

Cancer nanomedicine: progress, challenges and opportunities

Jinjun Shi¹, Philip W. Kantoff², Richard Wooster³ and Omid C. Farokhzad^{1,4}

Abstract | The intrinsic limits of conventional cancer therapies prompted the development and application of various nanotechnologies for more effective and safer cancer treatment, herein referred to as cancer nanomedicine. Considerable technological success has been achieved in this field, but the main obstacles to nanomedicine becoming a new paradigm in cancer therapy stem from the complexities and heterogeneity of tumour biology, an incomplete understanding of nano–bio interactions and the challenges regarding chemistry, manufacturing and controls required for clinical translation and commercialization. This Review highlights the progress, challenges and opportunities in cancer nanomedicine and discusses novel engineering approaches that capitalize on our growing understanding of tumour biology and nano–bio interactions to develop more effective nanotherapeutics for cancer patients.

Nanoparticle

(NP). Particle of any shape with dimensions in the 1–100 nm range, as defined by the International Union of Pure and Applied Chemistry (IUPAC). Despite this size restriction, the term nanoparticles commonly applies to structures that are up to several hundred nanometres in size, although key is that design of the nanostructure produces a unique function and property.

¹Center for Nanomedicine and Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA.

²Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA.

³Tarveda Therapeutics, Watertown, Massachusetts 02472, USA.

⁴King Abdulaziz University, Jeddah 21589, Saudi Arabia.

Correspondence to O.C.F. ofarokhzad@bwh.harvard.edu

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The growing interest in applying nanotechnology to cancer is largely attributable to its uniquely appealing features for drug delivery, diagnosis and imaging, synthetic vaccine development and miniature medical devices, as well as the therapeutic nature of some nanomaterials themselves^{1–6} (BOX 1). Nanotherapies that incorporate some of these features (for example, improved circulation and reduced toxicity) are already in use today, and others show great promise in clinical development, with definitive results expected in the near future. Several therapeutic nanoparticle (NP) platforms, such as liposomes, albumin NPs and polymeric micelles, have been approved for cancer treatment, and many other nanotechnology-enabled therapeutic modalities are under clinical investigation, including chemotherapy, hyperthermia, radiation therapy, gene or RNA interference (RNAi) therapy and immunotherapy (TABLE 1).

Along with enormous progress in the field of cancer nanomedicine (FIG. 1), we have also gradually realized the challenges and opportunities that lie ahead. Foremost, the complexity and the heterogeneity of tumours make it clear that careful patient selection is required to identify those most likely to benefit from a given nanotherapy. This is analogous to the targeted therapies approved or under development for use in specific biomarker-defined patient populations. **Most therapeutic NPs for solid tumour treatment are administered systemically; they accumulate in the tumour through the enhanced permeability and retention (EPR) effect^{7–10}**, which is generally thought to be the product of leaky tumour vasculature and poor lymphatic drainage. However, this interpretation of EPR is somewhat

oversimplified, as multiple biological steps in the systemic delivery of NPs can influence the effect, such as NP–protein interaction, blood circulation, extravasation into and interaction with the perivascular tumour microenvironment (TME), tumour tissue penetration and tumour cell internalization. In turn, NP properties (for example, size, geometry, surface features, elasticity, stiffness, porosity, composition and targeting ligand) can influence these biological processes, thus determining the EPR effect and therapeutic outcomes (FIG. 2). Nevertheless, it is important to point out that most of our current understanding of NP behaviour *in vivo* is based on animal data, and its translation to NP behaviour in humans remains largely unexplored. Although several studies have examined the pharmacokinetics (PK) of nanotherapeutics across species in preclinical and clinical studies^{11–13}, relatively few have correlated data across species to determine whether and how NP safety and efficacy in humans can be better predicted from preclinical animal models.

This Review aims to identify gaps in our understanding of why cancer nanomedicine has yet to fulfil its promise in prolonging patient survival, and to offer an overview of our current grasp of tumour biology and nano–bio interactions as they relate to maximization of the impact of cancer nanotherapeutics. Given the presumed crucial role of EPR, we present recent progress in exploring this effect and identifying markers to predict responses to nanotherapies, and in developing new strategies to enhance systemic NP delivery for more pronounced EPR and therapeutic benefit. We also examine the fundamentals behind the development of

Box 1 | Distinctive features of nanotechnology in oncological applications

- Improvement of the drug therapeutic index by increasing efficacy and/or reducing toxicities
- Targeted delivery of drugs in a tissue-, cell- or organelle-specific manner
- Enhancement of the pharmaceutical properties (for example, stability, solubility, circulating half-life and tumour accumulation) of therapeutic molecules
- Enabling of sustained or stimulus-triggered drug release
- Facilitation of the delivery of biomacromolecular drugs (for example, DNA, small interfering RNA (siRNA), mRNA and protein) to intracellular sites of action
- Co-delivery of multiple drugs to improve therapeutic efficacy and overcome drug resistance, by providing more precise control of the spatiotemporal exposure of each drug and the delivery of appropriate drug ratio to the target of interest
- Transcytosis of drugs across tight epithelial and endothelial barriers (for example, gastrointestinal tract and the blood–brain barrier)
- More sensitive cancer diagnosis and imaging
- Visualization of sites of drug delivery by combining therapeutic agents with imaging modalities, and/or real-time feedback on the *in vivo* efficacy of a therapeutic agent
- Provision of new approaches for the development of synthetic vaccines
- Miniaturized medical devices for cancer diagnosis, drug screening and delivery
- Inherent therapeutic properties of some nanomaterials (for example, gold nanoshells and nanorods, and iron oxide nanoparticles) upon stimulation

Enhanced permeability and retention (EPR) effect

The mechanism resulting from pathophysiological processes (for example, leaky tumour vasculature, poor lymphatic drainage and tumour microenvironment interactions) that leads to the accumulation and retention of nanoparticles or macromolecules in tumours.

Nano–bio interactions

The interactions between nanoparticles and biological systems (for example, serum proteins, extracellular matrix, cells and organelles) that determine the biological fates of nanoparticles, such as circulation half-life, biodistribution, tumour accumulation, tumour cell internalization and tumour microenvironment distribution.

Excipients

Substances other than the active pharmaceutical ingredient (API) that are included in the manufacturing process of a medication or are contained in a finished pharmaceutical product dosage form.

 C_{\max}

The maximum serum concentration that a drug or nanoparticle achieves after administration.

nanotechnologies to target the TME, which has such an important role in tumour progression and metastasis^{14,15}, and lastly, provide our perspective on challenges to the clinical translation of cancer nanomedicines.

Arsenal of nanomedicine platforms

Nanotechnology has made important contributions to oncology over the past several decades (FIG. 1; TABLE 1). Liposomes (for example, liposomal doxorubicin (LD); Doxil and Myocet) were the first class of therapeutic NPs to receive clinical approval for cancer treatment¹⁶, and along with other lipid-based NPs, still represent a large proportion of clinical-stage nanotherapeutics. **Although encapsulating drugs in liposomes has been broadly shown to improve PK and biodistribution, as yet no marketed liposomal therapeutic agents have exhibited an overall survival (OS) benefit when directly compared with the conventional parent drug¹⁷.** The recent phase III results of liposomal cytarabine–daunorubicin (Vyxeos; also known as CPX-351) compared with the standard of care regimen of cytarabine and daunorubicin in patients with high-risk acute myeloid leukaemia, showed improved OS of 9.56 months versus 5.95 months¹⁸. This is encouraging for the field of cancer nanomedicine and regulatory filing for the approval of Vyxeos is projected in late 2016. NP albumin-bound paclitaxel (nab-paclitaxel; Abraxane) was the second class of nanomedicines to be commercialized. The nab platform enables formulation of hydrophobic drugs while largely mitigating the need to use toxic excipients. The result may be a better-tolerated drug that can be used at higher doses and administered more quickly, thus enabling a higher drug C_{\max} and plasma area under the curve (AUC). Upon intravenous infusion, nab-paclitaxel rapidly dissociates into its albumin and paclitaxel constituents and has not been demonstrated to substantially alter the PK and biodistribution

of paclitaxel. Although the every-3-week dosing schedule of nab-paclitaxel is superior to paclitaxel in terms of response rate and time to progression for patients with breast cancer¹⁹, a once-per-week dosing schedule did not show similar trends in progression-free survival (PFS) or OS and furthermore, showed increased toxicity²⁰. Polymeric micelles (for example, Genexol-PM²¹ and NK105 (REF. 22)) and polymeric NPs (for example, CRLX101 (REF. 23), BIND-014 (REF. 11) and AZD-2811 Accurin²⁴) are two newer classes of cancer nanotherapeutic agent. Most recently, disappointing clinical results have been reported for BIND-014, CRLX101 and NK105, underscoring the need to rethink development strategies, including potential patient selection to identify those most likely to respond to nanotherapeutics. Inorganic nanomaterials (for example, gold nanoshell²⁵, iron oxide NP²⁶ and hafnium oxide NP²⁷) are also being investigated for use in cancer patients, with the iron oxide NP-based NanoTherm²⁶ already marketed in Europe for glioblastoma.

More intriguingly, our understanding of nano–bio interactions and the arsenal of nanomedicine platforms are expanding rapidly. The total number of papers related to ‘nanoparticle’ on PubMed nearly doubled every 2 years between 2000 and 2014, surpassing the remarkable rise of the number of publications on ‘monoclonal antibody’ (mAb) in the 1980s. In the case of mAb this translated to the development of important therapeutics, and we expect a similar transformative impact from the rise of nanomedicine in the years to come.

Beyond their widely reported use as carriers for chemotherapeutics, **NPs have shown potential for the delivery of various new anticancer therapeutic agents, including molecularly targeted agents²⁴, antisense oligonucleotides^{28,29}, small interfering RNA (siRNA)^{30–33}, mRNA³⁴ and DNA inhibitor oligonucleotides³⁵.** Furthermore, the use of viral NPs for therapeutic delivery has been facilitated by genetic and chemical engineering techniques³⁶. Examples include the use of adeno-associated virus, approved by the European Commission for lipoprotein lipase deficiency³⁷, lentivirus currently in various clinical trials for cell-based gene therapy and immunotherapy of various diseases including cancer³⁸, and engineered plant viruses (for example, tobacco mosaic virus and potato virus X) for cancer therapy in animal models^{39,40}. With their endogenous origin and organ tropism, exosomes have also been proposed for carrying anticancer payloads to target tumours⁴¹. Lastly, novel inorganic NPs such as nanodiamond^{42,43} and graphene^{44,45} have received considerable attention for cancer therapy.

We are also already seeing in-depth innovation in nanomedicine strategies. By integrating diagnostic and therapeutic functions into a single NP formulation, theranostic nanomedicine offers a promising strategy to monitor the PK and accumulation of therapeutics and the progression of disease, giving important insights into heterogeneities both within tumours and between patients for potential personalized treatment^{46,47}. By co-delivering multiple active pharmaceutical ingredients (APIs), NPs have also facilitated synergistic

Table 1 | Examples of clinical-stage nanomedicines for cancer therapy

Therapy modality	Generic name and/or proprietary name	Nanotechnology platform	Active pharmaceutical ingredients	Cancer type	Status	Refs
Chemotherapy: non-targeted delivery	Liposomal doxorubicin (Doxil)	Pegylated liposome	Doxorubicin	HIV-related Kaposi sarcoma, ovarian cancer, and multiple myeloma	Approved by FDA	6
	Liposomal daunorubicin (DaunoXome)	Liposome	Daunorubicin	HIV-related Kaposi sarcoma	Approved by FDA	6
	Liposomal vincristine (Marqibo)	Liposome	Vincristine sulfate	Acute lymphoblastic leukaemia	Approved by FDA	6
	Liposomal irinotecan (Onivyde or MM-398)	Pegylated liposome	Irinotecan	Post-gemcitabine metastatic pancreatic cancer	Approved by FDA	230
	Liposomal doxorubicin (Myocet)	Liposome	Doxorubicin	Metastatic breast cancer	Approved in Europe and Canada	6
	Mifamurtide (Mepact)	Liposome	Muramyl tripeptide phosphatidyl-ethanolamine	Nonmetastatic, resectable osteosarcoma	Approved in Europe	6
	Nab-paclitaxel (Abraxane)	Albumin NP	Paclitaxel	Breast, lung and pancreatic cancer	Approved by FDA	6
	SMANCS	Polymer conjugate	Neocarzinostatin	Liver and renal cancer	Approved in Japan	6
	Polymeric micelle paclitaxel (Genexol-PM)	Polymeric micelle	Paclitaxel	Breast cancer and NSCLC	Approved in Korea	6
	Liposomal cisplatin (Lipoplatin)	Pegylated liposome	Cisplatin	NSCLC	Phase III	231
	NK-105	Polymeric micelle	Paclitaxel	Metastatic or recurrent breast cancer	Phase III	232
	Liposomal paclitaxel (EndoTAG-1)	Liposome	Paclitaxel	Pancreatic cancer, liver metastases and HER2-negative and triple-negative breast cancer	Phase II	233–236
	Nab-rapamycin (ABI-009)	Albumin NP	Rapamycin	Advanced malignant PEComa and advanced cancer with mTOR mutations	Phase II	237,238
	CRLX-101	Polymeric NP	Camptothecin	NSCLC, metastatic renal cell carcinoma and recurrent ovarian, tubal or peritoneal cancer	Phase II	239–241
Chemotherapy: targeted delivery	MM-302	HER2-targeting liposome	Doxorubicin	HER2-positive breast cancer	Phase II/III	242
	BIND-014	PSMA-targeting polymeric NP	Docetaxel	NSCLC and mCRPC	Phase II	243–245
	MBP-426	TfR-targeting liposome	Oxaliplatin	Gastric, oesophageal and gastro-oesophageal adenocarcinoma	Phase I/II	246
	Anti-EGFR immunoliposomes loaded with doxorubicin	EGFR-targeting liposome	Doxorubicin	Solid tumours	Phase I	247
Chemotherapy: stimuli-responsive delivery	ThermoDox	Liposome	Doxorubicin	Hepatocellular carcinoma	Phase III	248
Chemotherapy: combinatorial delivery	Liposomal cytarabine–daunorubicin (CPX-351 or Vyxeos)	Liposome	Cytarabine and daunorubicin (5:1)	High-risk acute myeloid leukaemia	Phase III	249
	CPX-1	Liposome	Irinotecan and floxuridine (1:1)	Advanced colorectal cancer	Phase II	250

Table 1 (cont.) | Examples of clinical-stage nanomedicines for cancer therapy

Therapy modality	Generic name and/or proprietary name	Nanotechnology platform	Active pharmaceutical ingredients	Cancer type	Status	Refs
Hyperthermia	NanoTherm	Iron oxide NP	NA	Glioblastoma	Approved in Europe	6
	AuroLase	Silica core with a gold nanoshell	NA	Head and neck cancer, and primary and metastatic lung tumours	Pilot study	251,252
Radiotherapy	NBTR3	Hafnium oxide NP	NA	Adult soft tissue sarcoma	Phase II/III	253
Gene or RNAi therapy	SGT53	TfR-targeting liposome	Plasmid encoding normal human wild-type p53 DNA	Recurrent glioblastoma and metastatic pancreatic cancer	Phase II	254,255
	PNT2258	Liposome	DNA oligonucleotide against BCL-2	Relapsed or refractory non-Hodgkin lymphoma and diffuse large B-cell lymphoma	Phase II	256,257
	SNS01-T	Polyethylenimine NP	siRNA against eIF5A and plasmid expressing eIF5A-K50R	Relapsed or refractory B cell malignancies	Phase I/II	258
	Atu027	Liposome	siRNA against protein kinase N3	Advanced or metastatic pancreatic cancer	Phase I/II	259
	TKM-080301	Lipid NP	siRNA against PLK1	Neuroendocrine tumours, adrenocortical carcinoma and advanced hepatocellular carcinoma	Phase I/II	260,261
	DCR-MYC	Lipid NP	Dicer-substrate siRNA against MYC	Hepatocellular carcinoma	Phase I/II	262
	MRX34	Liposome	miR-34 mimic	Primary liver cancer, solid tumours and haematological malignancies	Phase I	263
	CALAA-01	TfR-targeting polymeric NP	siRNA against ribonucleotide reductase M2	Solid tumours	Phase I	227
	ALN-VSP02	Lipid NP	siRNAs against KSP and VEGFA	Solid tumours	Phase I	264,265
	siRNA-EPHA2-DOPC	Liposome	siRNA against EPHA2	Advanced cancers	Phase I	266
	pbi-shRNA STMN1 LP	Lipid NP	shRNA against stathmin 1	Advanced and/or metastatic cancer	Phase I	267
	Tecemotide	Liposome	MUC1 antigen	NSCLC	Phase III	268
	dHER2 + AS15	Liposome	Recombinant HER2 (dHER2) antigen and AS15 adjuvant	Metastatic breast cancer	Phase I/II	269
Immunotherapy	DPX-0907	Liposome	Multi-tumour associated antigens	HLA-A2-positive advanced stage ovarian, breast and prostate cancer	Phase I	270
	Lipovaxin-MM	Liposome	Melanoma antigens	Malignant melanoma	Phase I	271
	JVRS-100	Lipid NP	Plasmid DNA	Relapsed or refractory leukaemia	Phase I	272
	CYT-6091	Colloidal gold NP	TNF	Advanced solid tumours	Phase I	273

EGFR, epidermal growth factor receptor; eIF5A, eukaryotic initiation factor 5A; EPHA2, ephrin type-A receptor 2; FDA, US Food and Drug Administration; HLA-A2, human leukocyte antigen A2; KSP, kinesin spindle protein (also known as KIF11); mCRPC, metastatic castration-resistant prostate cancer; miR, microRNA; mTOR, mammalian target of rapamycin; MUC1, membrane-bound mucin 1; NA, not applicable; nab, nanoparticle albumin-bound; NP, nanoparticle; NSCLC, non-small-cell lung cancer; PEComa, perivascular epithelioid cell tumours; PEG, poly(ethylene glycol); PLK1, polo-like kinase 1; PSMA, prostate-specific membrane antigen; RNAi, RNA interference; shRNA, short hairpin RNA; siRNA, small interfering RNA; SMANCS, poly(styrene-co-maleic acid)-conjugated neocarcinostatin; TfR, transferrin receptor; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A.

cancer therapy and avoided some mechanisms of drug resistance, as evidenced by the large number of *in vivo* examples (TABLE 2). In addition to drug delivery, nanotechnology is gaining momentum in the area of cancer

immunotherapy. NPs have become increasingly attractive as potent antigen or adjuvant carriers for the development of synthetic vaccines, with enhanced tissue penetration and/or access to lymphatics, preferential uptake

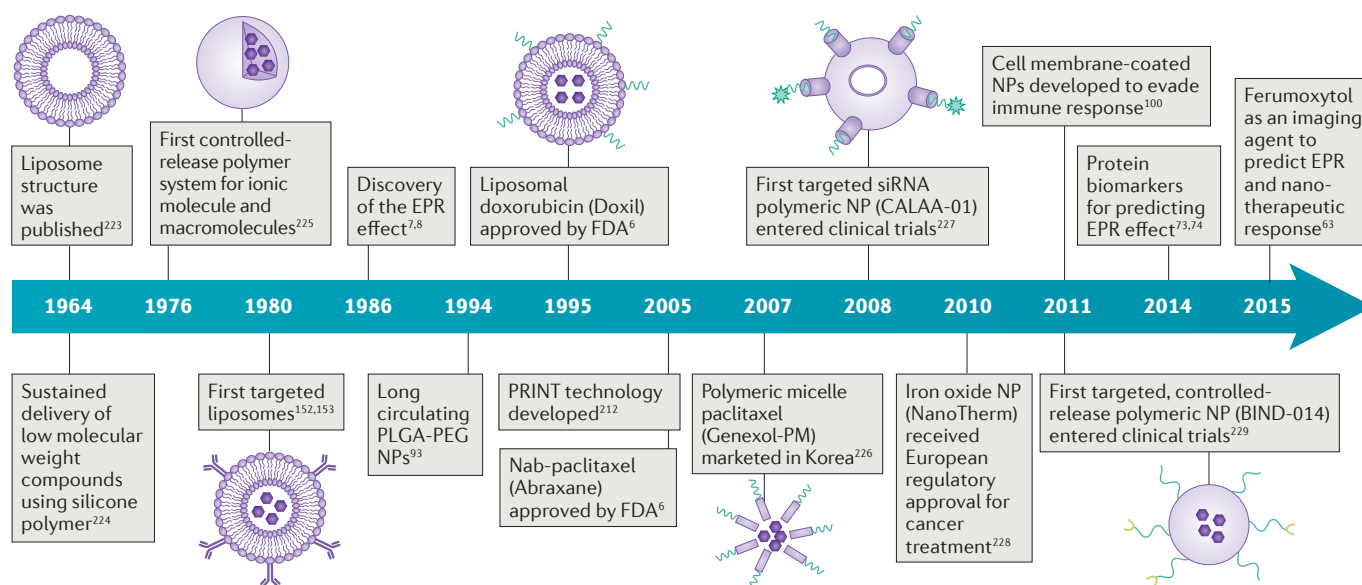


Figure 1 | Historical timeline of major developments in the field of cancer nanomedicine. EPR, enhanced permeability and retention; FDA, US Food and Drug Administration; nab, nanoparticle albumin-bound; NP, nanoparticle; PLGA-PEG, poly(D,L-lactic-co-glycolic acid)-b-poly(ethylene glycol); PRINT, particle replication in non-wetting template; siRNA, small interfering RNA.

by antigen-presenting cells, sustained release of antigens or adjuvants and NP-mediated phagosome escape of antigens for cross-presentation^{4,48–50}.

Nanotechnology may also hold great potential for addressing the shortcomings associated with biologics, including mAbs that are used for cancer immunotherapy. For example, the administration of biologic drugs can induce the formation of antidrug antibodies (ADAs) that may adversely affect their safety and efficacy⁵¹. Recently engineered tolerogenic NPs carrying rapamycin were shown to abolish the formation of ADAs for biologics in mice and non-human primates⁵², and human clinical trials are currently ongoing⁵³. Our expectation is that by gaining a deeper insight into nano–bio interactions and the personalization of nanomedicines, and through the application of nanotechnology to existing and emerging therapeutic modalities, we will begin to realize the true potential of nanomedicine in cancer and beyond.

The EPR effect in predictive nanomedicine

Despite efforts to develop non-invasive administration (for example, oral, pulmonary, nasal and transdermal) of NPs^{54–56}, most cancer nanotherapeutics are delivered intravenously for systemic transport to tumours. The preferential accumulation of NPs in tumours is generally ascribed to defective tumour vessels and impaired lymphatics in the tissue: enhanced permeability of the abnormal tumour microvasculature enables NPs to enter the tumour interstitial space, while suppressed lymphatic drainage causes retention within the tissue. The EPR effect^{7–10} has become the foundation of NP delivery to solid tumours. Nevertheless, it is increasingly clear that EPR varies substantially between both patients and tumour types, and even within the same patient or tumour type over time. However, little effort

has been made to address the effect of EPR on nano-therapeutic efficacy. Several preliminary clinical studies have already suggested the value of stratifying subpopulations of cancer patients according to their likelihood of accumulating NPs through EPR^{57–59}, implying that predictive markers for EPR may have a role in the clinical success of cancer nanotherapies.

In our previous review of EPR⁹, we discussed the parameters of the TME, some of which are well characterized for their interactions with NPs, whereas others are considered a ‘black box’ requiring extensive investigation. Recently, there has been growing emphasis on the role of tumour-associated macrophages (TAMs) in NP–TME interactions^{60–63}. TAMs have also been proposed as a reservoir of nanotherapeutics from which the payload is gradually released to neighbouring tumour cells⁶². Using high-resolution intravital imaging microscopy, a recent work systematically studied the extravasation and intratumoural distribution of two different types of NP⁶³: the clinically approved 30 nm magnetic NP (MNP) ferumoxytol⁶⁴ and a 90 nm polymeric NP composed of poly(D,L-lactic-co-glycolic acid)-b-poly(ethylene glycol) (PLGA-PEG)^{11,65,66}. Despite differences in both size and composition, MNP and polymeric NP exhibited similar PK after simultaneous intravenous injection, and colocalized to varying degrees in cancer cells and TAMs. Furthermore, after co-administration of MNPs and docetaxel-encapsulated PLGA-PEG NPs, tumour MNP levels showed a significant correlation with NP payload levels. Consequently, the MNP accumulation level successfully predicted the anticancer efficacy of the therapeutic polymeric NPs. A pilot clinical study was also recently initiated to assess ferumoxytol as a marker to predict tumour response to the nanoliposomal irinotecan MM-398 (REFS 57,61,67). Preliminary analysis

Area under the curve (AUC). The area between the curve and the x-axis in a plot of drug or nanoparticle blood plasma concentration against time.

Payloads
The therapeutic or diagnostic agents carried by nanoparticles.

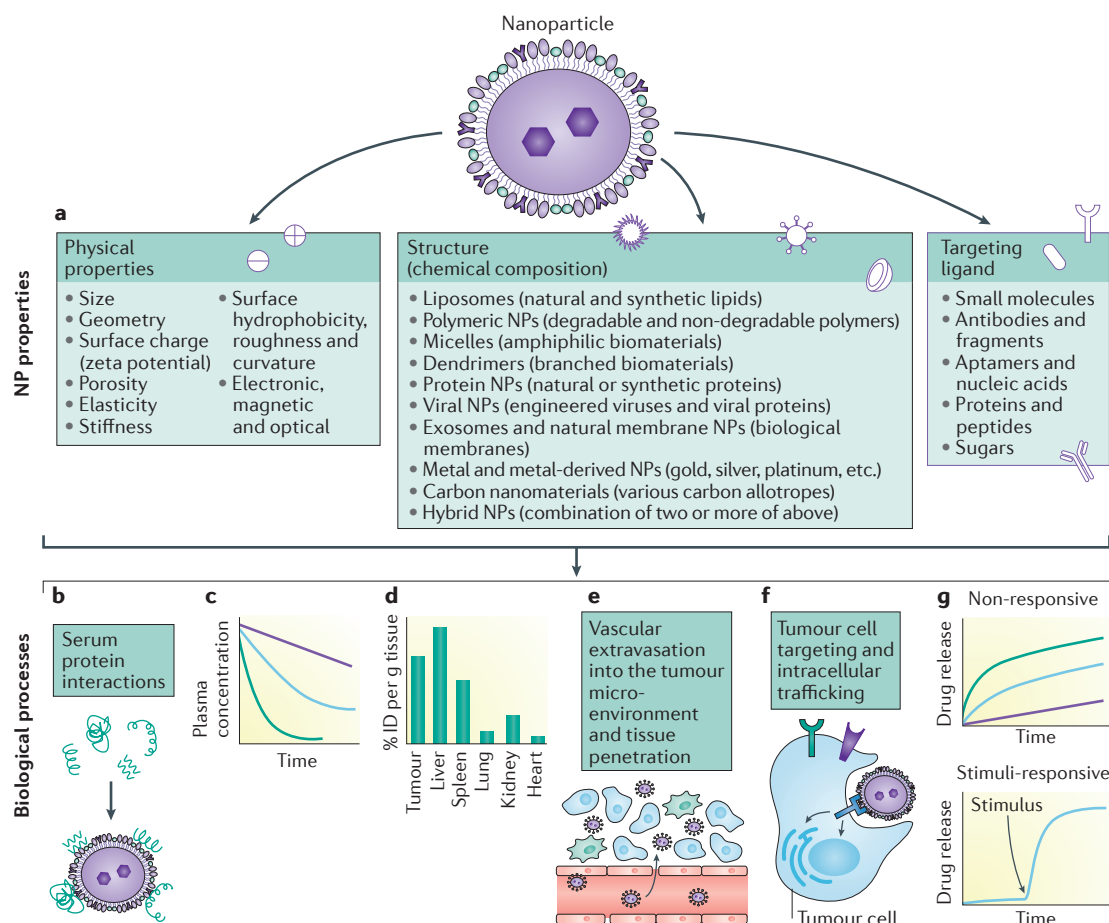


Figure 2 | The impact of nanoparticle properties on systemic delivery to tumours. Nanoparticles (NPs) can be made from different materials and have various physicochemical properties (for example, size, geometry, surface features, elasticity and stiffness, among others) and can be modified with a myriad of targeting ligands of different surface density (part **a**). NP properties affect the biological processes involved in the delivery to tumour tissues, including interactions with serum proteins (part **b**), blood circulation (part **c**), biodistribution (part **d**), extravasation to perivascular tumour microenvironment through the leaky tumour vessels and penetration within the tumour tissue (part **e**), and tumour cell targeting and intracellular trafficking (part **f**). NPs can also be designed to control the release profile of payloads (part **g**). ID, injected dose.

of lesion size reduction in six cancer patients suggests a positive association with ferumoxytol levels in the lesions at 24 hours⁵⁷, although a larger study is required for validation. We expect further similar findings to pave the way for companion imaging particles, such as ferumoxytol, to be used in patient selection and predictive nanomedicine (FIG. 3a).

Therapeutic NPs labelled with radioisotopes (for example, ¹¹¹In, ^{99m}Tc, ¹²³I and ⁶⁴Cu) have also been used to monitor biodistribution and tumour accumulation through non-invasive imaging techniques that include single-photon emission computed tomography (SPECT), computed tomography (CT) and positron emission tomography (PET)^{58,59,68–71}. A recent clinical study demonstrates that high tumour accumulation of LD as determined by quantitative imaging of [^{99m}Tc]LD, is positively associated with the response and survival of patients with unresectable pleural mesothelioma treated with a combination of LD and cisplatin⁵⁹. A high degree of heterogeneity in tumour accumulation was also revealed by PET–CT imaging of ⁶⁴Cu-loaded liposomes

in canine cancers, with six of seven carcinomas compared with only one of four sarcomas displaying high uptake of liposomes⁷². These results highlight the potential of radioisotope-labelled therapeutic NPs to assess patient suitability for nanotherapies (FIG. 3b). Although incorporation of contrast agents in therapeutic NPs can provide important insights into tumour heterogeneities and EPR, the development of such theranostic NPs may pose additional complexity in terms of design, synthesis, scaling and regulatory considerations⁴⁷.

Aside from developing imaging NPs as potential markers of therapeutic efficacy, few studies have aimed to identify EPR-predictive gene, protein or cell biomarkers (FIG. 3c). The ratio of matrix metalloproteinase 9 (MMP9) to tissue inhibitor of metalloproteinase 1 (TIMP1) in the circulation, as well as vessel wall collagen content, has been shown to predict EPR for liposomes^{73,74}. Various circulating biomarkers associated with angiogenesis, such as angiogenic factors (for example, vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2), MMP9, interleukin-8 (IL-8), IL-6 and hepatocyte

Table 2 | **In vivo examples of nanoparticle-mediated combination therapies for cancer treatment in mouse tumour models**

Nanotechnology platform	Active pharmaceutical ingredients	Therapeutic mechanism	Tumour model	Refs
Organic NPs				
Liposomes or lipid-based NPs	Irinotecan and cisplatin	Combination of chemotherapies	SCLC	274
	Combretastatin and doxorubicin	Combining anti-angiogenesis and chemotherapy	Melanoma	275
	Doxorubicin and antisense oligonucleotides	Combination of chemotherapy and antisense therapy (targeting MRP1 and BCL-2)	NSCLC	54
	Vorinostat and siRNA	Combination of chemotherapy and RNAi therapy (targeting MCL1)	Cervical cancer	276
	Docetaxel and DNA	Combination of chemotherapy and gene therapy using survivin suppressor	Hepatocellular carcinoma	277
	siRNAs	RNAi therapies against MDM2, MYC and VEGFA	NSCLC	278
	Oligonucleotide G3139 and D-(KLAKLAK) ₂ peptide	Combining antisense therapy (targeting BCL-2) and peptide-enhancing apoptosis	Melanoma	279
Polymeric micelles or NPs	Doxorubicin and paclitaxel	Combination of chemotherapies	NSCLC	280
	Doxorubicin and disulfiram	Combination of chemotherapy and anti-drug resistance	Drug-resistant breast cancer	281
	Paclitaxel and siRNAs	Combination of chemotherapy and RNAi therapy (targeting SNAIL and TWIST)	Breast cancer	282
	Camptothecin and DNA	Combination of chemotherapy and gene therapy using TRAIL-encoded plasmid	Colon cancer	283
	Paclitaxel and DNA	Combination of chemotherapy and gene therapy using IL-12-encoded plasmid	Breast cancer	284
	siRNAs	RNAi therapies against VEGFA and BCL-2	Prostate cancer	285
	Antisense oligonucleotides	Antisense therapies against miRNAs miR-10b and miR-21	Triple-negative breast cancer	286
Lipid-polymer hybrid NPs	Combretastatin and doxorubicin	Combining anti-angiogenesis and chemotherapy	Melanoma and Lewis lung carcinoma	197
	Cisplatin and siRNAs	Combination of chemotherapy and RNAi therapy (targeting REV1 and REV3L)	Prostate and breast cancer	287
	Doxorubicin and siRNA	Combination of chemotherapy and RNAi therapy against MRP1	Triple-negative breast cancer	288
	Doxorubicin and TRAIL	Combination of chemotherapy and cytokine-induced apoptosis	Breast cancer	289
	TGFβ inhibitor SB505124 and IL-2	Enhancing tumour immunotherapy	Melanoma	199
	siRNAs and miRNA	Combination of RNAi therapy (targeting MYC, MDM2 and VEGFA) and miR-34a-induced apoptosis	Lung metastasis	166
	Cisplatin and pyrolipid	Combination of chemotherapy and photodynamic therapy	Head and neck cancer	290
	Paclitaxel and yttrium-90	Combination of chemotherapy and radiotherapy	Ovarian intraperitoneal metastasis	291
Dendrimers	Doxorubicin and DNA	Combination of chemotherapy and gene therapy using TRAIL-encoded plasmid	Liver cancer	292
	Doxorubicin and CpG oligonucleotides	Combination of chemotherapy and immunotherapy	Prostate cancer	293
Inorganic NPs				
Iron oxide NP	Doxorubicin and curcumin	Combination of chemotherapies	Glioma	294
Graphene	Doxorubicin and TRAIL	Combination of chemotherapy and cytokine-induced apoptosis	NSCLC	44
Carbon nanotube	siRNA	Combination of hyperthermia and RNAi therapy	Prostate cancer	295
Gold nanorod	Doxorubicin	Combination of hyperthermia and chemotherapy	Cervical cancer	296
MoS ₂ nanosheet	Doxorubicin	Combination of hyperthermia and chemotherapy	Breast cancer	297

IL, interleukin; MCL1, myeloid cell leukaemia 1; MoS₂, molybdenum sulfide; miRNA, microRNA; MRP1, multi-drug resistance-associated protein 1 (also known as ABCB1); NPs, nanoparticles; NSCLC, non-small-cell lung cancer; RNAi, RNA interference; SCLC, small-cell lung cancer; siRNA, small interfering RNA; TGFβ, transforming growth factor-β; TRAIL, tumour necrosis factor (TNF)-related apoptosis-inducing ligand; VEGFA, vascular endothelial growth factor A.

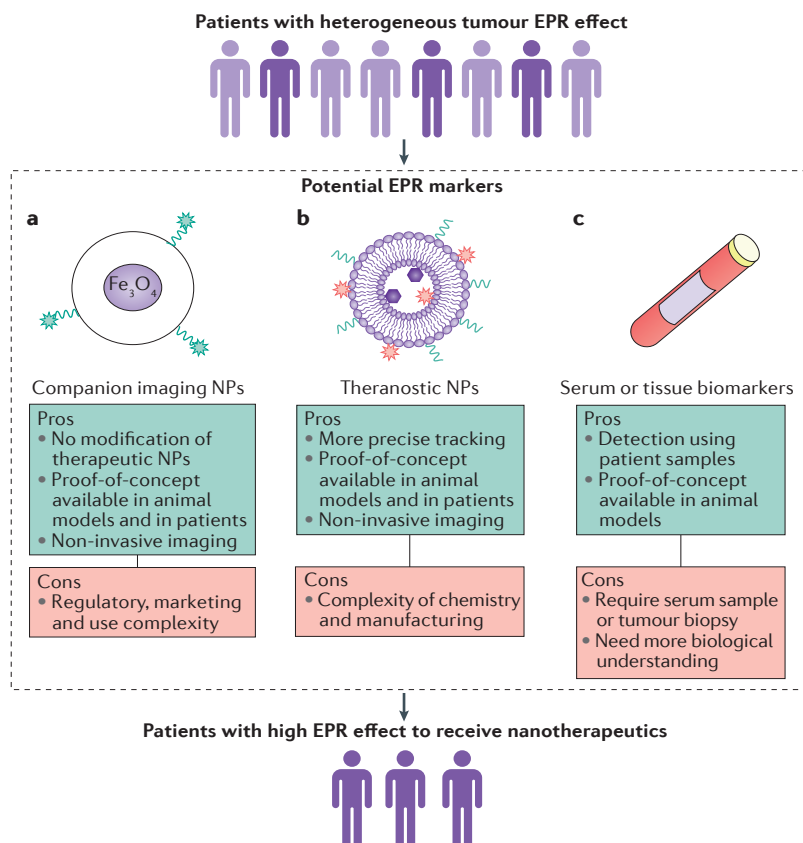


Figure 3 | Potential markers for predicting EPR effect and nanotherapeutic efficacy. **a** | Companion imaging agents (for example, ferumoxytol nanoparticle (NP)) have been applied to predict the accumulation of poly(D,L-lactic-co-glycolic acid)-*b*-poly(ethylene glycol) (PLGA-PEG) NP-encapsulated docetaxel and its anticancer activity in solid tumours, and ferumoxytol is currently in clinical trials to determine its feasibility as a predictive marker for the liposomal irinotecan MM-398. **b** | Therapeutic NPs labelled with imaging agents (for example, radioisotopes), also called theranostic NPs, have been used to monitor their biodistribution and tumour accumulation using various imaging techniques both preclinically and clinically. **c** | Serum and tissue biomarkers may also serve as surrogate markers for the enhanced permeability and retention (EPR) effect, as suggested by one recent example showing strong correlation of liposome accumulation in tumours with the relative ratio of matrix metalloproteinase 9 (MMP9) to tissue inhibitor of metalloproteinase 1 (TIMP1) in the circulation.

Opsonins

Plasma proteins (for example, immunoglobulins, complement proteins and fibrinogen) that coat a foreign particle to facilitate its uptake and destruction by phagocytic cells.

Mononuclear phagocyte system

(MPS). Part of the immune system composed of scavenging monocytes and macrophages, located in reticular connective tissue surrounding, for example, the liver, spleen, lung and bone marrow.

growth factor (HGF)), proteins and peptides (for example, endostatin and tumstatin), and endothelial cells and endothelial progenitor cells, have been described^{75,76}. However, their role, along with other potential biomarkers in predicting EPR, needs further investigation in preclinical and correlative clinical studies.

Enhancing drug delivery to the tumour

NP–protein interactions. When a NP enters a biological environment (for example, blood, interstitial fluid or extracellular matrix (ECM)), its surface is rapidly covered by various biomolecules (typically proteins), leading to the formation of a ‘corona’ (REFS 77–81) (FIG. 2b). The adsorption of proteins alters the particle size, stability and surface properties and, more importantly, provides the NPs with a biological identity that determines the physiological responses they elicit, ranging from cellular uptake and intracellular trafficking to PK, biodistribution and

toxicity (FIG. 2c–f). For instance, the binding of opsonins can trigger recognition and clearance by the mononuclear phagocyte system (MPS)⁷⁹. Conversely, it has also been suggested that a corona rich in dysopsonin proteins (for example, apolipoproteins and albumin), which inhibit phagocytic uptake, could contribute to the stealth effect of NPs^{82–84}. While ligand-functionalized NPs might lose targeting capability when a protein corona forms on their surface⁸⁵, decoration of NPs with some particular plasma proteins could improve delivery to specific organs. One recent example is the finding that apolipoprotein E is essential for some siRNA lipoplexes to target hepatocytes *in vivo*⁸⁶. In contrast, NP–protein interactions in clinical settings can also trigger hypersensitivity reactions in patients by activating the complement system⁸⁷.

Using various analytical techniques, several studies have extensively characterized the protein corona (for example, its composition, density, conformation, thickness, affinity and dynamics) on certain nanomaterials (for example, gold, silica and polystyrene NPs and liposomes)⁸⁸. It is now clear that NP–protein interactions are highly dependent on the NP physicochemical properties, exposure time as well as protein source and concentration. However, we still do not have a clear picture of how NP properties (FIG. 2a) and protein adsorption patterns (FIG. 2b) correlate with specific physiological responses (FIG. 2c–f). With high-throughput characterization of the serum protein corona fingerprint in a library of 105 different gold NPs, a quantitative multivariate model was developed to predict interaction of NPs with cells⁸⁹. Protein corona fingerprints and physicochemical properties of 17 liposomal formulations were recently used to predict multiple biological interactions including cellular uptake and viability of various tumour cells⁹⁰. Attention was also paid to the crucial role of human disease type on the composition of the protein corona and its effects on cellular uptake and toxicity of NPs⁹¹. Nevertheless, most of these studies were focused on NP–protein interactions *in vitro*, and little effort has been made to study protein corona formation *in vivo* and its correlation with PK, biodistribution and therapeutic efficacy. It is noteworthy that the very few *in vivo* evaluations of the protein corona demonstrated significant differences between *in vitro* and *in vivo* results⁹².

Moreover, we think that this field could be further advanced by addressing the following questions. Do we need specific protein-knockout mouse models to validate and explain the observations from *in vitro* studies and normal mice? In addition to the widely studied proteins in serum, how do the proteins in other biological environments, such as the TME, affect the corona, NP interactions with tumour cells and NP penetration across the tumour ECM? What new techniques will we need to more precisely characterize and quantify the *in situ* protein corona? We expect that by extending the methodology of quantitative structure–activity relationships to diverse NP platforms and biological responses, such as nanomics approaches could facilitate a deeper understanding and better control of the nano–bio interface and prompt more rational design of safe, effective and even patient-specific nanomedicines.

Blood circulation. There is a relationship between blood circulation half-life (FIG. 2c) and the efficiency with which a NP passively extravasates from the microvasculature into the TME. For tissues with relatively large blood flow and particles that efficiently extravasate from the microcirculation, a relatively short blood circulation half-life may be sufficient for the desired accumulation in the tumour. Conversely, for poorly perfused tissues or particles with low extravasation efficiency, a longer circulation half-life may be necessary to enhance exposure in the tumour microvasculature, enabling extravasation to occur progressively.

One major factor limiting circulation time is the non-specific interaction between NPs and serum proteins discussed above, which can promote opsonization and recognition by the MPS. Among various approaches to developing long-circulating NPs, the most widely used is PEG grafting on to the NP surface^{93,94}, such as Doxil, a 'stealth liposome' with a circulation half-life of approximately 2 days⁹⁵. Although pegylation can reduce protein adsorption through hydrophilicity and steric repulsion effects to avoid MPS clearance, such a simplistic view of 'stealth' long-circulating particles, which was coined more than 25 years ago ('Stealth' being a trademark of Liposome Technology, Inc., Menlo Park, California, USA), is becoming outdated. For example, increasing the density of PEG on a gold NP surface can decrease the amount and change the types of protein that bind to the NPs, reducing macrophage uptake *in vitro*⁸². More recently, pegylation of polystyrene NPs has been shown to selectively enrich the adsorption of clusterin to the NP surface, contributing to the decreased nonspecific macrophage uptake *in vitro*⁹⁶. The mechanical stiffness and elasticity of particles has also been recognized to influence MPS sequestration^{97,98}.

Another biologically inspired strategy to extend residence time in blood is to modify NPs with 'self' markers that prevent normal cells from activating the MPS. The bottom-up approach is chemical conjugation of self markers such as CD47 peptides⁹⁹ to the NP surface, which can inhibit phagocytosis. The top-down method is to coat NPs with a membrane of erythrocytes, leukocytes or thrombocytes, thus 'camouflaging' them to help reduce MPS elimination^{100–103}. Although the circulation half-life of cell membrane-coated NPs is longer than that of 'bare' NPs, it is still much shorter than that of the cells themselves. Therefore, more efforts are required to examine the changes in the cell membrane, including its components and elasticity, after the NP surface has been coated.

Extravasation to the TME. Extravasation of NPs from the systemic circulation to tumours (FIG. 2d,e) can be influenced by aberrant tumour vasculature, the perivascular TME and the NP itself. The metabolic demands of rapidly dividing cancer cells result in the formation of neovasculature that is architecturally abnormal and exhibits a 'leakiness' distinct from that occurring with inflammation. Unlike the endothelial lining of normal vasculature, which has a turnover of approximately 1,000 days, the endothelium in tumours can double approximately every 10 days¹⁰⁴, and the resulting microvasculature does not

have clearly defined morphology with distinct venules, arterioles or capillaries. In the case of inflammation, the extravasation of immune cells occurs primarily at the level of the venules¹⁰⁵. However, the exact contribution of various segments of the tumour neovasculature to permeability remains poorly understood. In addition to an arsenal of inflammatory mediators such as histamine^{106,107}, the interaction of tight junction modulators such as cationic polymers with endothelium can also induce endothelial contraction and tight junction disassembly, leading to vascular leakiness¹⁰⁸. For tumours, both vascular permeability and blood velocity are complex and kinetically variable from segment to segment¹⁰⁹. The spatiotemporal changes in vascular permeability can also be in part explained by the recent observation of transient opening and closing of pores, referred to as 'dynamic vents', in the walls of tumour vessels¹¹⁰. With an adequate NP circulation half-life, the dynamic vents could potentially improve delivery to tumours (in particular for large NPs). Furthermore, vascular mediators such as nitric oxide and angiotensin II could enhance tumour vascular permeability for more effective NP extravasation^{10,111}. There is also considerable variability in blood viscosity and oncotic pressure in various segments of the vasculature and TME, influencing the movement of NPs into and out of the tumour interstitium. Further studies will help to elucidate the mechanisms involved in NP extravasation into tumours, improving engineering and design schemes for efficient NP accumulation.

The impact of NP physicochemical properties on tumour extravasation and accumulation has also been examined. For example, in hyperpermeable murine colon adenocarcinoma, 30, 50, 70 and 100 nm polymeric micelles all demonstrated similar extravasation and anticancer activity, whereas in hypopermeable pancreatic tumours, only the 30 nm micelles showed sufficient accumulation¹¹². This study further indicates the influence of tumour heterogeneity on nanotherapeutic efficacy, underscoring the need for personalized nanomedicine. Compared with nanospheres, some elongated nanostructures (for example, the nanoworm¹¹³ and the nanorod¹¹⁴) improve tumour accumulation. Non-spherical particles also tend to accumulate and adhere to the endothelial cells that line vessel walls better than spherical or quasi-hemispherical particles, enhancing site-specific delivery^{115,116}. However, the effect of NP shape on extravasation can be very complicated, depending on the tumour models studied¹¹⁷.

Other unique strategies have also been proposed to enhance extravasation of NPs to the tumour interstitial space. Exploiting the 'tumour-tropic' property of certain cells (for example, mesenchymal stem cells, macrophages and monocytes)^{118–121}, therapeutic NPs can either be attached to the cell surface or loaded into the cells for homing to tumours. Recently, an innovative approach used two types of communicating NP to amplify tumour targeting and accumulation¹²²: the photothermal heating of 'signalling' gold nanorods disrupts tumour blood vessels to initiate extravascular coagulation, which can be recognized by the 'receiving' NPs in circulation, which bind to the resulting clot.

Nanomics

The collective study and characterization of the interactions between nanomaterials and biological systems.

Circulation half-life

The period required for drugs or nanoparticles in the blood to be reduced by one-half of a given concentration or amount.

Oncotic pressure

A form of osmotic pressure exerted by colloids in a solution, such as proteins in the plasma of a blood vessel.

Tumour penetration. Despite the emphasis on extravasation and accumulation in NP delivery, it is now known that, depending on the therapeutic payload, deep and uniform tumour penetration of nanotherapeutics may also be crucial for optimal outcomes. Studies of macromolecules (for example, dextrans¹²³ and antibodies¹²⁴) demonstrate that size and binding affinity affect both diffusion kinetics and depth of tissue penetration. For instance, higher-affinity antibodies that bind to target antigens on cancer cells penetrate tissue less efficiently than lower-affinity antibodies against the same target¹²⁴. This is because during tissue penetration higher-affinity antibodies tend to bind tightly to the target and become internalized, whereas lower-affinity antibodies tend to bypass their target and thus penetrate deeper. Much can be learned from antibody studies to aid in the design of nanotechnologies for cancer targeting, such as the pros and cons of adding targeting ligands on the NP surface⁴⁷; although this may enhance cellular uptake and lengthen tissue residence, it may also reduce the depth of tumour penetration.

Therapeutic NPs, nearly always larger than antibodies, tend to become trapped in the ECM around the microvessels from which they extravasate¹²⁵ (FIG. 2e). Challenges include the physiological barriers intrinsic to the TME, such as the dense interstitial matrix composed of collagen fibres and other proteins, and the elevated interstitial fluid pressure (IFP) induced by hyperpermeability of abnormal vasculature and lack of functional lymphatics deep in the tumour tissue^{125,126}. These in turn reduce convective transport of NPs across the vessel wall and into the interstitial space. Nonspecific uptake by perivascular stromal cells such as TAMs^{60–63} can further limit the diffusion of nanotherapeutics. It is also noteworthy that mean tumour intercapillary distances generally range from approximately 80 to hundreds of micrometres^{127–129}, presenting another obstacle to NPs reaching tumour cells that are further from vessels.

For enhanced tumour penetration, one possible solution is to tune the physicochemical properties of NPs to penetrate diffusional barriers in the interstitial matrix. Smaller NPs could more readily diffuse throughout the tumour tissue^{130–132}, but very small particles (for example, <5 nm) may be quickly cleared by renal filtration. Moreover, small NPs have a large surface area to volume ratio and a short diffusion distance for encapsulated drugs, limiting their drug-carrying and controlled-release capabilities. Nanorods (15 nm × 54 nm) have also exhibited more rapid tumour penetration than 35 nm nanospheres¹¹⁴, possibly related to their shorter dimension, although both have a 33–35 nm hydrodynamic diameter and nearly identical diffusion rates in water. In addition, surface modification with tumour-specific penetrating peptides, such as the cyclic peptide CRGDK/RGPD/EC (also called iRGD), has also been shown to substantially increase the depth of NP delivery into tumour parenchyma^{133,134}. Further systematic study of NP–TME interactions through real-time *in vivo* imaging techniques, such as intravital microscopy¹³⁵, may identify the optimal particle properties for rapid diffusion. TME-modification approaches, such as degrading the tumour ECM and

inhibiting the activity of tumour-associated fibroblasts to reduce their production of matrix components, which were previously overviewed¹³⁶, could likewise assist NPs in permeating tumour tissues.

Recently, an alternative novel multistage delivery strategy has been proposed to address the penetration problem¹³⁷. Small-molecule drug conjugates (SMDCs)¹³⁸ and miniaturized biologic drug conjugates (mBDCs), including peptide–drug conjugates¹³⁹, were developed to address the large size shortcoming of antibody–drug conjugates (ADCs), which limits their tumour tissue penetrability. SMDCs and mBDCs can also enhance retention and cellular uptake by tumours compared with free drug alone. However, their considerable drawbacks include poor PK, which may limit their tumour exposure and therapeutic impact. By incorporating SMDCs and mBDCs into controlled-release polymeric NPs for multistep delivery to tumours, it may be possible to combine the superior PK and tumour accumulation of NPs with the deep penetration and specific tumour cell targeting of released SMDCs and mBDCs for optimal targeted cancer therapy¹³⁷.

There have also been reports of similar multistage delivery platforms^{130,140} in which very small NPs (for example, approximately 10 nm quantum dots) are first loaded into large particles, such as approximately 3.5 µm hemispherical mesoporous silicon¹⁴⁰ or approximately 100 nm gelatin NPs¹³⁰. When the large particles reach the tumour vasculature or are exposed to the TME, the released smaller NPs can then readily diffuse throughout the interstitial space of the tumour. Other stimuli-responsive multistage delivery platforms have recently been developed for various hard-to-treat solid tumours^{141,142}.

Cellular uptake and intracellular trafficking. Effective cell internalization may also have an important role in enhancing NP retention, EPR and therapeutic efficacy, as many nanomedicines act on intracellular targets. This is particularly true for biomacromolecule payloads such as those involved in the RNAi pathway (for example, siRNA and microRNA (miRNA)), which require cytosolic delivery for bioactivity^{143–145}. To improve cellular uptake, one approach is to decorate the NPs with targeting ligands that recognize specific receptors on the tumour cell surface^{9,146,147} (FIG. 2f). Moreover, active targeting is of importance when tissue accumulation does not depend on EPR (for example, vascular targeting)¹⁴⁸ or when the delivery of therapeutic agents requires active transcytosis of physiological barriers such as the intestinal mucosa or the blood–brain barrier^{149–151}. Since the concept of active NP targeting was introduced more than 30 years ago^{152,153}, a few examples have made their way into clinical trials⁹, including targeted liposomes (for example, HER2 (also known as ERBB2) single-chain variable fragment (scFv)-targeted liposome (MM-302)¹⁵⁴), the first targeted and controlled-release polymeric NP (BIND-014)¹¹ and the first targeted siRNA NP (CALAA-01)³⁰. Even without targeting ligands, NPs can still be engineered for increased uptake by tumour cells by exploiting size- and shape-dependent cell internalization^{155,156}.

In addition, it may be important to investigate the effect of cancer cell mutations on NP internalization. Pancreatic cancer cells with KRAS mutations show elevated macropinocytosis of proteins such as albumin, and the ability of extracellular albumin to enhance the proliferation of cancer cells after glutamine starvation is also dependent on oncogenic KRAS expression¹⁵⁷. This study indicates that macropinocytosis might enhance the uptake of drugs such as paclitaxel when it is bound to albumin, partly explaining the recent success of nab-paclitaxel in treating advanced pancreatic cancer largely driven by oncogenic KRAS¹⁵⁸. Whether such an oncogenic mutation effect also applies to NP endocytosis remains unclear. It should also be noted that our current understanding of NP–cell interactions is generally based on *in vitro* studies, which may not reflect the heterogeneity of tumour cells *in vivo*. Recent advances in high-resolution cellular *in vivo* imaging methods have enabled the detailed analysis of single-cell PK and cell-to-cell variability in tumours^{159–162}, and are expected to provide insights into NP interaction with tumour cells and the TME *in vivo*.

After internalization, NPs must either release their therapeutic payload for diffusion through the cellular compartments to reach the target, or be directed through intracellular trafficking pathways to release therapeutics in the appropriate subcellular location. For cytosolic delivery of biomacromolecules such as siRNA, NP endosomal escape is crucial. Cationic lipid-, lipid-like material- and polymer-based NPs have shown great promise in siRNA delivery^{163–165}. Notably, most RNAi nanotherapeutic agents in clinical trials for cancer treatment are composed of liposomes or lipid NPs (TABLE 1). Although these lipid-based NPs are currently not functionalized with ligands for active targeting, targeted NP delivery could further enhance tumour accumulation and retention and cellular uptake of siRNA^{144,166}. Despite clinical trial success, the efficiency of lipid NP-mediated siRNA release from endosomes remains low (1–2%)¹⁶⁷, and approximately 70% of the internalized siRNA may undergo Niemann–Pick type C1-mediated exocytosis¹⁶⁸. Thus, alternative strategies will be necessary to develop NP platforms with highly efficient endosomal escape. Besides cytosolic delivery, targeting intracellular organelles such as the nucleus, mitochondria, endoplasmic reticulum and Golgi has also been pursued. Whereas some NPs have been developed for specific uptake by these subcellular compartments^{169–174}, the underlying barriers of organelle membranes to the transport of NPs need to be further explored.

Controlled drug release. An equally important yet often overlooked consideration is that systemically administered NPs may gradually release their payload during circulation (FIG. 2 g), such that long-circulating particles with slow tumour extravasation may hold relatively small payloads by the time they reach the TME. Therefore, simultaneous consideration of drug release, NP PK and NP extravasation is required to achieve optimal outcomes.

We think that the design of optimal NP systems requires a deep understanding of several complex parameters: the interplay between NP PK and drug PK, between

encapsulated drug and released drug in plasma, between drug C_{\max} and NP C_{\max} , between drug plasma AUC and NP plasma AUC; and factors that may differentially affect plasma versus tumour PK and AUC. Conventional small-molecule drugs generally reach their plasma C_{\max} during the intravenous infusion period, followed by a reduction in plasma drug concentration. Similarly, for drug-encapsulated controlled-release NPs, C_{\max} will be reached during infusion, yet the plasma concentration of the released drug will in general be very low initially and progressively increase, reaching its C_{\max} after some period of NP circulation. Importantly, the drug C_{\max} achieved through release from NPs is unlikely to ever reach the levels achieved with intravenous administration of free drug. Therefore, drug-associated toxicities related to C_{\max} may be mitigated using NPs. On the other hand, plasma AUC will be relatively similar for free drug and NP-released drug, with one key difference: the AUC of NP-released drug will be generally broad and flat, whereas the AUC for free drug is likely to be peaked, with a tail. The implications are that although certain toxicities may be reduced with the use of NPs, the AUC-related toxicity may be harder to overcome with NPs even though the total dose is released over a longer period of time. When drugs are delivered by NPs, the tumour PK and AUC are dramatically different from those of drugs given in the conventional form, in part because EPR results in differential tumour accumulation of NPs to a greater extent than free drugs and the drug released from NPs localized to tumours can lead to higher tumour drug concentrations over a longer period of time. Most publications demonstrate that NPs enhance delivery of drugs to tumours. A subset of studies show that over time, tumour drug C_{\max} , PK and/or AUC^{175–178} are improved compared with conventional dosage. However, these increases in tumour drug accumulation have not always translated into improved patient OS, begging several questions. Does the increase in tumour drug concentration broadly occur in patients or is patient selection needed to identify the subpopulation likely to accumulate NPs? Is increased tumour dose universally beneficial for all drugs or does that depend on the drug?

To precisely control drug release, various stimuli-responsive NPs have been developed and are summarized in a recent review article¹⁷⁹. In general, these NPs are designed to recognize subtle environmental changes associated with the TME and tumour cells (for example, pH, redox state and enzymes) or to be activated by external stimuli (for example, heat, light, magnetic field or ultrasound), triggering the release of the payload (FIG. 2 g). To some degree, external stimulation enables tailored drug-release profiles with temporal and spatial control. Thermosensitive liposomes (for example, the LD ThermoDox) for heat-mediated drug release are the most advanced clinical stage platform to date. In 2013, ThermoDox failed to meet its primary end point of PFS in a phase III study for hepatocellular carcinoma, and is now undergoing a phase III study with OS as the primary end point (see TABLE 1). More recent stimuli-responsive nanomaterials being investigated include pH- or redox-sensitive polymeric NPs^{142,180–182},

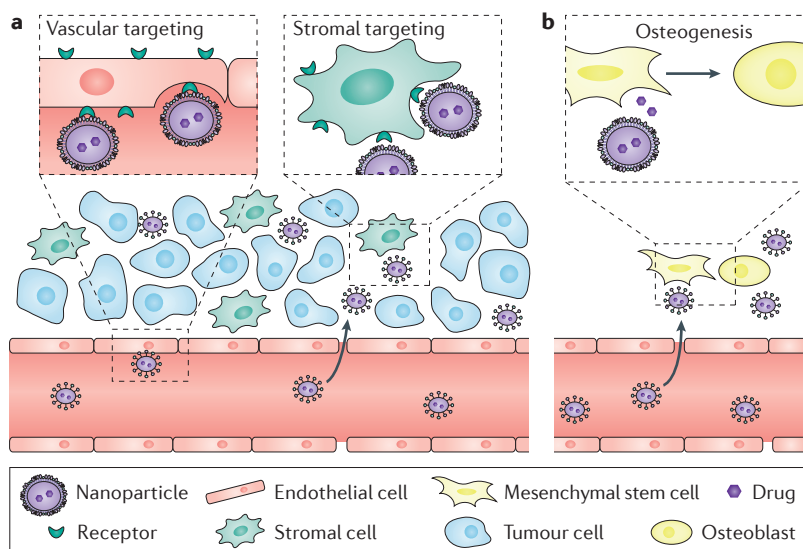


Figure 4 | Nanoparticle targeting of the tumour microenvironment and the premetastatic niche. Targeting of the tumour vasculature or stromal cells in the tumour microenvironment (part **a**) and the premetastatic microenvironments such as the bone marrow niche, where induction of the osteogenic differentiation of mesenchymal stem cells enhances bone strength and volume (part **b**). Cell-specific targeting can be achieved via the modification of nanoparticles (NPs) with ligands that bind to specific receptors (for example, $\alpha_v\beta_3$ integrin and mannose receptor) on the surface of tumour endothelial cells, stromal cells or other target cells. It should be noted that even without targeting ligands, NPs can be engineered for preferential cellular uptake. The payloads released from NPs localized in tumours or premetastatic tissues can also be nonspecifically taken up by these cells.

ultrasound-responsive polymer-grafted silica NPs¹⁸³ and near-infrared light-responsive graphene oxide nano-sheets¹⁸⁴, among others. The clinical potential of these newer systems remains to be determined.

Overall, with continuous improvements in our understanding of the biological steps in systemic NP delivery, a myriad of new strategies have been developed for enhancing drug delivery to tumours and therapeutic responses. As most of these results are from animal studies, further clinical validation is necessary.

Targeting the TME and the premetastatic niche

As the TME has an important role in tumour development, progression and metastasis and in the emergence of drug resistance, it has also been considered a target for cancer treatment^{14,15,185}. As discussed above, TME modification also offers an alternative strategy for enhancing the tumour accumulation and penetration of NPs^{107,136}. Compared with cancer cells, one advantage of targeting non-tumoural cells in the TME is that they are likely to be more genetically stable and thus less prone to develop drug resistance¹⁸⁵. However, targeting non-tumoural cells raises the challenge of achieving a therapeutic effect while minimizing toxicity to normal cells; how TME modification affects tumour growth and metastasis needs more careful examination. Beyond the TME of the primary tumour, the environmental conditions required for metastatic cells to survive and proliferate have also received considerable attention in the development of new therapeutic avenues¹⁸⁶. Early interference with the

formation of the premetastatic niche may be particularly beneficial in the treatment of malignancies that tend to metastasize.

Tumour vasculature. Much effort has focused on NP-mediated selective drug delivery to the tumour vasculature (FIG. 4a), which is crucial to tumour growth and metastasis¹⁴⁸. This is commonly achieved by coating NPs with ligands that bind specifically to over-expressed receptors such as $\alpha_v\beta_3$ integrin¹⁸⁷ on the surface of tumour endothelial cells. *In vivo* studies in mice revealed that inhibiting angiogenesis can cause regression of established tumours or suppression of metastasis^{188,189}. Besides targeted NPs, several non-targeted cationic lipid or polymeric NP platforms have been designed for preferential delivery of siRNA to vascular endothelium^{190–192}. A recent unique formulation called 7C1 specifically reduced the expression of target endothelial genes at low siRNA doses without substantially reducing their expression in pulmonary immune cells, hepatocytes or peritoneal immune cells¹⁹². By silencing VEGF receptor 1 (VEGFR1) or delta-like protein 4 (DLL4) involved in angiogenesis, 7C1 RNAi NPs reduced growth and metastasis of Lewis lung carcinoma in mouse models *in vivo*¹⁹². This interesting system could also be used to study how interactions with serum proteins direct nanomaterials to endothelial cells *in vivo*.

Stromal cells. Targeting stromal cells such as tumour-associated fibroblasts and macrophages has also been proposed for cancer treatment (FIG. 4a). A unique docetaxel-conjugated NP platform called Cellax significantly depleted α -smooth muscle actin (α -SMA)-expressing fibroblasts, reducing tumour ECM and IFP, increasing vascular permeability and suppressing metastasis¹⁹³. This effect is presumably through the adsorption of serum albumin on Cellax, followed by specific interaction with α -SMA⁺ fibroblasts that also express elevated levels of the albumin-binding protein, secreted acidic cysteine-rich glycoprotein (SPARC). Differentiation of TAMs to a pro-tumorigenic or immunosuppressive (M2-like) phenotype has commonly been associated with tumour progression and poor patient outcome¹⁹⁴. By inhibiting the activity of signal transducer and activator of transcription 3 (STAT3), hydrazinocurcumin-loaded NPs can ‘re-educate’ TAMs to transform from an M2-like into an antitumorigenic M1 phenotype for inhibited tumour growth¹⁹⁵. PEG-sheddable, mannose-modified NPs have also been developed to efficiently target TAMs that have elevated expression of mannose receptors, while minimizing uptake by macrophages of the MPS¹⁹⁶. Furthermore, NP-based co-delivery of multiple agents targeting both TME and tumour cells has produced synergistic anticancer effects^{197–199}. However, TME-targeting strategies must be pursued with care, as tumour stroma exhibits bipolar activity in tumorigenesis²⁰⁰.

Metastatic microenvironment. NP delivery to the major sites of metastasis (for example, lungs, liver, lymph nodes, brain and bone) and metastatic tumour cells themselves have been comprehensively discussed

elsewhere²⁰¹. A newly developed system of polymeric micelles formulated from polymer–drug conjugates has shown promising therapeutic efficacy in a mouse model of colon cancer with lung metastasis²⁰², and in a pilot study of one patient with castration-resistant prostate cancer with lung and bone metastases²⁰³. Comparatively little effort has been devoted to exploiting nanotechnology to modify the premetastatic microenvironmental niche and suppress tumour growth. In a recent study, a bone-homing polymeric NP platform was engineered for spatiotemporally controlled delivery of therapeutic agents²⁰⁴ (FIG. 4b). After pretreatment with alendronate-conjugated, bortezomib-loaded polymeric NPs, mice showed significantly slower myeloma tumour growth and prolonged survival. The application of such pretreatment strategies for protecting the organs vulnerable to metastasis could be accelerated by revealing which microenvironmental factors control the intravasation, adhesion and growth of metastatic tumour cells and how this is achieved.

Challenges in clinical translation

Controllable and reproducible synthesis. The determination of optimal physicochemical parameters is crucial for the successful development of therapeutic NPs. A considerable amount has been learnt regarding individual factors that can confer effective immune evasion, tumour extravasation and diffusion, cell targeting and internalization, and controlled drug release^{65,66,78,205,206}. Nevertheless, systematic parallel screening of the myriad of NP properties remains difficult, owing to the challenge of rapid, precise and reproducible synthesis of NP libraries with distinct features. Compared with traditional bulk techniques, which generally form NPs with high polydispersity, microfluidic technologies have recently attracted attention for high-speed self-assembly of NPs with narrower size distribution, tunable physical and chemical characteristics and greater batch-to-batch reproducibility^{207–211}. Similarly, particle replication in non-wetting template (PRINT) technology has enabled the synthesis of monodisperse NPs with precise control over size, shape, chemical composition, drug loading and surface properties^{212,213}. Such advances could eventually facilitate NP discovery, analogous to the way high-throughput screening of small molecules advanced drug discovery.

Evaluation and screening. With the rapid emergence of NPs composed of novel biomaterials or nanostructures, *in vitro* evaluation is important to identify biocompatible candidates before animal testing is pursued. *In vitro* assays can also improve our understanding of NP–cell interactions. However, as conventional *in vitro* models using cells cultured in multiwell plates lack the complexity of biological tissues and control over fluid flow, such platforms may not capture the intricate interplay of NPs with physiological barriers. Recent efforts to develop biomimetic ‘organ/tumour-on-a-chip’ tools^{214–216} may avoid the limitations of current *in vitro* models. The incorporation of tumour-like spheroids into a microfluidic channel could offer insights into the effects of interstitial flow,

cell binding and particle size on NP accumulation and diffusion²¹⁶. Comparison of NP behaviours in such chip systems with animal models may offer a preview of the potential of these biomimetic microdevices.

To assess *in vivo* NP performance (for example, PK, biodistribution, efficacy and safety), the use of animal models is obligatory. Whereas some studies have demonstrated PK scaling across different species (including humans) for different nanotherapeutics^{11–13,32}, one well-recognized obstacle is the discrepancy between the efficacies obtained in preclinical studies and the outcomes from clinical trials, in large part owing to the lack of tumour models that can recapitulate human cancers^{217,218}. Diverse animal models are currently available, including cell line-based subcutaneous and orthotopic xenografts, patient-derived xenografts (PDXs) and genetically engineered mouse models (GEMMs). However, no single model can fully reproduce all aspects of human malignancy, and EPR is generally more consistent in animal models than in cancer patients⁹. Furthermore, considering the major contribution of tumour metastases to cancer mortality, models of human tumour metastasis will be invaluable for the evaluation of EPR and NP penetration and targeting in metastatic tissues compared with primary tumours. The translation of nanotherapeutics may be greatly improved by the development of animal models that mimic closely the heterogeneity and anatomical histology of human tumours, such as high-fidelity PDXs²¹⁹, humanized mouse models²²⁰ and GEMMs with aggressive metastasis²²¹.

Scalable manufacturing. Another challenge to clinical development stems from the escalating complexity in the chemistry, manufacturing and controls (CMC) and good manufacturing practice (GMP) requirements as NP technologies transition from preclinical to clinical development, subsequent commercialization and beyond, as long as the product is on the market. Although the shared goal of CMC and GMP is to assure that a product consistently meets a predetermined standard of quality, they involve different but overlapping approaches and regulation. The scale-up of simple NPs, including liposomes and polymeric systems with small-molecule APIs that have desirable physicochemical properties, can be achieved using manufacturing unit operations readily available and widely used in the pharmaceutical industry. The scale-up of more complex nanomedicines may pose additional CMC and GMP challenges, and require modification of existing unit operations or development of novel manufacturing processes. Examples include nanomedicines that integrate biological targeting ligands or biological components, carry a combination of two or more therapeutics, are formulated through layer-by-layer assembly or comprise multiple functional units such as theranostics or multistage systems.

In general, large-scale and reproducible synthesis will be more difficult when NP formulation involves multiple steps or complicated technologies. Indeed, the transition from laboratory to clinic is nearly always accompanied by the optimization of formulation parameters, or even a change in formulation methods, making

Polydispersity

The heterogeneity of particle or molecule size in a mixture.

forward thinking of scale-up considerations an important aspect of early NP design and engineering. The PRINT technology is amenable to reproducible fabrication of NPs²¹³, yet scaling to kilograms remains to be demonstrated. A coaxial turbulent jet mixer technology, which has the advantages of homogeneity, reproducibility and tunability normally accessible only in microscale mixing techniques such as microfluidics, has recently been developed for mass production of polymeric NPs (potential of ~3 kg/day per channel)²²². Although today the mainstay of NP manufacturing remains bulk synthesis, robust and versatile approaches such as PRINT and turbulent jet mixer technologies, which can prepare NPs at throughputs suitable for industrial-scale production, may accelerate clinical translation.

Conclusion

Like most other scientific advances that have revolutionized medicine over the past decades, cancer nanomedicine must also mature before its full impact can be realized. Improving our understanding of tumour heterogeneity and identifying EPR markers will enable selection of patients maximally responsive to

nanotherapies. A full understanding of nano–bio interactions, systemic transport of NPs to tumour cells and targeting of NPs to the TME or premetastatic niche will lead to safer and more efficacious nanotherapeutics. Addressing the challenges of controllable, reproducible and scalable NP synthesis, as well as NP screening and evaluation, will facilitate clinical development. Although most approved nanomedicines have used existing drugs as payloads, we expect the next generation of nanomedicines to increasingly incorporate new molecular entities (for example, kinase inhibitors²⁴) and novel classes of therapeutic agent (for example, siRNA, mRNA and gene editing).

In summary, we are rapidly acquiring a much deeper understanding of the challenges and opportunities presented by cancer nanomedicine. This Review has explored the importance of the convergence of nanotechnology and tumour biology for more successful development and clinical translation of nanotherapeutics. We expect that nanomedicines will shift the paradigm of cancer treatment, and that the true goal of cancer nanomedicine — dramatic improvement in patient survival — will become a reality in the foreseeable future.

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Competing interests statement

The authors declare **competing interests**: see Web version for details.