Cancer nanomedicine

Sangeeta N. Bhatia, Xiaoyuan Chen, Marina A. Dobrovolskaia and Twan Lammers

The field of cancer nanomedicine seeks to overcome the inherent shortcomings of conventional cancer diagnostics and therapies. Yet despite the surge of interest in and attractive attributes of nanotechnologies, challenges remain in their clinical translation, prompting some to argue that they have not yet reached their true potential. In this Viewpoint article, we asked four experts for their opinions on how we can fulfil the great promise of nanomedicine for the detection, diagnosis and treatment of patients with cancer.

There is a large body of literature describing different nanoparticles as potential cancer therapies, yet only a handful has been US Food and Drug Administration (FDA) approved. Why is there such a disconnect?

Sangeeta N. Bhatia. This question has been in the air for years — and I fundamentally disagree with its framing. Let me explain: if we ask ourselves what new modalities have been introduced to patients to tackle human disease over time (for example, monoclonal antibodies, small interfering RNA (siRNA), gene therapy, CRISPR), we never judge the impact of the modality based on the timescale of regulatory approvals in a single disease area, such as cancer. Instead, we invest in the science and technology broadly and celebrate what emerges — often unexpectedly. In the United States, monoclonal antibodies were first approved for use in transplant rejection, siRNA for use in peripheral nerve disease and gene therapy in the eye, and CRISPR clinical trials are advancing in applications across several disease areas from haematology to liver disease. These new modalities have gone on to transform many areas of medicine, including (but not limited to) oncology.

If nothing else, the COVID-19 pandemic has taught us that long-standing investments in nanoparticle technology were well worth it. Decades of foundational science enabled the ability to package mRNA into four-component lipid nanoparticles that could be designed optimally, manufactured at scale, be accepted by regulators and, ultimately, be delivered as vaccines to patients with unprecedented speed. Without nanotechnology, in my opinion, we would be in a very different public health situation today. Many of us first envisioned that nanoparticles would deliver cytotoxic chemotherapeutic drugs with an improved therapeutic index as 'smart bullets' or improve cancer imaging. Where we stand today is with an updated vision that has broadened to include many new cancer applications including synergizing with immune checkpoint inhibition, potentiating adoptive cell therapy, enabling cancer vaccines, intraoperative margin detection, ultrasensitive detection of tumour-associated analytes and more. And whereas there were already several approved nanoparticle-based therapies[1], we stand at a moment in time where nanoparticles have now been administered safely to millions of people around the world. The future for cancer nanotechnology is brighter than ever.

Xiaoyuan Chen. It is true that translation success does not equate to academic output in the field of nanomedicine because nanomedicine faces several scientific challenges in terms of unclear toxicities and nano–bio interactions, reproducibility and transparency, and complexity (cost) of nanoparticle manufacturing:

- Unclear toxicities and nano–bio interactions. Nanoparticles are composite structures. The components, mode of assembly, surface, ligand, rigidity, charge and so on affect the behaviour of the structures. The complexity of nanoparticles makes it rather challenging to identify their potential toxicity profiles. With respect to nano–bio interactions, especially nano–immuno interactions, these have not been studied in proper model systems.
- Reproducibility and transparency. The determination of optimal physico-chemical parameters is crucial for the successful development of therapeutic nanoparticles. However, ensuring reproducibility and transparency restricts systematic and large-scale screening of nanoparticles, owing to the difficulty of rapid, precise and reproducible synthesis of nanoparticles with distinct properties.
- Complexity and cost of nanoparticle manufacturing. Another challenge in clinical translation is the complexity in chemistry, manufacturing and controls (CMC), and in good manufacturing practice (GMP) requirements. Despite the predetermined standards for small molecules and biologics, the requirements for nanomedicine involve different but overlapping regulations. Although promising, nanoparticles are usually costly in terms of labour and material expenses, and product release criteria are difficult to standardize.

Marina A. Dobrovolskaia. This 'disconnect' is a vivid example of the risks associated with the clinical translation of a previously untried technology. For example, today's market size of liposomal doxorubicin (which includes the original brand product Doxil and several of its generic versions) in the US is more than US $1 billion, and the projection for the global market in 2028 is US $2.1 billion with an 8% compound annual growth rate[2]. The global market of all liposomal drugs is also growing at a 12.8% compound annual growth rate[3]. But the history of this success is complex. In the early 1980s when liposomal technology was proposed for drug delivery, the initial scientific excitement about it captured big pharma's attention, and investors flocked to the field. As described in a 1990 *New York Times* article, the advent of this technology was followed by a big disappointment 9 years later when "all the miraculous things it was supposed to do" did not happen[4]. The translational activities simmered, and many industrial players left

the field[4]. It then took 10–20 years for the technology to mature enough to enter the next growth phase, as exemplified by the approval of two other liposomal cancer nanomedicines, Vyxeos and Onivyde.

Similar stories are repeated time and time again with every single new technology. To name a few, after the initial proof of concept using mRNA as a source of protein therapeutics in 1990 (REF.[5]), the technology was shelved until 2020 before gaining momentum again just recently with the successful use of COVID-19 vaccines. Likewise, siRNA technology was in trials for more than 20 years until Alnylam Pharmaceuticals finally got their first product, patisiran (an siRNA targeting *TTR*, which encodes transthyretin, a transport protein), approved by the FDA in 2018. This was followed by approval of two other products — givosiran (targeting 5-aminolevulinic acid synthase 1 (*ALAS1*)) and lumasiran (targeting 2-hydroxyacid oxidase 1 (*HAO1*)) — both within just a few years following the approval of the first siRNA product. I intentionally used mRNA and siRNA technologies as examples because products that utilize these technologies owe their success to nanotechnology — particularly to lipid nanoparticles and lipid nanocomplexes — used for delivery.

Besides the substantial scientific expertise, instrumentation and characterization methods needed for every new technology to translate into a viable drug product, the development is also hampered by high costs, regulatory gaps, investors' uncertainty and societal refusal, which collectively create the so-called 'valley of death'. The more complex the technology, the longer it takes and the more it costs to bring it to the clinic. For example, the cost of the 20-year journey of patisiran from bench to clinic was approximately US $2.5 billion[6]. The tangled story of mRNA COVID-19 vaccines further demonstrates the intellectually and emotionally complex environment for translating new technologies to the clinic[7]. Therefore, this 'disconnect' teaches us to be more patient with new technologies, not to expect too much too soon, and to appreciate and respect the plurality of opinions and the role of cognitive dissonance in public perception of new technologies.

Twan Lammers. A handful is not correct. More than 15 cancer nanomedicines have received regulatory approval, which is comparable with the number of approved antibody–drug conjugates. It is interesting to note in this regard that there has been much more criticism of the translation of cancer nanomedicines than of antibody–drug conjugates. This discordance likely results from the fact that there are thousands of papers every month on novel nanomedicines, oftentimes in high impact-factor (material science, not cancer) journals, with strong statements on improving the treatment of 'high-hanging fruit' malignancies, such as glioblastoma and pancreatic cancer. Conversely, there are much fewer papers on novel antibody–drug conjugates, and they tend to be in less high impact-factor journals, regarding less difficult-to-treat cancers and with less strong claims.

Among the main reasons why non-nanomedical anticancer drugs have had more impact on clinical cancer care than nanomedicine formulations is that biomarkers for patient stratification are more readily available for the former, contributing to their clinical success. Patients to be treated with antibodies can be stratified fairly easily, by histopathologically assessing receptor overexpression in tumour biopsy samples. Patient stratification is significantly more complicated for nanomedicines, particularly in the case of formulations not targeted to a certain receptor[8]. Non-invasive positron emission tomography (PET)- or single-photon emission tomography (SPECT)-based assessment of nanomedicine tumour targeting seems to be the go-to strategy in this regard, but this is not very pragmatic, and not cost- and time-efficient. Consequently, to promote the translation of traditional tumour-targeted nanomedicines, a key current challenge is to establish simple and straightforward biomarkers for patient stratification, for example based on tumour tissue or liquid biopsies.

Q *Is there an opportunity for nanotechnology to play a key role in cancer detection and diagnosis?*

S.N.B. An essential challenge in cancer detection is diagnosing lethal cancer (that is, not cancer one may die with but cancer one will die from) at earlier stages when intervention can have the greatest impact on outcomes[9]. At the heart of this challenge is our still nascent understanding of early cancer pathogenesis in different tissues. Meanwhile, detection by clinical imaging or molecular methods estimates that tumours can remain undetectable for more than a decade after tumour initiation (the sensitivity problem). Equally important is the specificity problem (identifying a lethal cancer while minimizing a false positive result). All of this must occur on a vast landscape of signals emanating from normal human biology with natural variations across a heterogeneous population. Nanotechnology can amplify signals to increase sensitivity, multiplex to improve specificity, and leverage artificial intelligence to precisely identify lethal cancers earlier.

The contributors

Sangeeta Bhatia is the John J. and Dorothy Wilson Professor of Engineering at Massachusetts Institute of Technology (MIT), the Inaugural Director of the Marble Center for Cancer Nanomedicine and a Howard Hughes Medical Institute Investigator. Her laboratory leverages micro- and nanotechnology for medical innovation with applications in liver disease, cancer, and infectious diseases. Bhatia was the 25th person in history to be an elected member of all three US National Academies: NAS, NAM, and NAE, and she is a passionate advocate for diversity in science and engineering and a serial biotech entrepreneur.

Xiaoyuan (Shawn) Chen is Nasrat Muzayyin Chair Professor in Medicine and Technology, Yong Loo Lin School of Medicine and Faculty of Engineering, National University of Singapore. His current research interests are mainly theranostics (radiotheranostics, nanotheranostics, immunotheranostics, magnetotheranostics, phototheranostics and so on) that can be clinically translatable. He has published more than 900 papers and numerous books (total citations >105,000, H index 169 based on Google Scholar).

Marina Dobrovolskaia leads the Nanotechnology Characterization Laboratory (NCL), supporting the extramural nanotechnology research and development community to advance the translation of promising nanotechnology concepts from bench to clinic. She has pioneered the field of preclinical nano-immunotoxicology by establishing the NCL immunology assay cascade, sharing her knowledge in more than 100 publications, and making seminal contributions to international standards development initiatives.

Twan Lammers is professor of medicine and head of the Department of Nanomedicine and Theranostics at the Institute for Experimental Molecular Imaging (ExMI) at RWTH Aachen University Clinic. His group aims to individualize and improve disease treatment by combining drug targeting with imaging. To this end, image-guided drug delivery systems are being developed, as well as theranostic materials and methods to monitor tumour growth, angiogenesis, inflammation, fibrosis and metastasis. He is a co-founder of SonoMAC GmbH, and serves on the scientific advisory board of BiOrion, Sense Biopharma and Cristal Therapeutics.

It is worth noting that many of the most exciting emerging analytes are themselves nanoparticles, such as circulating tumour DNA that exists as an 11 nm nucleosome blood-borne particle, extracellular vesicles such as exosomes, and 'synthetic biomarkers' that are engineered to liberate nanoscale analytes[10]. Thus, the foundational knowledge of nanoscale material properties and analytical infrastructure that we have established in nanotechnology is poised to be leveraged for cancer detection and diagnosis.

Notably, most global cancer deaths occur in low and middle-income countries[11]. The infrastructure for facing this emerging challenge is just barely being established, and the integration of bespoke innovative technologies from the outset may present an opportunity to leapfrog current paradigms that are infrastructure intensive (for example, stool tests rather than colonoscopies, point-of-care paper tests rather than laboratory tests). Global oncology represents an enormous opportunity for nanotechnology to broaden its impact[12].

X.C. Yes, nanostructures have unique features that small molecules may not have. When properly designed, nano-formulas and nanotechnologies can be applied for high-sensitivity and high-specificity detection of biomarkers in vitro and for non-invasive multimodality imaging of biological events in vivo:

- Nanotechnology for in vitro diagnostics. Nanotechnology offers high selectivity and sensitivity and the ability to conduct simultaneous measurements of multiple targets. Biosensors can be improved with nanoparticles and nanomaterials to provide specific targeting. In addition, the use of nanoparticles provides an increased surface to volume ratio, which makes nano-biosensors highly sensitive in detecting circulating tumour cells, proteins, nucleic acids and so on. Quantum dots (which are colloidal fluorescent semiconductor nanocrystals)[13], gold nanoparticles[14] and polymer dots (which are π-conjugated organic polymers)[15] are three nanoparticle probes commonly used preclinically and clinically in diagnosing cancer.
- Nanotechnology for in vivo imaging. Nanoparticle probes can preferentially accumulate in tumour tissues through active and/or passive targeting, thereby allowing imaging and diagnosis of cancer in vivo[16]. For example, radiolabelled nanoparticles are uniquely suited for PET or SPECT mapping of sentinel lymph nodes. T_2-weighted magnetic resonance imaging (MRI) probes are mostly iron oxide nanoparticles. For contrast-enhanced ultrasound, microbubbles (spheres containing gas and filled with a contrast agent) and nanobubbles have significantly improved the resolution and sensitivity of ultrasound images. Nanoparticles with multiple functions can also be applied to multimodality imaging, which is otherwise not possible with small molecules[17].

M.A.D. Nanotechnology offers many opportunities for cancer detection and diagnosis. One of my favourites is the ability of nanoparticles to shift the sensitivity of diagnostic methods to otherwise hard to achieve femtogram levels. Unfortunately, this fantastic property has not yet found its broad applicability because of the lack of clinically acceptable biomarkers that would reliably signal the disease at such low levels.

In the 17 years since the launch of the Nanotechnology Characterization Laboratory (NCL) Assay Cascade in 2005, we have noticed a progressive decrease in the number of diagnostic imaging agents, with most materials submitted to the NCL for characterization being therapeutics and vaccines. This trend perfectly aligns with the experience of the National Cancer Institute (NCI), which has observed a similar decline in cancer imaging agent grant applications[18]. The reasons underlying the dominance of nano-enabled drugs versus diagnostic agents are not completely understood, but among others include challenges with achieving selective tissue targeting; accumulation and persistence in tissues; inherent toxicities; higher safety standards for clinical trials; and low overall return on investment.

T.L. Absolutely. But not with 20–200 nm multifunctional nanoparticles for multimodal functional and molecular imaging[19]. Although nanoparticles in this size range typically do possess long circulation times, they are not needed for vascular and perfusion imaging, as there are multiple clinically approved alternatives for angiography. When aiming to molecularly image receptors overexpressed on cancer cells, nanoparticles' long circulation times and their propensity to passively accumulate in tumours play against them, resulting in false positives. For localizing tumours and metastases, nanoparticles are unable to compete with established MRI, computed tomography (CT), ultrasound and PET probes and protocols, not least because of the high variability in nanoparticle tumour accumulation, which will result in false negatives.

There are several exceptions, related to the detection of tumour margins, sentinel lymph nodes and lymph node metastases. Gold nanoparticles and pH-responsive fluorescent micelles have been shown to facilitate tumour delineation and metastatic lesion localization via surface-enhanced Raman spectroscopy and intraoperative optical imaging, respectively. Peritumourally injected ^{99m}Tc-labelled sulfur and albumin colloids with a size of 6–600 nm are routinely used in nuclear medicine to localize sentinel lymph nodes. Also, intravenously injected ferumoxtran-10 iron oxide nanoparticles with a size of 20–40 nm have been showing promising results for the detection of small lymph node metastases in patients with prostate cancer.

Beyond in vivo imaging, significant progress is expected in the next couple of years in the development of nanotechnological materials and methods for enhancing the sensitivity and specificity of ex vivo diagnostic tools, enabling the detection of cells, proteins or nucleic acids at very low concentrations, in tiny volumes of bodily fluids or tissue specimens. In parallel, technologies will be increasingly established to extract and analyse endogenous nanoparticles (such as exosomes, microparticles and apoptotic bodies; collectively referred to as extracellular vesicles) from liquid and tumour tissue biopsies, in order to use the information contained within them for diagnostic purposes, such as cancer screening, staging, therapy selection and treatment monitoring.

How can we improve nanoparticle delivery?

S.N.B. A significant body of work in the past decade has helped shed light on the journey a nanoparticle takes to reach its destination; from the formation of protein corona in the bloodstream to overcoming subsequent physiological barriers at the organ, tissue, cellular and subcellular levels[20]. This improved insight has informed a newer generation of targeting technologies to enhance binding to specific tissues and cell populations; for example, by leveraging serum absorption of apolipoproteins to target the liver[21]; endogenous trafficking processes such as albumin hitch-hiking to increase accumulation in lymph nodes[22]; or other biologically inspired processes such as decorating nanoparticles with peptides that leverage active transport pathways akin to viruses for deeper penetration into tumour tissues[23]. To complement these bio-inspired

approaches, unbiased models of nanoparticle drug delivery systems have also been established. For example, high-throughput combinatorial screening of large nanoparticle libraries via barcoding systems enables rapid and functional screening in vivo[24], and universal formulation strategies have been devised to systematically alter nanoparticle delivery behaviour[25]. Thus, both rational design based on evolutionary winners and unbiased approaches leveraging high-throughput science and data analytics offer paths forward to improve nanoparticle delivery to sites of interest.

X.C. To improve nanoparticle delivery, we need a better understanding of the journey that nanoparticles take in the body after different routes of administration[26]. In the past, we have overemphasized the concept of leaky tumour vasculature and the so-called enhanced permeability and retention (EPR) effect. This often works quite nicely in preclinical xenograft models but not necessarily in the clinic. The nano-formula will circulate in the blood, extravasate to the interstitial space, penetrate the tumour microenvironment, bind to the target on the tumour cells, internalize into the intracellular compartment, release the cargo and, finally, act to kill or provide an insult to the cells of interest[27]. Every step of this process can be considered in the design of nano-formulas to improve delivery, reduce unwanted reticuloendothelial system uptake, and lead to safer and more efficacious treatment outcomes.

For example, patients can be stratified (for instance, by contrast-enhanced MRI using nanoparticles) according to their tumours' propensity to passively accumulate nanomedicines. Furthermore, nanomedicines can be made into smart formulas that can transform in terms of size, charge, shape and so on by external (for example, temperature, magnetism, light and X-ray) or internal (for example, pH and enzyme) stimuli for more efficient tumour delivery. Nanomedicines can also be designed to target not only tumour cells but also various stromal components in the tumour microenvironment to modulate the immune system to recognize cancer cells, or to target and potentiate tumour-infiltrating myeloid cells[28]. For some applications, nanomedicines such as cancer nano-vaccines can be designed to be administered locally (for instance, via topical or intramuscular routes), which can drastically reduce off-target effects[29]. We can also further improve encapsulation and targeting strategies to maximize the amount of drug or drug combinations delivered to the tumour region.

M.A.D. The key to improving drug delivery is understanding the relationship between nanoparticle physico-chemical properties and the desired type of biological responses. Although a lot has already been achieved in this direction, many areas are still unclear owing to the structural complexity of nanoparticles and the limitations of current methods for nanoparticle physico-chemical characterization. The significant barrier in this area is that not all physico-chemical characterization methods and characterization frameworks apply to all nanoparticle types. Moreover, using a nanocarrier successfully to deliver one drug does not equate to success in the delivery of another drug. One dream for a formulation scientist is a computer algorithm that would take an active pharmaceutical ingredient (API) structure and information about the delivery target site, and select a nanoparticle carrier with optimal properties for achieving such delivery. Many bioinformatics attempts are currently underway but, to my knowledge, none of them so far is broadly applicable to all types of drugs and all types of nanocarriers.

On another important note, according to one recent study, the female menstrual cycle dramatically changes the delivery of nanoparticle-formulated drugs to tumours in various locations in the body[30]. Therefore, another approach for delivery improvement is advancing the fundamental understanding of how biological processes such as circadian rhythms and hormonal fluctuations influence nanoparticle pharmacokinetics, efficacy and safety.

T.L. We have to realize that several clinically used drug delivery platforms, particularly antibodies (that is, nature's own targeting vectors) and PEGylated liposomes, already present with very good tumour-targeting capabilities. If tumour targeting with such formulations does not work out well in certain patients, then this is typically not due to the delivery system but, rather, to tumour-specific pathophysiological constraints, such as poor tumour perfusion and high stromal density.

There are multiple means to improve nanoparticle delivery to tumours. These include pharmacological and physical priming approaches, using systemically administered agents to induce vascular normalization or stromal remodelling, as well as locally applied co-treatments, such as hyperthermia, radiotherapy and ultrasound[31]. A downside of the latter is that they are locally constrained, and thus not very helpful in the case of metastasis. A downside of priming treatments in general is that it is nearly impossible to non-invasively, quantitatively and repetitively monitor tumour-directed drug delivery in patients. Consequently, we typically do not know how good or bad tumour-directed drug delivery actually is, and we will have to come up with ways to rationalize which pharmacological or physical co-treatment may work at which dose for which tumour in which patient.

An important misconception with regard to improving nanoparticle delivery to tumours relates to the added value of 'active' targeting. It seems that people fail to realize that active ligand-mediated targeting depends heavily on passive targeting principles, particularly on a long nanoparticle circulation time, efficient tumour perfusion, high tumour vascular permeability and proper tumour tissue penetration. None of these principles profit from the presence of a targeting ligand, and some of them may even suffer from it; for example, a shorter circulation time resulting from more rapid phagocytic capture, or less efficient tumour penetration because of the presence of the binding site barrier. What may change upon active targeting is that cancer cell uptake (versus otherwise predominant uptake in tumour-associated macrophages) and tumour retention increase a little, but certainly not massively. Active targeting should therefore not be used to enhance overall tumour-directed drug delivery but, rather, as a means to ensure uptake by specific cells in tumours. When considering the use of nanoparticles for anti-oncogene siRNA therapy, then active targeting may be a must, as siRNA really needs to be delivered into cancer cells. This is different for small-molecule drug delivery, for example liposomal doxorubicin, which upon predominant uptake in and processing by tumour-associated macrophages will become available to neighbouring cancer cells.

In the era of cancer (nano-) immunotherapy, we have furthermore realized that targeted delivery to tumours and tumour cells is not at all a must. Using nanoparticles to deliver immunomodulatory agents to antigen-presenting cells in the spleen or to myeloid progenitor cells in the bone marrow may be at least equally promising[32,33]. This is arguably easier to achieve, as it targets tissues with a natural propensity for nanoparticle accumulation, and therefore suffers much less from issues

related to heterogeneity in nanoparticle tumour targeting.

What are the advantages of nanotherapeutics over conventional drugs?

S.N.B. The ability to manipulate and tune nanomaterials at a biological scale is critical to overcome physiological barriers for many therapeutic modalities, but genetic medicines especially (for example, genome editing, prime editing and RNA therapeutics). Trafficking through the body from the bloodstream to target cells and to subcellular compartments where these therapies act is an obstacle course that requires a shepherd. Running this gauntlet is the superpower of nanotechnology — whether by controlling for physiochemical surface properties, architecture or affinity groups[34].

X.C. Nanotherapeutics have a plethora of advantages over conventional drugs. Nanotechnology has been applied to increase absorbability. Nanomedicine is also applicable for drugs that are absorbed too quickly and removed from the body as waste before treatment can be effective. Furthermore, the controlled release of active components from nanomedicine can increase the time period in which a drug remains active in the body.

Nanotechnology also offers the means to target chemotherapies directly and selectively to cancer cells, guide in surgical resection of tumours and enhance the therapeutic efficacy of radiation-based and other current treatment modalities. All of these can add up to a decreased risk of toxicity to the patients and an increased probability of extended survival.

Research on nanotechnology for cancer therapy extends beyond drug delivery into the creation of new therapeutics available only through the use of nanomaterial properties. Although small compared with cells, nanoparticles are large enough to encapsulate many small-molecule compounds, which can be of multiple types. At the same time, the relatively large surface areas of nanoparticles can be functionalized with ligands, including small molecules, DNA or RNA strands, peptides, aptamers or antibodies. These ligands can be used for therapeutic effect or to direct nanoparticle fate in vivo. These properties enable combination drug delivery, multimodality treatment and combined therapeutic and diagnostic action, known as 'theranostics'. The physical properties of nanoparticles, such as energy absorption and re-radiation, can also be used to disrupt diseased tissue, as in laser ablation and hyperthermia applications.

Integrated development of innovative nanoparticle packages and APIs will also enable exploration of a wider repertoire of active ingredients, no longer confined to those with acceptable pharmacokinetics or biocompatibility behaviour. In addition, immunogenic cargo and surface coatings for nanoparticles are being investigated as adjuvants to traditional radiotherapy and chemotherapy as well as stand-alone therapies. Innovative strategies in this regard include the design of nanoparticles as artificial antigen-presenting cells[35] and in vivo depots[36] of immunostimulatory factors that exploit nanostructured architecture for sustained antitumour activity.

M.A.D. There are many advantages, including, but not limited to, improved solubility of hydrophobic drugs, prolonged circulation and controlled release, targeted delivery, API stabilization and protection from rapid clearance by mononuclear phagocytic cells. As a scientist focusing on drug safety, I want to concentrate on reduced toxicity. For example, hypersensitivity reactions to the Cremophor EL-based formulation of the otherwise highly efficacious anticancer drug paclitaxel (Taxol) is the number one reason for switching patients' treatment to a nano-albumin-based formulation, Abraxane[37]. Likewise, reduction of cardiotoxicity has led to the success of liposomal doxorubicin (Doxil) over its traditionally formulated counterpart, Adriamycin[38]. The maximum tolerated dose (MTD) of recombinant cytokine tumour necrosis factor (TNF), which failed clinical trials owing to systemic toxicity, was 200 $\mu g\,m^{-2}$, whereas PEGylated gold nanoparticle-formulated TNF successfully passed a phase I clinical trial at the threefold higher dose (600 $\mu g\,m^{-2}$) without reaching the MTD[39].

T.L. Nanotherapeutics are traditionally developed to improve pharmacokinetic features. By decreasing metabolism and excretion, nano-formulations prolong drug presence in the bloodstream, thereby enhancing their ability to accumulate in tumours. At the same time, nano-formulations typically reduce the volume of distribution, aiding in attenuating drug localization in healthy off-target tissues. Together, these features contribute to a better control over side effects, as well as — at least in some cases — to better therapeutic outcomes.

Beyond such traditional incentives, more recently explored advantages of nano-formulations include: (1) the ability to efficiently protect labile and/or immunogenic cargo, such as mRNA, not only in COVID-19 vaccines but also in cancer vaccination and immunotherapy set-ups; (2) the propensity to controllably engage with (subsets of) immune cells, particularly also outside tumours, providing novel opportunities for cancer nano-immunotherapy[32,33]; (3) the capacity to co-formulate multiple drugs in one nanoparticle, thereby enabling the delivery of optimal ratios of two (or more) agents to tumours and metastases for better combination therapy outcomes; and (4) the possibility to integrate nanosize-dependent physical features into therapeutics that cannot be conveyed by small-molecule analogues, for example for use in magnetic fluid hyperthermia (NanoTherm) or for enhancement of radiotherapy responses (Hensify, AGuIX).

How can we better conduct preclinical testing of nanotechnologies to ensure efficacy and safety?

S.N.B. It is no secret that preclinical testing in oncology is challenging. Cancer nanotechnology carries the same burden. The best way forward is broad engagement across disciplines and sectors to share learnings, data and models. At the Massachusetts Institute of Technology (MIT) Koch Institute, several faculty members have worked together to launch and sustain a community of interest through the Marble Center of Cancer Nanomedicine. The Center has been a platform for discussing all aspects of clinical translation related to nanotechnology, as well as engaging with basic scientists and clinical partners across various institutions. More recently, we have launched a new initiative at the Center — along with several industry partners — to enable breakthrough innovations in all areas impacted by nanomedicine, from drug delivery to gene editing, biomedical imaging and diagnostics[40]. We embrace the philosophy that convening a broader community of experts is the best way to accelerate delivery of safe and effective solutions to patients with cancer.

X.C. Key issues related to the clinical development of nanomedicines include biological challenges, large-scale manufacturing, biocompatibility and safety, government regulations and overall high cost in comparison with the current therapies. These factors

can impose significant hurdles limiting the appearance of nano-formulas on the market, irrespective of whether they are therapeutically efficacious or not.

As most nanomaterials are immunogenic, properly designed in vitro and in vivo studies accounting for immunotoxicity and haematotoxicity tests are necessary. Moreover, the effect of the immune system on the ability of nanoparticles to perform targeted drug delivery needs to be better understood.

In terms of tumour models to test the nano-formulas, use of subcutaneous xenograft models is a good first step to support an experimental hypothesis. However, such artificial tumour models grown in immunodeficient mice do not recapitulate the human cancer situation. Spontaneous tumour models and humanized mouse models with patient-derived xenografts would be better suited to study the immunological side effects and potential efficacy of the desired nano-formulas.

Imaging can play an essential role in the preclinical evaluation of nanomedicine-based drug delivery systems and has provided important insights into their mechanism of action and therapeutic effect. For clinical translation, suitably labelled nano-formulas can be injected at microdose level for phase 0 study of pharmacokinetics, biodistribution and target site accumulation of nanomedicine-based drug delivery systems, which can help pave the way for standard dose-escalation phase I studies and more advanced phase II/III efficacy studies.

M.A.D. I propose applying the 3M concept — mechanisms, markers, models — to improve preclinical testing. First, restructure and supplement traditional studies with studies focusing on 'liability' arising from toxicities common or expected for the given API, carrier and indication. This requires understanding the mechanisms of nanoparticle and drug toxicities both alone and in the context of the final product. Second, gain a fundamental understanding of how genetic variability in key elements of the immune response (for example, human leukocyte antigen (HLA) types, pattern recognition receptors (PRRs), complement and tryptase) and underlying conditions (for example, common variable immuno-deficiency, asthma, mastocytosis (a rare disorder characterized by abnormal accumulation and activation of mast cells) and myelodysplastic syndrome (a common haematological malignancy)) influence the body's response to the same type of nanocarrier. This would aid in the personalization of nanotechnology-based applications (therapies, diagnostics and vaccines) and improve their safety (for example, see the recent insights gained from the use of lipid nanoparticle vaccines)[41] by creating a panel of reliable markers for each 'liability'. Third, overcome the barrier arising from the common notion that animal models used in preclinical research do not accurately recapitulate what happens in humans. This is especially true for immune responses. Humanized mice are utilized with limited success. One alternative, thought-provoking idea would be to consider the use of naturalized models (that is, environmentally, and therefore immunologically, 'wild' strains of mice)[42] and, when applicable owing to the shared toxicity with humans, not widely accepted models (for example, pigs for prediction of infusion reactions)[43]. Although using these models would come at the expense of greater costs, complexity and data variability, they may be more realistic and, thus, more relevant to humans.

T.L. Standardization is important and comparative analyses are key. Reporting guidelines towards standardization in bio–nano research have recently been postulated, but have pros and cons[44,45]. The billion-dollar question in this regard is what needs to be demonstrated preclinically to ensure efficacy and safety in the clinic. Convincing proof of concept is required for tolerability and mechanism of action, ideally in multiple established preclinical model systems. Conceptually comparable with the NCI-60 human tumour cell lines screen[46], the implementation of a standardized panel of laboratory animal model systems for the cancer in question, in which novel (nano-) drug formulations are controllably compared head to head against the current clinical standard-of-care treatment, would help a lot to properly ascertain the potential of (nano-) drugs at the preclinical stage.

Specifically for nanomedicines, it will be important to start identifying biomarkers and developing companion diagnostics already at the preclinical stage. This has become standard for almost all other oncology drugs, and has proven to be crucial for the majority of agents that made it to the market in the past 10–20 years. Remarkably, however, this is still hardly ever considered for cancer nanomedicines.

Not specifically for nanomedicines — but broadly applicable to the development of (anticancer) drugs) — it would be extremely helpful if academic and industrial scientists would more readily publish and openly discuss reasons for failure. Many advanced-stage preclinical and phase I–III clinical trials do not meet expectations and end points. However, these negative results are hardly ever communicated, although they are potentially providing very valuable pieces of information, to various types of stakeholders. Because of this, (nano-) drug development and clinical progress are significantly slowed down, in multiple different regards. Institutionalized measures to close these gaps in transparency and translation are urgently needed.

Sangeeta N. Bhatia[1,2,3,4,5]✉, *Xiaoyuan Chen*[6,7,8,9,10,11]✉, *Marina A. Dobrovolskaia*[12]✉ *and Twan Lammers*[13]✉

[1]*Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA, USA.*

[2]*Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA, USA.*

[3]*Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA.*

[4]*Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.*

[5]*Howard Hughes Medical Institute, Chevy Chase, MD, USA.*

[6]*Department of Diagnostic Radiology, Yong Loo Lin School of Medicine and Faculty of Engineering, National University of Singapore, Singapore, Singapore.*

[7]*Department of Surgery, Yong Loo Lin School of Medicine and Faculty of Engineering, National University of Singapore, Singapore, Singapore.*

[8]*Department of Chemical and Biomolecular Engineering, Yong Loo Lin School of Medicine and Faculty of Engineering, National University of Singapore, Singapore, Singapore.*

[9]*Department of Biomedical Engineering, Yong Loo Lin School of Medicine and Faculty of Engineering, National University of Singapore, Singapore, Singapore.*

[10]*Clinical Imaging Research Centre, Centre for Translational Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.*

[11]*Nanomedicine Translational Research Program, NUS Center for Nanomedicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.*

[12]*Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD, USA.*

[13]*Department of Nanomedicine and Theranostics, Institute for Experimental Molecular Imaging (ExMI), Center for Biohybrid Medical Systems (CBMS), RWTH Aachen University Clinic, Aachen, Germany.*

✉*e-mail: sbhatia@mit.edu; chen.shawn@nus.edu.sg; marina@mail.nih.gov; tlammers@ukaachen.de*

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Competing interests

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