were less susceptible to intravasation[124]. In another study, a gelatinase-responsive molecule, P18-PLGVRGRGD, which can self-assemble into fibrous nanostructures within tumours, led to increased retention[125]. Interestingly, an opposite, ‘size reduction’ approach has also been explored to aid nanoparticle delivery. The idea is to design relatively large nanoparticles that can ‘smartly’ reduce their dimensions in tumour areas, thereby migrating deeper into tumours. For example, a composite nanosystem was developed that contained a 100 nm gelatin core and multiple ~10 nm quantum dot satellites. These nanoparticles were degraded by MMPs within tumours, releasing quantum dots to the surroundings[126]. In another example, ~100 nm polymeric clustered nanoparticles were used, which were disassembled in the acidic

tumour extracellular environment, releasing ~5 nm platinum prodrug-conjugated poly(amidoamine) dendrimers[127]. Other examples include porphyrin microbubbles that can burst into nanoparticles in tumour areas upon *in situ* ultrasound stimuli and an injectable nanoparticle generator consisting of micrometre-sized porous silicon particles encapsulated with doxorubicin–polymer conjugates, which, once released within tumours, self- assemble into nanoparticles that can be internalized by cancer cells to induce cell death[128].

Increasing the EPR effect for optimal tumour uptake

As mentioned above, many factors, including low micro-vascular permeability, high IFP and thick stroma, may prevent efficient delivery of nanoparticles to and perfusion within a tumour. Choosing the right nanoparticle formulation and targeting strategy may improve the delivery, but opposing factors in some tumours may simply be too strong. However, in such cases it may be possible to artificially modify the tumour microenvironment and tip the balance in favour of nanoparticle extravasation and penetration[3], as summarized in TABLE 1.

One such example is IFP management. IFP is determined by several factors, including hyperpermeable and tortuous tumour vasculature, increased transvascular fluid flow, high concentrations of plasma proteins, compromised lymphatic drainage, and compression of interstitial space by fast-proliferating tumour and stroma cells. Instead of addressing these individual factors, a more sophisticated approach is to ‘normalize’ the abnormal tumour vasculature that underlies the high IFP. For example, treating tumours with an anti-angiogenesis agent (an anti-vascular endothelial growth factor (anti-VEGF) monoclonal antibody) combined with radiation was shown to lower the IFP in tumours by up to 74%[129]. In a different study, treatment with an anti-VEGF was found to markedly increase the penetration distance of bovine serum albumin from tumour vessels[130]. This strategy was also tested in the clinic with bevacizumab, which is a humanized anti-VEGF monoclonal antibody. In patients with rectal carcinoma, the treatment led to a decrease of IFP by 73%[131]. In patients with NSCLC, bevacizumab treatment was found to enhance tumour vasculature and blood perfusion in some patients, improving therapy efficacy with carboplatin and abraxane[132]. Such a normalization treatment can be used in conjunction with nanomedicines to enhance intratumour delivery and therapeutic efficacy[133].

Another barrier to efficient nanoparticle delivery is insufficient tumour vascular leakiness. Tumours often possess discontinuous endothelial layers, but fenestration sizes range widely, probably from 50 to 4,700 nm (REF. 134). Clearly, the dimensions of the fenestra may greatly affect the extravasation of nanoparticle drugs, especially relatively large ones, and it is hoped that an external stimulus can enlarge the endothelial gaps to augment the EPR effect and promote nanoparticle accumulation. This can be achieved by vasculature-targeting photodynamic therapy, which causes cell rounding and contraction, leading to enlarged endothelial gaps. The approach was previously demonstrated with small-molecule photosensitizer-mediated PDT[135] and with ferritin-mediated vascular targeting PDT[136]. Moreover, ultrasound has been explored as a weapon against poorly penetrable endothelium. This often involves the use of microbubbles, which, in response to an external ultrasound stimulus, collapse within blood vessels, generating mechanical stress that can disrupt cell

membranes and enhance capillary permeability. This strategy has been used in preclinical and clinical studies to temporarily loosen the blood–brain barrier, improving extravasation of macromolecules and nanoparticle drugs[137,138].

Owing to a relative abundance of nutrients and oxygen, tumour perivascular regions often have a dense layer of cancer cells that may restrict penetration of nanoparticles and, by compressing interstitial space, cause increased IFP. These perivascular cancer cells have also been investigated as a target for enhanced EPR. Radiotherapy, which preferentially kills oxygenated cells, can be applied to eliminate perivascular cells, leading to increased nanoparticle accumulation. For example, a single radiation dose led to a transient increase of tumour uptake of polyamidoamine dendrimers[139], and immunophototherapy as an EPR-enhancing method was demonstrated to enhance tumour accumulation of nanoparticles by an order of magnitude[140]. Unlike vasculature-targeting methods, which need to be carefully gauged to avoid thrombus formulation and vessel collapse, perivascular targeting is potentially associated with a lower risk of occluding circulation.

Poor blood pressure regulation in tumours represents another challenge. This is attributable to several factors, including the lack of a smooth muscle layer around tumour blood vessels and a high level of nitric oxide[126]. In the 1980s, the vasoconstrictor angiotensin II (AT-II) was explored as an agent to increase EPR[141]. AT-II causes vasoconstriction and a systemic increase in blood pressure. However, owing to a reduced expression of AT-II receptors, tumour blood vessels barely respond to AT-II treatment. This leads to an increased blood flow and enlarged microvascular lumen, which favours deposition of macromolecules and nanoparticles into tumours. It was found that AT-II can increase the tumour uptake of poly(styrene-co-maleic-acid)–neocarzinostatin conjugate several-fold, in contrast to healthy tissues, the uptake into which was unchanged, or even reduced[142]. Recently, losartan, an angiotensin inhibitor, was studied to modulate the tumour microenvironment[143]. Losartan reduced solid stress in tumours, leading to increased vascular perfusion, as well as improved delivery of drug and oxygen.

Several other methods to increase the EPR effect have been investigated, including the use of hyperthermia, PTT, radiofrequency energy and convection-enhanced delivery[144–147]. Despite great promise, more studies are needed to assess the side effects of these treatments, including toxicity to normal tissue and circulation occlusion. Just as the EPR effect is affected by intertumour and intratumour heterogeneities, the EPR increase is expected to differ among tumours, which again underscores the importance of imaging guidance. The impact of EPR enhancement is often transient, and therefore it is important to understand the time window of the effect by prognostic imaging, and to inject nanomedicines within the optimal interval. The combination of diagnosis and therapy is crucial.

Therapeutic efficacy versus systemic toxicity

The premise of nanotherapy is to enhance treatment efficacy against tumours and reduce toxicity to normal tissues. Although most of the research focus has been directed toward the former, the benefits of the latter are just as important. A survey of the nanomedicine literature from the past decade highlighted the overall dismal delivery of injected nanoparticle dose to solid tumours[1]. But the tumour delivery efficiency is neither the sole

factor that contributes to therapeutic index nor a parameter that governs regulatory approval of nanoformulations[148]. Reducing drug toxicity and side effects is as important as enhancing tumour uptake efficiency, if not more so. Indeed, with the goal to benefit patients, nanomedicine is an excellent platform to shift from off-target to on-target drug accumulation[149].

Thus, instead of just looking at the absolute tumour accumulation numbers, we should examine the relative increase of drug accumulation in tumours relative to normal tissues and the relative decrease of accumulation in healthy tissues, which is equally important. For example, the benefits of Doxil, relative to free doxorubicin, in tumour uptake are often marginal. However, Doxil can universally reduce cardiotoxicity, which is a dose-limiting side effect of doxorubicin (the altered pharmacokinetics may, however, lead to increased incidence of palmar–plantar erythrodysaesthesia). This allows for better tolerance and prolonged dosing, which are important factors in Doxil's clinical success. A similar story applies to Abraxane. It is now clear that Abraxane nanoparticles quickly disassemble in the blood and thus are not necessarily delivered efficiently to tumours. Nevertheless, it is a blockbuster drug in the clinic, and a key reason is, again, improved patient tolerance. Chemotherapy-induced toxicities, including those to the pulmonary system, cardiovascular system, liver, bowel and pancreas, are well-documented and may be detected by conventional imaging methods. Future nanotherapy should take treatment-induced side effects into consideration for prognosis, regimen selection and dosage escalation.

In view of vast inter-patient heterogeneity, individualized cancer nanotheranostics are desirable for optimal therapeutic efficacy and minimal toxicity. In this regard, nanotheranostics holds great potential in '*N*-of-1' trials (in which a single patient constitutes the whole trial)[150] and in interim imaging-guided treatment (to decide between standard chemotherapy and a more intensive, but more toxic, regimen). Under the concept of *N*-of-1 trials, one patient obtains a series of prognoses using nanotheranostics for regimen selection. After a period of treatment using the selected regimen, another prognosis is performed for regimen adjustment. In this pattern, therapeutic outcomes can be constantly monitored. Although there are some *N*-of-1 clinical trials to improve patient management for osteoarthritis[151], chronic pain[152] and cancers[153], there have been no attempts to use this strategy to study nanoparticle drugs.

Nanotheranostics for cancer immunotherapy

Immune nanotheranostics are an important class of cancer nanotheranostics[154–156]. Cancer immunotherapy treats cancer by means of the immune system[157], and the companion diagnostics and prognostics allow cancer diagnosis, patient stratification, and therapy response monitoring. Unlike chemotherapeutic nanotheranostics, in which the diagnostics/prognostics and therapeutics both target tumour, immune nanotheranostics may target peripheral lymphocytes, tumour-infiltrating lymphocytes or tumour cells. Therefore, immune nanotheranostics is an example in which diagnostics/prognostics and therapeutics are separately incorporated. Because the diagnostics/prognostics of immune nanotheranostics are similar to nanotheranostics discussed above, we focus our discussion on nanotherapeutics, specifically on cancer immunotherapeutic nanovaccines.

The development of subunit vaccines is partially hampered by their limited half-life. Depot-forming water-in-oil emulsions, such as incomplete Freund's adjuvant, can prolong the half-life of vaccines and potentiate the immunogenicity of antigen, but they failed to elicit a robust T cell response upon boosting vaccination and yielded limited clinical outcome[158]. Nanovaccines[155,159–165] can penetrate tissue barriers, co-deliver antigen and adjuvant, and efficiently deliver vaccine into antigen-presenting cells (APCs) for antigen cross-presentation. For example, for a nanovaccine made of a gold nanoparticle core and a shell of CpG oligonucleotides (CpG ODN), a Toll-like receptor 9 (TLR9) agonist was used for delivering CpG to inhibit tumour growth[166]. Liposome was also studied to improve lymph-node-targeted delivery of cyclic di-GMP (cdGMP), a potential robust immunostimulatory adjuvant[167,168]. In addition to synthetic nanocarriers, naturally derived nanovaccines have been explored because of their good biocompatibility. For example, lipid-modified molecular vaccine can hitchhike endogenous albumin to deliver vaccine to lymph nodes, thus eliciting a robust anti-tumour T cell response. Compared with exogenous nanocarriers, nanovaccines assembled *in vivo* from exogenous and chemically defined molecular vaccines are attractive for potential good manufacturing practices[169]. In another example, high-density-lipoprotein (HDL)-mimicking nanodisks co-delivered adjuvant and cancer-specific neoantigen for personalized cancer immunotherapy[162]. Use of cancer-cell or erythrocyte membranes[170–172] to cloak nanoparticles can also transplant the biomolecular signature of the membrane onto the nanoparticle and impart the biocompatibility of the host cell membrane. Nanovaccines also confers optimal immunization by efficient intracellular delivery of many molecular adjuvants[162,166,173] and antigens. Specifically, intracellular delivery of antigen is pivotal to elicit robust antigen-specific T cell responses, because antigen needs to be internalized and processed by intracellular protease machinery in APCs, and transported into specialized intracellular compartments to bind with newly synthesized major histocompatibility complex (MHC) molecules for antigen cross-presentation. In this regard, antigen delivered by exogenous nanoparticles is internalized by APCs, processed, loaded onto MHC class I and cross-presented to $CD8^+$ T cells[174,175].

Nanovaccines are also expected to aid the development of neoantigens, which are derived from somatic mutation in tumours, and are thus expressed exclusively in tumour cells. The signature of tumour mutations (termed the mutatome) of each patient is unique, making neoantigens ideal for personalized cancer immunotherapy. However, the natural frequency of neoantigen-specific T cells is often small. Thus, it is desirable to deliver exogenous neoantigen — which nanovaccines can do efficiently, as discussed above[162] — to increase the frequency of tumour-specific T cells. Despite the hope of neoantigen-based nanovaccines, several challenges remain. First, the load of naturally occurring neoantigen is extremely low in some tumour types, such as glioblastoma, and pancreatic and breast cancer, which hinders the identification of effective neoantigen[176]. Second, current technology takes ~3 months to identify and manufacture synthetic neoantigen peptides for vaccination. Nanotechnology can also improve the delivery of mRNA vaccines. Antigen-encoding, *in vitro* transcribed modified mRNA vaccine generally has low risk of latent viral infection and potent T cell response[177–180]. Delivery of mRNA to APCs is prerequisite for optimal therapeutic efficacy but is challenged by nuclease susceptibility, inefficient intracellular delivery, and endosome mRNA trapping. Nanovaccines can be explored to address these

challenges[181,182]. For example, liposome was used to deliver neoantigen-encoding mRNA, resulting in efficient mRNA delivery to lymphoid dendritic cells in the spleen and potent T cell responses in mice and in human[181,182]. Worth noting, one mRNA can be engineered to encode multi-epitope neoantigens, thus conferring a broad spectrum of T cell responses for optimal cancer therapy. Several ongoing clinical trials are likely to unveil detailed potential and challenges of nanovaccines.

Conclusion

Cancer nanomedicine has evolved considerably in the past two decades, beginning with a great expansion of the materials repertoire, characterized by diversity and multifunctionality. Some of these nanomaterials were then further explored for scaled-up manufacturing and clinical translation to address unmet oncological needs. Tumour targeting has been a central theme to nanomedicine that has also gradually evolved. This started with the community concentrating on pure EPR-based tumour uptake, focusing almost entirely on extending nanoparticle circulation half-lives, and largely ignoring intertumour variations. The concept of active targeting was then introduced, which to a certain degree touches upon the issue of tumour variation. Now, entering the era of personalized medicine, modern nanomedicine is focusing more than ever on tumour heterogeneity and tailoring of regimens for individual patients. Nanotheranostics, which had been considered an interesting but not necessarily mainstream concept in nanomedicine, may find many opportunities in this campaign. Its utility is highly application-oriented, so it is important to break the dogma and embrace all possibilities that could make cancer treatment more efficient. A physically integrated diagnostic-plus-therapeutic nanoparticle may be advantageous in some applications but cumbersome in others. Hence, we suggest extending the concept of nanotheranostics to a broad approach that uses diagnosis to aid or guide nanoparticle therapy. The take-home messages are summarized in BOX 2.

Box 2

Take-home messages for the future developments of nanotheranostics

Nanotheranostics

We suggest extending the concept of nanotheranostics beyond its current meaning of a nanomedicine that affords both diagnostic and therapeutic functions, to a broad approach that uses diagnosis to aid or guide nanoparticle therapy procedures.

Tumour heterogeneity and patient stratification

Conventional nanomedicine is founded on the enhanced permeability and retention (EPR) effect. This effect is, however, an oversimplified model because it largely neglects heterogeneity within and among tumours. Future nanomedicines should treat each tumour as a unique and complex organ. Instead of putting all factors under the EPR effect, it is worthwhile to consider each individual factor and combine the information for comprehensive prognosis, and to stratify patients based on prediction of nanotherapeutic efficacy. Nanotheranostics can have an important role in this new campaign.

Nanoparticle accumulation in target tissues

For tumour-targeting nanotheranostics (for example, chemotherapy) based on comprehensive tumour characterizations, it is possible to choose or even tailor nanoparticles for optimal tumour targeting; it is also possible to artificially modulate the tumour microenvironment to favour accumulation of nanoparticles within tumours. For nanotheranostics (for example, nanovaccines) that target secondary lymphoid organs, nanoparticle accumulation in these organs can be optimized.

Nanoparticle–body interactions

It is crucial to understand the interactions between nanoparticles and the human body (for example, tumour, healthy tissues, blood cells and proteins), to ultimately enhance nanoparticle delivery to tumour cells, prevent opsonization (chemical modification that makes the nanoparticle more readily identified by phagocytes) and reduce distribution in healthy tissues.

High efficacy and low toxicity

Although tumour uptake is a very important parameter when evaluating a nanomedicine, it is not the sole criterion. Reducing systemic toxicity and improving patient tolerance, for example, are crucial benefits of nanoparticle delivery and critical benchmarks in the clinical translation and implementation of nanotechnology.

Clinical translation

Clinical translation of cancer nanotheranostics requires commitment in scaled-up synthesis, detailed understanding of the interactions between cancer nanotheranostics and the human body, long-term assessment of toxicity, and establishment of regulatory protocols for cancer nanotheranostics.

In addition to the delivery of traditional chemotherapeutics, there is great promise in the delivery of immunotherapeutics using nanocarriers. Immunotherapy induces durable and systemic antitumour immunity, which is especially beneficial for the treatment of metastatic cancer. Many types of immunotherapeutics, including cancer therapeutic vaccine, can be efficiently delivered to targeted tissues for optimal therapeutic efficacy with reduced side effects. Moreover, companion diagnostics is critical for patient stratification and for the design of neoantigen-based personalized cancer immunotherapy. In this sense, almost all future nanomedicine may be considered nanotheranostics, given the increasing emphasis on the combination.

Despite the promise of nanotheranostics, there are multiple barriers to successful clinical translation. The community should seek in-depth understanding of nanoparticle–tumour interactions and cooperation between diagnosis and therapy. More efforts should also be directed at scaled-up synthesis, long-term assessment of toxicity and establishment of regulatory protocols for nanotheranostics[183]. Only then can we deliver the technology at the patient's bedside for effective and personalized therapy.

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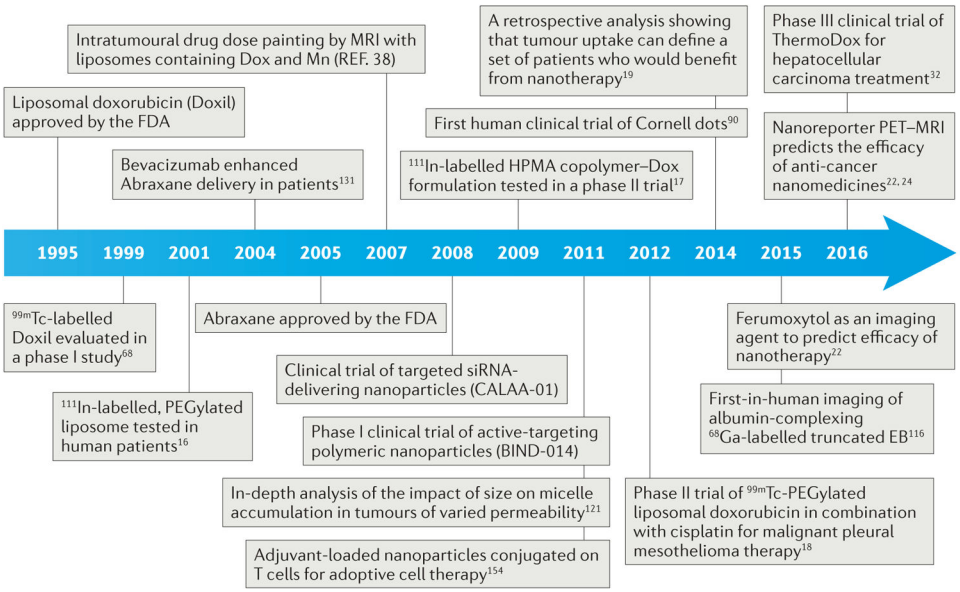

Figure 1. Historical timeline of key advances in cancer nanotheranostics

EB, Evans blue; FDA, US Food and Drug Administration; HPMA, *N*-(2-hydroxypropyl)methacrylamide; MRI, magnetic resonance imaging; PEG, polyethylene glycol; PET, positron emission tomography; siRNA, small interfering RNA.

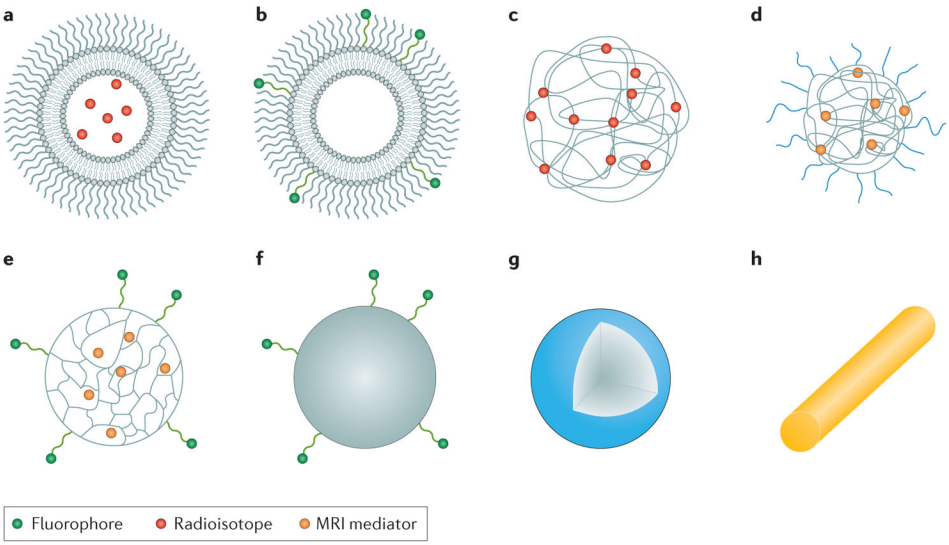

Figure 2. Nanotheranostics for cancer diagnosis

In nanotheranostic agents, imaging functions are imparted to nanomedicines by adding moieties that are readily detected by imaging methods. These nanotheranostic agents have been exploited for tumour diagnosis and subsequent patient stratification, for understanding the pharmacokinetics and pharmacokinetics of nanomedicines, and for monitoring therapy response. **a** | Liposome with radioisotope in the core. **b** | Liposome labelled with fluorophores on the surface. **c** | Polymeric conjugate labelled with radioisotopes. **d** | Polymeric micelle loaded with T_1 MRI mediators. **e** | PLGA nanoparticle with T_1 MRI mediators loaded inside and fluorophores labelled on the surface. **f** | Iron oxide nanoparticle labelled with fluorophores on the surface. **g** | Iron oxide nanoparticle coated with photoacoustic or photothermal material. **h** | Gold nanorod. MRI, magnetic resonance imaging; PLGA, poly(lactic-co-glycolic acid); T_1, longitudinal relaxation time.

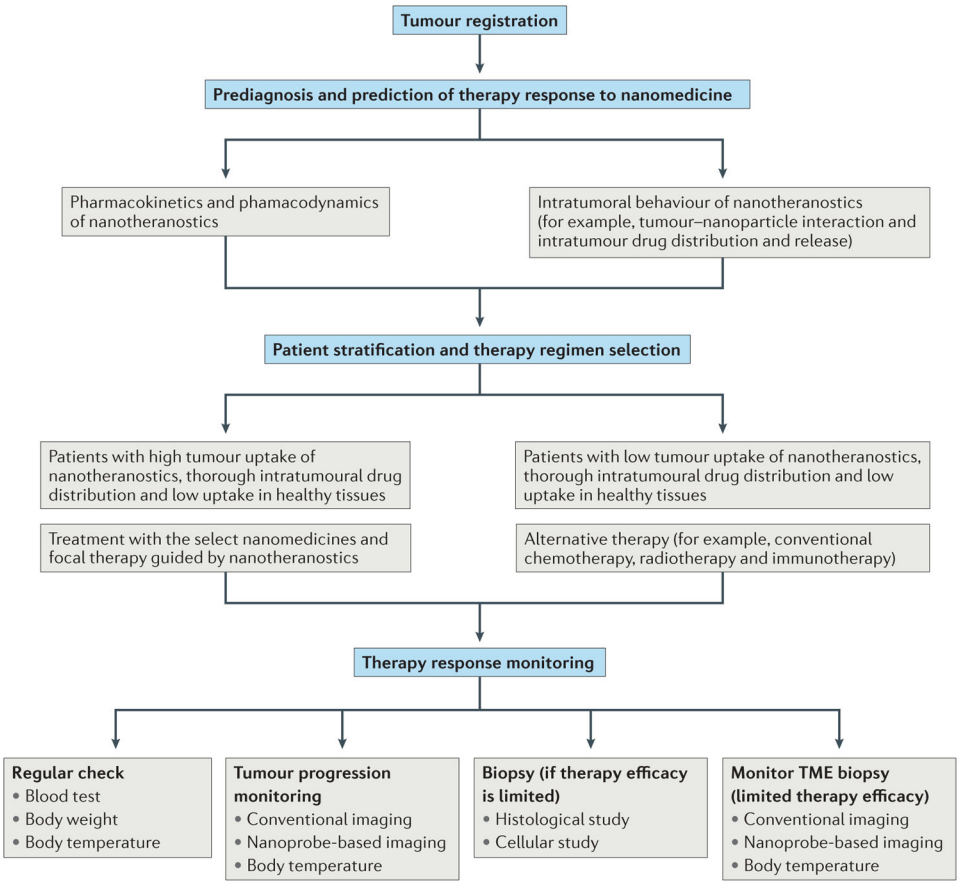

Figure 3. Applications of nanotheranostics in cancer therapy

Patients go through pretreatment imaging to understand the pharmacokinetics and pharmacodynamics of the nanomedicines as well as intratumoural distributions and drug release. Based on the imaging results, prognoses can be made, along with selection of patients who are likely to benefit from the nanotherapy. Next, the select patients will receive the nanomedicine and — from the earliest stages — receive monitoring of therapy responses, the feedback from which will in turn guide the evaluation of therapeutic efficacy and, if necessary, adjust future treatment regimens for optimal therapy outcome. TME, total mesorectal exision.

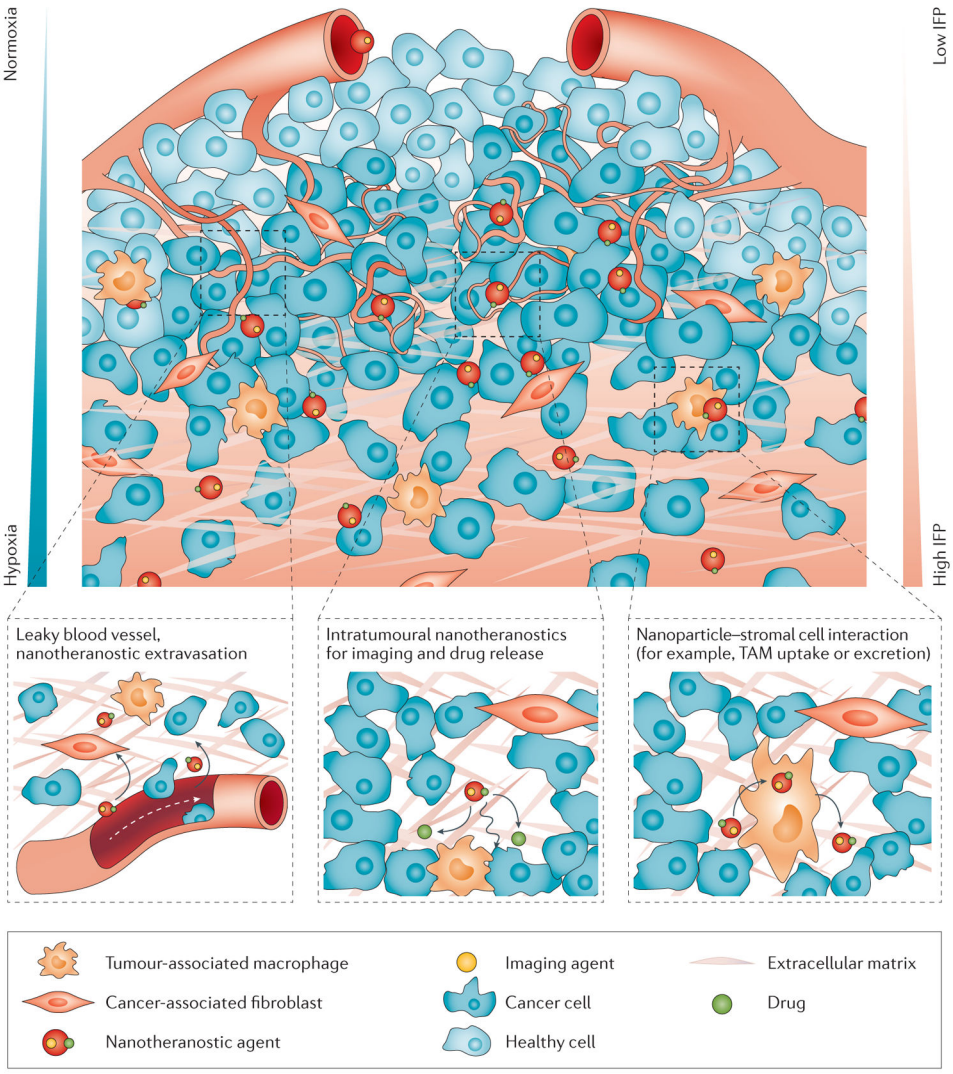

Figure 4. Tumour characteristics that affect the intratumoural fates of nanotheranostics

Slow blood flow may affect the extravasation of nanoparticles in a size-dependent manner. Leaky blood vessels affect the extravasation of nanoparticles in a size-dependent manner. This vasculature leakiness varies considerably between tumours of different types and stages. Dense blood vessels typically enhance tumour accumulation of nanotheranostics. A dense extracellular matrix (ECM), especially in the tumour periphery, may restrict the tumour penetration of nanotheranostics. An increased interstitial fluid pressure (IFP) in many tumours represents a barrier for transcapillary transport of nanotheranostics. Nonspecific uptake by stromal cells, such as tumour-associated macrophages (TAMs), may negatively affect deep tumour penetration and delivery of nanoparticles to cancer cells, but, on the other hand, may serve as an intermediate reservoir[22].

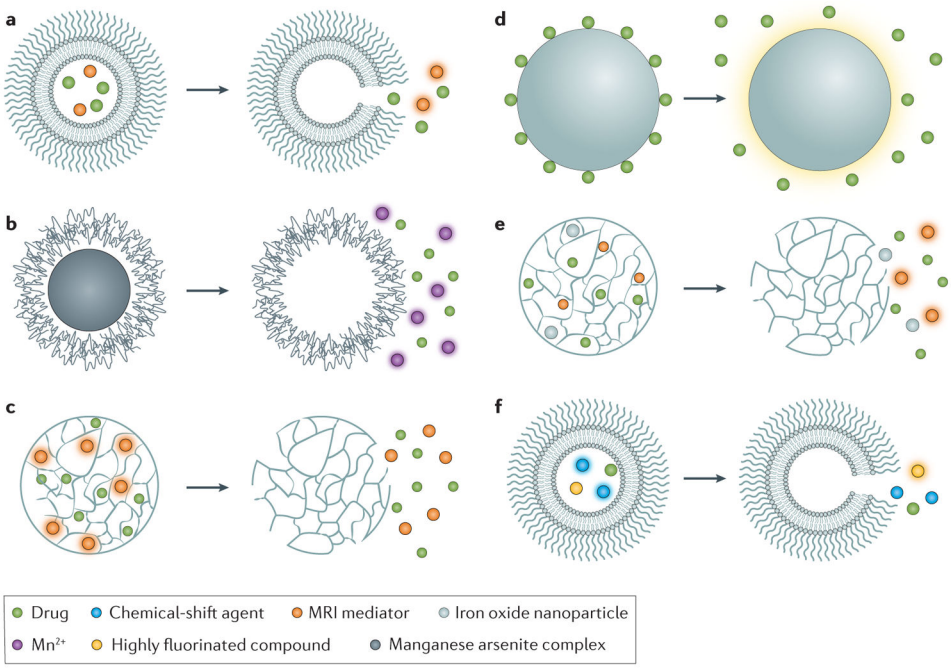

Figure 5. Nanotheranostics for drug-release monitoring

Designer nanotheranostics have been developed to monitor the intratumoural drug release from nanomedicines by various mechanisms. **a** | T_1 MRI mediators co-released with drug molecules from a liposomal carrier, which generates T_1 hyperintensity in magnetic resonance scans. **b** | Mn^{2+} and drug molecules ($HAsO_3^-$)are released on decomposition of Mn^{2+}-doped arsenic trioxide nanoparticles from the mesoporous silica shell, resulting in T_1 hyperintensity in magnetic resonance scans. **c** | T_1 mediators are co-released with drug molecules from the polymeric micelle, resulting in T_1 hyperintensity in magnetic resonance scans. **d** | Drugs are released from the surface of iron oxide nanoparticles, resulting in T_2 hyperintensity in magnetic resonance scans. **e** | Iron oxide nanoparticles, Gd-DTPA and drug molecules (5-FU) are loaded in PLGA nanoparticles. On drug release, T_1 hyperintensity is generated in the magnetic resonance scans owing to deshielding. **f** | Chemical-shift agents (for ^1H CEST detection) and highly fluorinated compounds (for ^{19}F detection) are loaded into liposomes together with drug molecules. Before release, signal enhancement is generated in ^1H CEST magnetic resonance images; after drug release, hyperintensity results in the ^{19}F magnetic resonance images. CEST, chemical exchange-dependent saturation transfer; DTPA, diethylenetriamine pentaacetate; MRI, magnetic resonance imaging; PLGA, poly(lactic-co-glycolic acid); 5-FU, fluorouracil. T_1, longitudinal relaxation time; T_2, transverse relaxation time.

Table 1

Enhancing tumour uptake of nanoparticles through nanoparticle engineering and tumour microenvironment modulation

Approach		Details
Engineering nanoparticles		
Tumour penetration	Small particle size	In general, small nanoparticles penetrate relatively deep into tumours
	Minimized TAM uptake	Appropriate surface engineering (for example, PEGylation) can minimize nanoparticle uptake by TAMs
	In situ size shrinkage	Smart nanoparticles of relatively large sizes reduce their dimensions in response to a stimulus in tumours, leading to improved penetration[126–128]
	Tumour-specific nanoparticle screening	Based on imaging results and tumour characterizations, nanoparticle formulations that would afford the best penetration can be selected or designed[108,120,121]
Tumour retention	Large nanoparticle size	Large nanoparticles prolong retention in the tumour
	In situ nanoparticle size expansion	Building-block molecules or small nanoparticles self-assemble into relatively large nanostructures within tumours, leading to prolonged tumour retention[125]
	Active targeting of nanoparticle	Through binding with a tumour biomarker, intravasation is reduced to prolong tumour retention[108,119]
	Magnetic guidance	Use of an external magnetic field can enrich magnetic nanoparticles in tumours
Modulating the tumour microenvironment		
Increase blood vessel leakiness		Use photodynamic therapy or ultrasound to enlarge gaps on endothelial walls[134–138]
Vascular normalization		Prune tumour blood vessels to enhance drug delivery and lower tumour IFP[129–133]
Kill perivascular cancer cells		Use radiotherapy or immunophototherapy to selectively eliminate these relatively oxygenated cells[139,140]
Break down ECM		Melt down ECM or reduce the deposition of the related components
Eliminate CAFs or other stroma cells		Specifically kill, inactivate or quiesce CAFs
Blood pressure regulators		Modulate the constriction or tension of blood vessels[141–143]

CAF, cancer-associated fibroblasts; ECM, extracellular matrix; IFP, interstitial fluid pressure; PEG, polyethylene glycol; TAM, tumour-associated macrophages